

**Abstracts**

# **CROI** 2016

Conference on Retroviruses  
and Opportunistic Infections

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 **IAS-USA**  
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# ORAL ABSTRACTS

## 1 Program Committee Workshop for New Investigators and Trainees: Session Summary

**Moderators:** **Scott M. Hammer**, *Columbia Univ, New York, NY, USA* and **John W. Mellors**, *Univ of Pittsburgh, Pittsburgh, PA, USA*

Formidable progress has been made in our understanding of HIV pathogenesis, epidemiology, treatment, and prevention in the past 3 decades. However, much more research must be done if we are to realize the goal of ending the HIV/AIDS pandemic. To address the persistent need for new ideas and innovation, the Program Committee (PC) Workshop for New Investigators and Trainees has been an annual kick-off event of the Conference on Retroviruses and Opportunistic Infections (CROI). Its purpose is to engage young investigators in identifying the scientific questions to be answered and hurdles to be faced. This is an opportunity for PC members to interact directly with attendees and provide insights into state-of-the-art basic and clinical research areas while providing guidance and orientation for the meeting that is about to unfold. The 2016 PC Workshop New Investigators and Trainees will feature the following speakers and topics: (1) Dr Wesley Sundquist will address selected topics in molecular virology, specifically advances in understanding of HIV-1 Nef function and viral assembly and packaging; (2) Dr Richard A. Koup will discuss the state of HIV vaccine research focusing on human immune response in relation to the microbiome and the role of neutralizing and nonneutralizing antibodies in control and prevention of HIV infection; (3) Dr Huldrych F. Günthard will describe current knowledge of HIV-1 reservoir(s) and the challenges faced in quantifying and eliminating the replication competent component; (4) Dr Sharon L. Hillier will discuss the most recent advances in HIV prevention, including the importance of developing and deploying a variety of biomedical and behavioral options and the particular challenge of reaching special populations at high risk of infection; and (5) Dr Judith S. Currier will address pathogenesis of and interventions for long-term complications of treated HIV infection, focusing on cardiovascular disease, bone metabolism, renal disease, and frailty. These concise state-of-the-art presentations by PC members are designed to inform and inspire new investigators and to maximize their CROI experience.

## 2 Panel Discussion on Stigma, Trauma, and Stress: Considerations for HIV Research and Programs

**Moderator:** **Morenike Ukpong-Folayan**, *Obafemi Awolowo Univ, Ile-Ife, Nigeria*

In the last several years, significant progress has been made with HIV treatment and prevention research. Access and use of research outcomes have been slow however, and so has the translation of research outcomes to policy and action within the most severely affected regions of the world. Many successes and landmark efforts in research translations have resulted through strategic partnerships established between researchers, community members and other relevant stakeholders. Community visibility in the research process can have significant impact on community ownership of research outcomes and advocacy for uptake and use of study products. The panel discussants will share lessons learned from the field of HIV research implementation, translation to policy and programs, and final uptake and use by communities; highlight the impact of stigma in the design and implementation of HIV research and its implication for uptake and use of study products; discuss the place of human rights in making decisions about research planning and implementation (lessons from hormonal contraception advocacy); and the influence of history, culture and power disparities on research management. Also, the panel will discuss the implications of stigma rights and power disparities for community recruitment in HIV research, and uptake and use of study products. The implications of these for trauma and stress of communities will also be highlighted. Facilitating Community involvement in HIV research, as well as the translation and dissemination of research findings and products, is critical to ending the epidemic. Such efforts should promote a sense of empowerment, mental health, and spiritual well-being for people infected and affected with HIV. This requires negotiating boundaries about how identities are defined and relationships are established. Stigma rights and power disparities shape these identities and influence community engagement in research uptake of research outcomes.

## 3 Key Considerations in Studies of Antiviral Pharmacokinetics and Pharmacodynamics

**Jennifer Kiser**, *Univ of Colorado, Denver, CO, USA*

Clinical pharmacology determines drug efficacy and toxicity. Knowledge of structure-activity relationships, mechanisms of action, absorption, distribution, metabolism and excretion, drug localization, and concentration-effect associations are critical for informed and improved use of drugs. New technologies and analytical approaches allow for an enhanced understanding of antiviral pharmacology. These measures, which can be readily incorporated into clinical trials, allow researchers to capitalize on opportunities to provide context to trial outcomes. This talk will focus on strategies for incorporating pharmacokinetic/pharmacodynamic measures into the current research agendas for HIV prevention, cure, and viral hepatitis. The application of statistics and mathematical modeling (population pharmacokinetics, physiologic-based pharmacokinetic modeling) to analysis of pharmacology data will also be discussed.

## 4 Using Big Data to Improve the HIV Care and Prevention Continuum

**Patrick Sullivan**, *Emory Univ, Atlanta, GA, USA*

This presentation will explore how big data can be used to improve the HIV care and prevention continuum. We will discuss the use of multiple data sources to depict the distribution of HIV in communities, and to illustrate how existing prevention and treatment resources relate to needs. Data from the synthesis of surveillance data, Census data, and service provider data will be illustrated, and use cases will be presented to show how synthesized data have been used to identify resource gaps and improve program impact. We will also review efforts to link data from social media and other sources to develop an "early warning" system that could identify areas in need of increased HIV prevention efforts while risk is ongoing. Examples will be provided of the types of approaches that are being evaluated, and of preliminary results of such studies. We will explore the utility of data from medical records systems and applications in public health settings, and discuss future opportunities to utilize unstructured data to develop new knowledge. The presentation will highlight examples of current studies, will provide resources on types of data available, and will share information on how to access key existing sources of data relevant to HIV prevention and care.

## 5 HIV Transmission Networks and HIV Prevention in the Era of TasP and PrEP

**Martina Morris**, *Univ of Washington, Seattle, WA, USA*

As we move into the era of scaling up biomedical HIV prevention and care, it remains important to understand how the underlying HIV transmission network will influence the population level efficacy of these prevention efforts. The existing disparities in HIV prevalence and incidence may widen, as some communities successfully fall below the persistence threshold, while other communities struggle with declining but continuing endemic transmission. This talk will review the types of data needed for estimating the underlying patterns of network connectivity and their impact on HIV transmission and prevention dynamics. Recent advances in statistical and epidemic modeling now make it possible to exploit simple egocentric sampling designs that can be used in a variety of survey settings. We will give examples of how such network data is being used for HIV prevention planning purposes, and discuss priorities for future research.

**6 Deciphering Mucosal Barrier Functionality and HIV Immunity by Mass Spectrometry****Adam Burgener**, *Univ of Manitoba, Winnipeg, MB, Canada*

Advances in proteomic techniques, such as mass spectrometry, allow for an unprecedented ability to study immunological systems, by characterizing thousands of proteins simultaneously in biological samples with high sensitivity and dynamic range. Mass spectrometry can be utilized to study whole proteomes, their modifications, location, and even interactions, which has led to the growing adoption of this technology in biomedical research and furthered our understanding of human diseases and immunity. In HIV infection, mucosal immune systems are an intense area of research given the critical roles it plays in both HIV transmission and disease. Understanding mucosal immune systems has significant relevance for HIV prevention efforts and vaccine design, where their success rest upon their ability to elicit or maintain protective mucosal immunity while simultaneously avoid inflammation processes which enhance HIV susceptibility. However, mucosal immune correlates of HIV infection largely remain undefined, largely driven by comprehensive toolsets to study mucosal surfaces, of which mass spectrometry technology can afford. In this presentation, I will first introduce the mass spectrometry workflow and the opportunities this technology provides to understand complex biological samples and host immunity, with particular consideration for clinical studies. I will then provide examples relevant to the HIV field, by discussing how mass spectrometry has provided novel mechanistic insight into mucosal immune system changes associated with HIV risk factors, such as hormonal contraceptives, genital tract inflammation, and microbial dysbiosis. Finally, I will show how this can be integrated with large-scale clinical trials, by discussing our ongoing studies of proteome profiling of >800 women at high risk for HIV from South Africa (w/ CAPRISA), the generation of proteome libraries spanning thousands of host and microbial factors, and the potential this information has to understand host pathogen interactions, HIV transmission, and reproductive health. This presentation will provide a broad overview of how to incorporate mass spectrometry into clinical/lab studies, how it can be integrated with other immunological platforms (microbiome, cellular, cytokines, etc.), and how it can contribute to HIV prevention technology development and reproductive health studies for women.

**7 Drop-Based Microfluidics for Single-Cell Studies****David A. Weitz**, *Harvard Univ, Boston, MA, USA*

This talk will report on the use of drop-based microfluidics to investigate the behavior of single cells. The method entails the creation of small aqueous drops, each containing picoliters to nanoliters of fluid, immersed in a continuous, inert oil phase. The oil phase provides the fluidic control, enabling each drop to be manipulated and studied. The drops are the optimal size to control a single cell, or a single cell plus a second, target cell. In addition, each drop can contain an arbitrary number of viruses or other infectious agents; this number can be as small as a single virus. Interactions among the virus, the target cell and the probe cell can be probed, with all components trapped within the confines of the drop. Each drop represents an independent experiment, and up to hundreds of millions of drops can be probed in a single experiment. This enables very high throughput screening of either the probe cells or the behavior of the virus. The readout can be either optical or through bar-coded DNA, read through next generation sequencing. Alternatively, if specific cells can be identified, they can be selected and sorted from the rest of the sample for further use or analysis. This talk will explore applications of this method to probe up to millions of cells at single-cell resolution. The probes include full transcriptome measurement at single-cell resolution, viral neutralization through antibody secretion and cell-cell interactions.

**8 Integration of Systems Biology with Tissue Engineering and Organs-On-Chips****Linda Griffith**, *Massachusetts Inst of Tech, Boston, MA, USA*

Move over, mice! Systems biology – data-driven modeling of extracellular and intracellular communication networks linked to phenotypic outcomes at the cell, tissue, or systemic level in patients – is yielding new insights into mechanisms of HIV infection, variability in patient responses to infection, and responses to therapies. For example, CAPRISA investigators have shown a complex role for inflammation in the female reproductive tract on HIV susceptibility, with elevated levels of certain cytokines associated with barrier disruption and increased susceptibility. In vitro human tissue engineered models that recreate mucosal barrier tissues in long term culture, with the ability to probe local paracrine cytokine and growth factor networks operating between epithelia, stromal and immune cells longitudinally over time in culture, offer the potential to parse mechanisms and test efficacy of interventions. The first part of this presentation will describe approaches to recapitulate 3D barrier mucosal tissues using synthetic extracellular matrix that supports long term reconstitution of tissues and can be dissolved on demand to (a) measure communication networks via multiplex luminex-type assays and (b) release cells without alteration of cell surface proteins or disruption of cell-cell junctions; synthetic matrix approaches can be tailored to specific epithelial barriers. Such approaches work in parallel with explant cultures, allowing polarized tissues with defined compositions to be created. The second part of the presentation will address development of “organs-on-chips” approaches to analyze systemic interactions between epithelial barrier tissues (gut, reproductive tract) and the liver, with respect to how inflammation cross talk influences responses to therapeutics. Emphasis will be placed on the practical design of platforms that enable quantitative control of PK/PD of drugs and endogenous biological regulators, particularly lipophilic compounds such as sex steroids (e.g estradiol) and antiretroviral drugs, which commonly have log D values of 2-4 and thus strongly partition into the popular PDMS (silicone rubber) microfluidic systems, making alternate approaches to fluidic systems necessary for quantitative analysis. Further, challenges with designing platforms to measure inflammation crosstalk and potential applications of technologies arising from the DARPA/NIH Microphysiological Systems program will be described.

**9 Treatment for HCV Genotype 1-Infected Patients With HIV/HCV Coinfection****Anne F. Luetkemeyer**, *Univ of California San Francisco, San Francisco, CA, USA*

Treatment of HCV is a priority in HIV/HCV co-infected patients due to the morbidity, mortality and potential for more rapid disease progression that HCV incurs in HIV infection. Choice of DAA treatment is complicated by drug-drug interactions with HIV antiretrovirals. This interactive session will review currently available DAA treatment options for HIV/HCV individuals with HCV genotype 1 with attention to drug interactions and consideration of any impact that HIV infection may have on treatment duration and outcome.

**10 HCV Genotype 3 Infection: Treatment for Patients With Cirrhosis and Treatment Failure****Karine LaCombe**, *Sorbonne Univs, Paris, France*

Genotype 3 (GT3) HCV chronic infection has long been considered as difficult-to-treat. Mainly transmitted through intravenous drug use, GT3 HCV presents certain conformational peculiarities, rendering the activity of common direct antiviral agents less potent than other genotypes. It also negatively interacts with lipid metabolism, which increases the risk of developing steatosis and in turn has a synergistic and deleterious effect on the evolution of liver fibrosis. All these factors together explain why many GT3 HCV patients have failed previous antiviral treatments and have now reached advanced stages of liver fibrosis and cirrhosis, becoming increasingly difficult to treat. During this presentation, we will review the clinical and therapeutic history of a GT3 HCV mono-infected patient and highlight the issues that clinicians must face in care and management, including the assessment of drug-drug interactions and evaluation of liver cirrhosis before treatment and beyond cure.

**11 HCV Infection Treatment Before and After Liver Transplant****Elizabeth C. Verna**, *Columbia Univ, New York, NY, USA*

Hepatitis C (HCV) remains the most common indication for liver transplantation (LT) in the United States, and graft outcomes have traditionally been inferior to patients without HCV due to recurrent disease. The recent transformation in our ability to eradicate chronic HCV infection with well-tolerated, potent interferon-free regimens has dramatically changed our management of HCV before and after LT. The dramatic improvement in the efficacy and tolerability of the direct acting antiviral (DAA) agents now renders the goal of treatment for all patients with decompensated liver disease, either before or after LT, a potential reality. Treatment for cure on the LT waiting list to potentially avoid LT or to eliminate the risk of recurrent disease in the allograft would be the ideal approach if feasible and risk-free for all. In addition, data have emerged from post-LT trials revealing high

rates of SVR even in the setting of chronic immunosuppression. However significant questions remain about the optimal timing of treatment in individual patient scenarios. Thus, HCV treatment should remain individualized.

## 12 Treatment of HCV Infection in Groups at High Risk for Reinfection

**Oluwaseun Falade-Nwulia**, *Johns Hopkins Univ, Baltimore, MD, USA*

The availability of efficacious oral HCV treatments of short duration with limited side effects has removed a key barrier to HCV treatment in high risk groups. These groups such as injection drug users with potential to transmit HCV to others are high priority for HCV treatment. Several challenges however remain with respect to delivery of treatment and eventual control of HCV at the population level. HCV reinfection in particular represents a major obstacle to control of HCV in injection drug using populations. HIV infected men who have sex with men are also at risk for HCV reinfection. By presenting a case of HCV in an HIV/HCV co-infected injection drug user, we will review data to support treatment of HCV in injection drug users. Current knowledge of rates and predictors of HCV reinfection after successful treatment and potential strategies for HCV reinfection prevention will also be highlighted.

## 13 T Cell Control of HIV: Implications for Vaccines and Cure

**Bruce D. Walker**, *Ragon Inst of MGH, MIT, and Harvard, Cambridge, MA, USA*

HIV infection results in progressive and ultimately profound immune suppression in the absence of treatment; moreover, there is no evidence that the infection is ever eradicated by host defenses. However, the remarkable ability of some HIV infected persons to maintain viral loads below the limits of detection in the absence of antiretroviral therapy provides evidence that the immune system can achieve the upper hand in this infection. Since the discovery of HIV-specific CD8 T cells in 1987, numerous laboratories have contributed to a convincing array of data from patients indicating that these cells are the main contributors to controlling acute and chronic HIV infection. Massive induction of HIV-specific CD8 T cells occurs following onset of viremia in hyperacute infection, the rapidity and magnitude of which are associated with set point viral control. However, in most persons dysfunction and dysregulation of these responses as well as immune escape rapidly ensue. Emerging data provide insights to harnessing and maintaining the antiviral efficacy of CD8 T cells, which will be key to functional and sterilizing cure strategies.

## 14 N'Galy-Mann Lecture: Confronting HIV and TB From the Bronx, NY, to Tugela Ferry, South Africa

**Gerald H. Friedland**, *Yale University School of Med, New Haven, CT, USA*

Among the most striking features of the global pandemic of HIV/AIDS has been its early unrecognized and subsequent explosive arrival and its entwined disastrous relationship with tuberculosis, particularly among vulnerable populations locally and globally. In the 1980's, early in the pandemic, this relationship featured prominently in the AIDS urban epicenter of NYC and its most impoverished borough, the Bronx, and particularly among people who inject drugs and their sexual partners and children. Both HIV and TB and their complex interaction required appreciation of and attention to their shared personal, clinical and epidemiologic characteristics and their social, political and human rights context. They raised issues, as well, of how health professionals, governments and civil society respond to such unanticipated, explosive and challenging threats. The occurrence these two diseases, in this setting, presaged the repetition of these issues globally in similar impoverished global communities. Twenty years later, 8,000 miles distant, eerily similar ingredients resulted the explosive rise of in both HIV and TB and of multiple and extensively drug resistant (M/XDR) TB in urban and rural Sub Saharan Africa. This talk will explore the differences and also the similarities in the etiology and the social and human rights contexts of the entwined epidemics, focusing on the recurring themes and events in the Bronx, NY and more recently in Tugela Ferry in rural KwaZulu-Natal, South Africa. Particular attention will be focused on success and challenges of strategies elaborated to control and reverse the epidemics and their resultant morbidity and mortality in both settings. Although distinct in time and place these may have more general relevance to recent, current and inevitable future epidemics and to the challenges of the full realization of the goals of both HIV and TB elimination.

## 15 Harnessing Antibodies for HIV-1 Prevention and Treatment

**John R. Mascola**, *VRC, NIAID, NIH, Bethesda, MD, USA*

Passive immunization with polyclonal or monoclonal IgG antibodies has been used to for prevention or early post-exposure treatment of numerous infectious pathogens, particularly viruses such as Hepatitis A and B, Varicella-Zoster, Rabies and Respiratory Syncytial virus. Thus, the recent isolation of broadly neutralizing monoclonal antibodies (mAbs) against the HIV-1 has engendered interest in testing these antibodies for prevention or treatment of HIV-1 infection. Numerous preclinical studies in non-human primates demonstrate the ability of neutralizing mAbs to completely block SHIV infection when administered prior to, or shortly after, viral exposure. For prevention of infection in humans, potential advantageous characteristics of passive immunization with human mAbs include likely clinical safety and prolonged plasma concentrations, with potential to mediate protection for weeks or months after a single dose. In contrast to prevention, where mAbs need to interrupt a viral transmission event, mAbs administered in the setting of established HIV-1 infection face the obstacle of viral escape variants. Thus, combinations of mAbs may be required. A key question is whether antibody Fc-mediated effector functions, such as antibody-dependent cellular cytotoxicity (ADCC), can lead to killing of HIV-1 infected cells and impact the cell associated viral reservoir. Clinical trials to address questions of HIV-1 prevention and treatment are underway.

## 16 Antiretroviral Therapy: Where Are We Now? Where Are We Going?

**Joseph J. Eron**, *Univ of North Carolina at Chapel Hill, Chapel Hill, NC, USA*

Antiretroviral therapy has dramatically changed the course of HIV-1 infection for those infected persons who have access. As of 2014 over 13 million people world-wide have received antiretroviral therapy. In many centers in developed and developing countries 80% or more of patients on ART have plasma HIV RNA suppressed to below the limit of detection and in clinical trials of initial ART success rates are greater than or equal to 90% at a year. Later lines of therapy have increasingly higher success rates. Life expectancy for people on therapy has increased dramatically and in countries with a high percentage of infected people on therapy new HIV-1 infections have declined. Antiretroviral therapy has also become simpler and safer with better tolerated regimens, frequently including integrase inhibitors, becoming standard in many treatment guidelines. The durability of these simple, safe and effective regimens has increased and virologic failure with emergent resistance has become less common. For many patients the future of antiretroviral therapy is now.

Patients with virologic failure and multi-drug resistant virus are uncommon although there exists a much larger pool of patients with multi-drug resistant virus who are currently suppressed on more complex and perhaps fragile regimens. Therefore new agents that have activity against resistant variants will continue to be needed and those inhibiting HIV-1 via a new mechanism of action will be more likely to have activity. For other patients adherence to oral ART presents a substantial challenge. Long-acting antiretroviral agents may provide a means to serve a greater proportion of infected people either long-term or through chaotic periods in their lives. Long-acting injectable ART holds promise for the near future and alternatives such as once weekly oral therapy or implantable sustained release combination ART or vector delivery of broadly neutralizing antibody combinations may be considerations in the future. With our current therapies and new strategies in development we will have an array of antiretroviral therapies that will serve virtually all people with HIV infection and support the treatment goals of the WHO.

## 17 Rare Host Genetic Variation Influencing Risk of Heterosexual HIV-1 Acquisition

**Romel D. Mackelprang**<sup>1</sup>; Mary Emond<sup>1</sup>; Michael J. Bamshad<sup>1</sup>; Xuanlin Hou<sup>1</sup>; Jessica Chong<sup>1</sup>; Kati Buckingham<sup>1</sup>; Nelly R. Mugo<sup>2</sup>; Jared M. Baeten<sup>1</sup>; Connie M. Celum<sup>1</sup>; Jairam R. Lingappa<sup>1</sup>; for the Partners in Prevention HSV/HIV Transmission and Partners PrEP Studies  
<sup>1</sup>Univ of Washington, Seattle, WA, USA; <sup>2</sup>Kenya Med Rsr Inst, Thika, Kenya

**Background:** Since CCR5-D32, no common host genetic variant has consistently been associated with HIV-1 acquisition. However, whole genome sequencing may identify rarer novel variation that alters HIV-1 acquisition risk. We applied this approach to samples from Africans with high HIV-1 exposure to increase statistical power to detect associations.

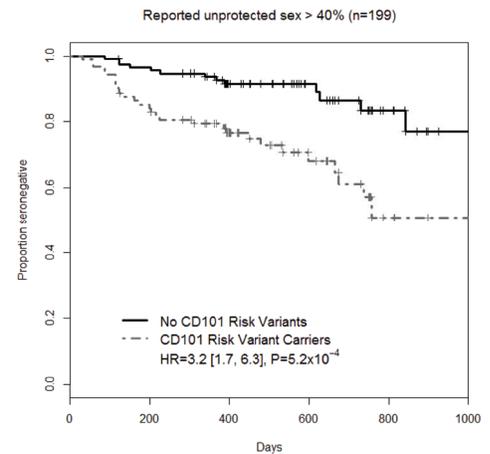
**Methods:** This analysis included Discovery and Replication stages. In the Discovery stage, whole genome sequences were compared between 50 HIV-1 seroconverting (SC) cases and 50 HIV-1 exposed seronegative (HESN) controls with the highest HIV-1 exposure among ~4000 eligible HESN. Exposure was quantified based on unprotected sex acts and plasma HIV-1 RNA of the infected partner. Comparisons were done for each gene by aggregating variants "by-gene" using RVT1 burden tests. Discovery phase genes were sequenced in 199 HESN with high HIV-1 exposure and 1030 HESN with low exposure; 179 of these HESN acquired HIV-1. We used survival analysis to determine if having  $\geq 1$  rare variants in the Discovery genes was associated with HIV-1 acquisition in the Replication sample.

**Results:** Discovery: Rare variants in CD101 and UBE2V1 were most strongly associated with HIV-1 acquisition ( $p=3.6 \times 10^{-5}$  and  $4.5 \times 10^{-5}$ , respectively). Specifically, 30 (60%) cases had 2-5 rare CD101 missense variants compared to 12 (24%) controls. Each additional rare variant raised HIV-1 infection risk by 2.6-fold (95% CI: 1.5-4.5). For UBE2V1, 27 (54%) cases had 1-2 rare variants vs 10 (20%) HESN controls; each additional variant raised HIV-1 risk 3.7-fold (95% CI: 1.7-8).

**Replication:** Associations of rare variation in CD101 and UBE2V1 replicated in the high HIV-1 exposure subset (Fig 1). Specifically,  $\geq 1$  specific rare CD101 variants identified in the Discovery stage yielded a hazard ratio (HR) for SC of 3.2 (95% CI: 1.7-6.3) among participants reporting unprotected sex at  $>40\%$  of study visits,  $p=5.2 \times 10^{-4}$ . This association decreased with lower HIV-1 exposure among the entire cohort (HR=1.4,  $p=0.02$ ). UBE2V1 variants were associated with a HR=3.2 (95% CI: 1.2-8.2;  $p=0.02$ ) in the increased exposure Replication sample.

**Conclusions:** Using whole genome sequencing, we discovered and replicated associations of rare genetic variation in CD101 and UBE2V1 with elevated HIV-1 acquisition risk. CD101 could influence HIV-1 susceptibility by altering regulatory T cell function, and UBE2V1 through immune activation. These findings may be valuable for HIV-1 vaccine development and therapeutics.

Figure 1: Kaplan-Meier curve comparing HIV-1 acquisition among participants with and without a CD101 missense variant in the Replication cohort. Participants included in this analysis reported unprotected sex at a frequency of greater than 40%.



## 18LB HIV-1 Laboratory Contagion During Recombination Procedures With Defective Constructs

**Claudia Alteri**<sup>1</sup>; Alessandro Soria<sup>2</sup>; Ada Bertoli<sup>1</sup>; Alessandra Bandera<sup>3</sup>; Gabriella Scarlatti<sup>4</sup>; Monica Tolazzi<sup>4</sup>; Emanuela Balestra<sup>1</sup>; Andrea Gori<sup>3</sup>; Francesca Ceccherini-Silberstein<sup>1</sup>; Carlo Federico Perno<sup>5</sup>

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**Background:** An accidental contagion during the production of theoretically non-infectious HIV-1 laboratory-recombinant viruses, used only for research purpose, is here described.

**Methods:** HIV-1 infection was diagnosed by ELISA assay, confirmed by Western Blot, and HIV-RNA/DNA test. Baseline plasma samples were used for routine *pol* and V3 sequences, PBMC for the whole HIV-1 genome sequencing and for *in-vitro* isolation. Phylogenetic analyses using Neighbor Joining (NJ) and Maximum Likelihood (ML) methods revealed the source of infection. Ethics committee approval and signed informed consent were obtained.

**Results:** A lab worker resulted HIV-1 positive during a routine screening HIV test. In the 6 months preceding HIV diagnosis, he/she exclusively worked on the production of a recombinant *nef*-defective HIV, starting from a *nef/**env*-defective NL4.3 vector and JRFL *env*-encoding plasmid. In the same laboratory other HIV-1 constructs were handled by other researchers at the same time. A thorough investigation did not evidence any laboratory accident during the whole period. At diagnosis, CD4 were 392 cells/ $\mu$ L, HIV-RNA 3.30 log cps/mL, and HIV-DNA 157 cps/ $10^6$  PBMC. Virus was R5-tropic. After 25 months of stable HIV-1 RNA and CD4-cells, a sudden 1-log HIV-RNA increase occurred; TDF/FTC/RPV cART was started, with full viremia control.

NJ trees revealed a B-subtype virus, totally unrelated with other 629 HIV-1 clinical strains collected at the same hospital, but clustering exclusively with NL4.3 for *pol* sequence, and with JRFL for V3 (bootstrap: 96% and 100%, respectively). Genetic distance confirmed the homology of *pol* and V3 with NL4.3 and JRFL, respectively ( $0.002 \pm 0.002$  and  $0.000 \pm 0.000$ ). Full-length viral sequence, performed in a different laboratory, confirmed the results and revealed a NL4.3/JRFL recombinant strain surprisingly expressing *nef*. A primary isolate obtained from his/her PBMC culture confirmed the *nef*-expressing NL4.3/JRFL recombinant strain. By inferring ML trees, the entire HIV-1 genome clustered again with NL4.3 (bootstrap $>99\%$ ), with the exception of *env* (strongly linked with JRFL, bootstrap=99.7%). To date, how *nef* gene (absent in both vectors) entered into the recombinant virus, and mode of contagion, remain both unclear.

**Conclusions:** This clinical case highlights that *in-vitro* recombination procedures with *per se* non-infectious vectors, in a laboratory handling multiple HIV constructs, may still represent a risk of HIV contagion notwithstanding increasing biosafety efforts.

## 19 Identification of a Highly Functional DC Subset in Controllers by Single-Cell RNA-Seq

**Enrique Martin-Gayo**<sup>1</sup>; Michael Cole<sup>2</sup>; Kellie E. Kolb<sup>3</sup>; Zhengyu Ouyang<sup>1</sup>; Sam W. Kazer<sup>3</sup>; Bruce D. Walker<sup>1</sup>; Nir Yosef<sup>2</sup>; Alex K. Shalek<sup>1</sup>; Xu G. Yu<sup>1</sup>

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**Background:** Highly-efficient T cell-mediated immune responses are crucial for antiviral immune defense in HIV controllers, but the mechanisms supporting the development of T cell immunity in these persons are not well understood. One possibility is that conventional dendritic cells (cDCs) from these Elite Controllers (ECs) may contribute to antiviral immune defense through induction of potent type I IFN responses with improved antigen-presenting properties upon exposure to HIV-1. Here, we use single-cell transcriptional profiling to analyze cell-intrinsic immune responses to HIV-1 in isolated, individual cDCs from ECs.

**Methods:** Single-cell RNA-seq profiling of HIV-1-exposed cDCs from an EC was used to identify gene expression programs associated with cell-intrinsic HIV-1 immune recognition and type I IFN signatures in cDCs. Potential surface markers for cDC subsets associated with improved functional properties were validated by flow cytometry. Functional properties of sorted cDC subsets were analyzed by co-culture with allogeneic T cells in mixed leukocyte reaction assays.

**Results:** Single-cell RNA-seq identified three distinct cDC subpopulations from an EC after exposure to HIV-1. These cDC subsets differed in expression of genes related to IFN signaling, immune activation, cytokine signaling and HIV-1 replication, and were phenotypically distinguishable by two membrane markers. Importantly, among these cDC subsets, we identified a highly functional population, preferentially induced in ECs ( $n=8$ ,  $p=0.007$ ), in contrast to progressors ( $n=8$ ) or healthy individuals ( $n=8$ ), that had distinct and potent upregulation of interferon-stimulated genes and remarkably effective functional antigen-presenting properties. Importantly, induction of this highly functional cDC subset in response to HIV-1 exposure could be facilitated by specific TLR agonists in HIV-1 negative individuals ( $n=8$ ,  $p=0.008$ ).

**Conclusions:** This study identified a previously unrecognized subpopulation of cDCs induced preferentially in ECs with improved ability to mount cell-intrinsic immune responses to HIV-1 and expand antiviral T cell immune responses. Manipulation of this dendritic cell subpopulation by TLR agonists may provide novel opportunities for improving immune-based approaches for HIV treatment and prevention in a broader patient population.

## 20 The Sooty Mangabey Genome Sequence Reveals New Insights in Natural SIV Infections

**David J. Palesch**<sup>1</sup>; Steven E. Bosinger<sup>1</sup>; Gregory Tharp<sup>2</sup>; Yue Liu<sup>3</sup>; Muthuswamy Raveendran<sup>3</sup>; Donna Muzny<sup>3</sup>; Richard Gibbs<sup>3</sup>; Kim C. Worley<sup>3</sup>; Jeffrey Rogers<sup>3</sup>; Guido Silvestri<sup>1</sup>

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**Background:** In contrast to HIV infection in humans and experimental SIV infection of rhesus macaques, SIV infection of natural host sooty mangabeys (*Cercocebus atys*) is typically non-pathogenic despite high levels of virus replication. To identify host genetic factors that allow sooty mangabeys (SM) to avoid SIV-related pathogenesis, we produced a *de novo* whole genome assembly for the SM and performed comparative analysis with AIDS-susceptible species.

**Methods:** DNA from a female SM was sequenced at the Baylor Human Genome Sequencing Center using Illumina short read technology and Pacific Biosciences long reads. The assembly was produced using ALLPATHS-LG to generate contigs and scaffolds, then Atlas-Gapfill and PBJelly for filling gaps and extending contigs. Annotation was performed through the NCBI Genome Annotation Pipeline. To identify immune molecules with divergence between SM and AIDS-susceptible primates, we aligned multiple protein sequences using the MultAlin tool. To identify assembly/annotation errors, we used RNA-seq data from multiple SM tissues. Raw reads were aligned to the SM assembly Caty1.0 and to the rhesus assembly MacaM using the STAR Aligner tool.

**Results:** The final assembly has deep coverage (mean 192x) across 2.85 Gb of the SM genome with contig N50 of 113 kb and scaffold N50 of 12.8 Mb. We identified several candidate genes with major sequence differences between human, rhesus macaque and SM, which were confirmed by RNA-Seq. A key phenotypic feature of SMs is the significantly lower innate responses to lipopolysaccharide and other components of gram-negative bacteria compared to AIDS-susceptible species. This may help to limit overall immune activation after SIV infection. Of interest, we identified a C-terminal frame shift mutation in the toll-like receptor 4 (TLR4) locus of SM, a crucial sensor of bacterial products that induces a proinflammatory cytokine response. The same frame shift mutation was also observed in the coding region of TLR4 of African Green Monkeys. Lastly, we also identified major structural variations in exons 3 and 4 of the immune regulatory protein intercellular adhesion molecule 2 (ICAM2).

**Conclusions:** We identified several mutations predicted to have substantial effect on protein function in SM and AGM relative to non-natural SIV hosts. Identification of a TLR4-specific mutation in the SM genome provides the mechanistic basis for attenuated responses to Gram-negative bacteria, an important aspect of the natural host phenotype.

## 21 Hyperacute Microbial Translocation During Pathogenic SIV Infection

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**Background:** During chronic human immunodeficiency virus (HIV) infection, microbial products migrate into the blood from the gastrointestinal tract concomitant with systemic immune activation. Whether this pathological microbial translocation occurs during acute immunodeficiency virus infection remains unknown, but given the immunomodulatory activity of microbial products, acute-phase translocation could drive early virus replication.

**Methods:** Using simian immunodeficiency virus (SIV)-infected cynomolgus macaques, we performed 16S ribosome deep-sequencing and quantitative PCR to investigate SIV-associated perturbation to the composition and abundance of microbial products within stool and blood plasma. ELISA-based assays were used to monitor fluctuations in peripheral inflammation (MCP-1 and SAA-1), the bacteria-specific host response (sCD14 and EndoCab), and intestinal permeability (IFABP). We used flow cytometry to track changes to peripheral blood lymphocyte populations.

**Results:** We found that within the first week of infection, prior to the peak of viremia, plasma levels of bacterial DNA increased as much as 1,390-fold over baseline while plasma levels of soluble CD14 (sCD14) correlated with set-point levels of virus replication. Translocation was accompanied by peripheral inflammation and an increase in CD4+CCR5+ T cells, which are the primary targets of virus replication.

**Conclusions:** Altogether, our results identify hyperacute microbial translocation as one of the earliest pathological phenomena to occur during immunodeficiency virus infection, and suggest that it may promote early virus replication. Prophylactic strategies may benefit from better understanding the hyperacute-phase relationship between host commensals and incipient immunodeficiency virus.

## 22 CD8 T Cells Are Required to Suppress Viremia in SIV-Infected ART Treated Macaques

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**Background:** HIV infection persists despite suppressive antiretroviral therapy (ART) and treatment interruption results in rapid viral rebound. Previous studies using antibody-mediated CD8+ lymphocyte depletion in simian immunodeficiency virus (SIV)-infected rhesus macaques (RM) showed that these cells suppress viremia in ART-naïve animals. However, the role of CD8+ lymphocytes during ART-mediated virus suppression is unknown. Using in vivo CD8+ lymphocyte depletion, we sought to elucidate whether CD8+ lymphocytes are required to maintain virus suppression in ART-treated SIV-infected RM.

**Methods:** 13 rhesus macaques were infected IV with 3000 TCID50 of SIV<sub>mac239</sub>. At 8 weeks post SIV infection animals were put on a 4 drug ART regimen consisting of Tenofovir, Emtricitabine, Raltegravir, and Darunavir for the duration of the study. Once virus was undetectable, we administered the anti-CD8 antibody MT-807R1 intravenously (50 mg/kg). Plasma viral load and quantification of cell-associated SIV<sub>mac</sub> gag DNA was determined by RT-PCR. A next-generation, ultra-sensitive RNA *in situ* hybridization technology, RNAscope was used to visualize SIV-RNA+ cells in lymph nodes. Multiparametric flow cytometry was performed to monitor the immunological effects of the used interventions.

**Results:** We found that in SIV-infected, ART-treated RMs, depletion of CD8+ lymphocytes results in increased virus production in both plasma and lymph nodes in 100% (13 out of 13 RM) of the treated animals. We also show that repopulation of CD8+ T cells (but not CD8+ NK cells) is associated with reestablishment of virus control. In addition, we found that the level of SIV-specific CD8+ T cells pre-CD8 depletion correlate with virus production after CD8 depletion. While the levels of SIV-DNA-positive cells remained unchanged after CD8+ lymphocyte depletion and reconstitution, the frequency of SIV-infected CD4+ T-cells pre-depletion positively correlates with both peak and area-under-the-curve of viremia post-depletion.

**Conclusions:** This study is the first to examine the effects of CD8+ lymphocyte depletion on SIV-infected rhesus macaques undergoing continuous ART. Our findings reveal a previously underappreciated role of CD8+ T cells in cooperating with ART to maintain virus suppression during therapy. We believe this study provides important rationale to further explore the potential effect of therapeutic vaccinations and check-point blockade inhibitors in ART-treated HIV-infected humans.

## 23 Lack of CTL Attenuates, but Does Not Ablate Compartmentalization of SIV Replication

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**Background:** In chronic, asymptomatic SIV disease, SIV RNA+ cells are concentrated within B cell follicles. As CTL are abundant in extrafollicular regions (EF) of secondary lymphoid tissues, but scarce inside the follicle (F), we hypothesized that in acute infection prior to CTL development or in chronic infection after CD8 depletion, compartmentalization of SIV RNA+ cells would diminish.

**Methods:** Rhesus macaques were infected IV with SIVmac239. On day 7 (acute infection), 6 animals were sacrificed and lymph nodes (LN), spleen, and rectum harvested and snap frozen in OCT. Three animals underwent LN biopsy (day 42), treatment with CD8 depleting antibody (day 60), and necropsy on day 84. *In situ* hybridization for SIV RNA and immunostaining for Ki67, CD4, and CD20 were performed to determine frequencies of SIV RNA+ cells and target cells within F and EF. Wilcoxon matched-pairs signed rank test was used to determine significance.

**Results:** In acutely infected animals, frequencies of SIV RNA+ cells did not differ significantly in F vs EF of LN (median F:EF 1.2,  $p=0.6$ ), but were higher in F vs EF in spleen (median F:EF 3.7,  $p=0.03$ ) and rectum (median F:EF 17;  $p=0.03$ ). After adjusting for Ki67+CD4+ cells in each compartment, there was a trend in all tissues for more SIV RNA+ to be in F vs EF (medians F:EF: LN 2.2;  $p=0.06$ ; spleen 1.7;  $p=0.12$ ; rectum 2.3,  $p=0.06$ ). In chronically infected animals prior to CD8 depletion, frequencies of SIV RNA+ cells in LN were 2.8- to 60-fold higher in F vs EF. After controlling for Ki67+CD4+ cells, SIV RNA+ cells were 11- to 40-fold higher in F vs EF. Following documented CD8 depletion of LN, differences in F vs EF largely abated with the LN F:EF ratio ranging from 0.7-1.8. This was primarily due to increases in SIV RNA+ cells in EF (range, 4- to 56-fold more vs pre-depletion LN), while increases in F were smaller (range, 1.2-1.9 fold more vs pre-depletion LN). Ki67+CD4+ cell frequencies were not substantially altered by CD8 depletion. After adjusting for Ki67+CD4+ cells, frequencies of SIV RNA+ cells were consistently higher in F vs EF of LN post CD8 depletion (range, F:EF 1.4 to 7.2).

**Conclusions:** In the absence of virus-specific CTL, the concentration of virus-producing cells in B cell follicles is attenuated, but not eliminated. These data support the hypothesis that virus-specific CTL play a key role in virus compartmentalization, and further suggest that T follicular helper cells are inherently more permissive to SIV than other cells.

**24 Sooty Mangabey CD4+ T Cells Express the SIVsmm Coreceptor CXCR6**

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**Background:** Nonpathogenic SIVsmm infection of sooty mangabeys (SM) is characterized by limited infection of certain CD4+ T cell subsets, such as central memory T cells. One mechanism that may contribute to limited infection of certain cells is extremely low expression of CCR5 by SM and other natural hosts. However, SM exhibit high viral loads despite little CCR5. Our lab has demonstrated that SIVsmm infection occurs in vivo in the absence of CCR5 and that smCXCR6 supports SIVsmm infection in vitro and in smPBMC ex vivo. We hypothesize that CXCR6 is a principal coreceptor in SM, and that CXCR6 expression by expendable CD4+ T cell subsets enables viral replication in CCR5-low natural hosts without either disruption of CD4+ T cell homeostasis or pathogenesis. Our aim was to define expression patterns of CXCR6 on CD4+ T cells of SM, as well as rhesus macaques (RM). We also examined GPR15, which has modest coreceptor activity in vitro.

**Methods:** Because anti-human CXCR6 reagents do not cross-react with smCXCR6 or rmCXCR6, we generated a novel monoclonal antibody by immunizing mice with B78H1 cells transduced to express smCXCR6. This antibody specifically reacts with all primate CXCR6 molecules tested. Using flow cytometry, we defined expression patterns of CXCR6, GPR15 and CCR5 on both resting and ConA/IL-2-stimulated CD4+ T cells from uninfected SM and RM.

**Results:** Both CXCR6 and GPR15 were expressed on resting memory CD4+ T cells of SM and RM. Notably, CXCR6, GPR15 and CCR5 expression defined three distinct populations of resting CD4+ T cells in both species, with few cells expressing multiple coreceptors. Upon stimulation, the proportion of CXCR6+ CD4+ T cells increased in both SM and RM. In stark contrast, CCR5+ CD4+ T cells increased in RM but not SM following stimulation, as previously reported. As a result, CXCR6+ cells substantially outnumbered CCR5+ cells among SM CD4+ T cells.

**Conclusions:** CXCR6 defines a CD4+ T cell population in SM that is distinct from CCR5-expressing cells, and may identify a unique subset that can support SIVsmm replication without pathogenic consequences. The divergent changes in CCR5 and CXCR6 expression in response to stimulation suggest that CXCR6 and CCR5 expression are controlled by distinct mechanisms. Delineating the specific T cell subsets that express CXCR6 in blood as well as in tissues will help determine how CXCR6 targets SIV in SM, and in the setting of very low CCR5 expression, contributes to nonpathogenic consequences of infection.

**25 Safety, Immunologic and Virologic Activity of Anti-PD-L1 in HIV-1 Participants on ART**

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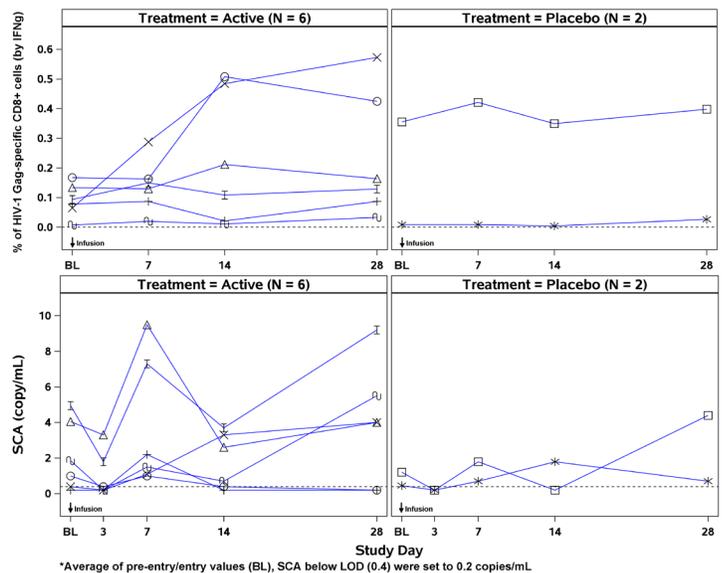
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**Background:** HIV-1 infected persons with viremia suppressed on antiretroviral therapy (ART) have persistent T-cell exhaustion that may limit clearance of HIV-1 expressing cells. Monoclonal antibodies (mAbs) targeting the PD1:PD-L1 axis have shown clinical activity in cancer studies. Blocking this axis in patients suppressed on ART may improve HIV-1 specific immune responses.

**Methods:** In a pre-specified analysis of an initial dosing cohort of ACTG 5326, 8 HIV-1 infected participants on ART with HIV-1 RNA <40 c/mL and ≥0.4 c/mL by single copy assay (SCA) received one infusion (double-blind) of anti-PD-L1 mAb (BMS-936559) at 0.3 mg/kg (N = 6) or normal saline (NS) (N=2). Anti-PD-L1 pharmacokinetics and receptor occupancy (RO) on CD3+, CD4+ and CD8+ T cells were measured and safety was assessed. HIV-1 Gag-specific CD8+ T cell responses, plasma HIV-1 RNA by SCA, and CD4+ T cell-associated (CA) RNA and DNA by qPCR were measured at pre-entry, entry and over 28 days post infusion. In participants who received active mAb, pre-entry/entry (BL) and all post-infusion values were averaged and compared (paired t-test).

**Results:** BMS-936559 was well tolerated; all treatment-related adverse events were mild/moderate. Plasma half-life of BMS-936559 was 3.7 days. RO peaked at 80-100% within 2 hours of dosing and was <20% in 5 of 6 participants by week 4. The average percentage of HIV-1 Gag-specific CD8+ cells (by IFNg (Figure) or CD107a) increased over the 28 days post infusion ( $p=0.14$  and  $0.09$ , respectively), as did polyfunctionality of the Gag-specific CD8+ T cell response, all driven by two strong responders in the anti-PD-L1 treated group. SCA levels appeared to decrease at 3d then increase at 7d (Figure). There was no change in the average SCA HIV RNA or CA-HIV RNA from pre-infusion through Day 28 ( $p=0.69$  and  $0.53$ ). CA-RNA:DNA ratio did not change. At 36 weeks post-infusion of anti-PD-L1 mAb or NS, an asymptomatic participant with a previously normal cortisol had an abnormally low AM level and was diagnosed with pituitary insufficiency.

**Conclusions:** This is the first prospective study of a PD1:PD-L1 axis inhibitor in HIV-infected participants on ART. Despite the low anti-PD-L1 dose, there was a trend toward increased HIV-1 Gag specific CD8+ T cell responses over 28 days post-infusion, including increased CD107a expression consistent with reversal of CD8+ T cell exhaustion. Responses were larger in a subset, consistent with responses to immune checkpoint mAbs in cancer treatment and animal models of viral infections.



**26LB Effect of Sequential Vacc-4x/GM-CSF Immunization and Romidepsin on the HIV Reservoir**

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**Background:** Immune priming prior to reversal of latency may facilitate killing of infected CD4+ T cells and could be a component of an HIV cure. To clinically assess this concept, we evaluated whether therapeutic HIV immunization followed by latency reversal would impact measures of viral transcription, plasma viremia, and reservoir size in HIV patients on suppressive antiretroviral therapy (ART).

**Methods:** This single-arm phase Ib/Ia trial enrolled 20 HIV-infected adults who received 6 intradermal immunizations with Vacc-4x adjuvanted with GM-CSF prior to receiving intravenous romidepsin 5 mg/m<sup>2</sup> once weekly for 3 weeks while maintaining ART. This “shock & kill” approach was followed by a monitored antiretroviral pause (MAP). Co-primary endpoints were changes from baseline in total HIV DNA in CD4+ T cells (by ddPCR) and infectious units per million (IUPM) by quantitative viral outgrowth assay (qVOA) as markers of the reservoir size. HIV transcription was quantitated by cell-associated unspliced HIV RNA in CD4+ T cells. Plasma HIV RNA was analyzed by the Cobas Taqman assay. Safety was evaluated at each study visit.

**Results:** Seventeen of 20 enrolled participants (3 females, 17 males, median age 48.5 years, median CD4 670 cells/mm<sup>3</sup>) completed all Vacc-4x/GM-CSF immunizations and romidepsin infusions. Reductions in the latent reservoir were observed with both assays. Total HIV DNA was reduced by 40% (95% CI: 11–59, p=0.012). qVOA was evaluable at baseline and at least one follow-up time point in 6/17 participants. The 6 evaluable participants had a median reduction in IUPM of 40% (p=0.019). As previously observed, HIV transcription increased rapidly within the first hours after each romidepsin administration. Eight participants had at least one quantifiable plasma HIV RNA (range: 21–619 c/ml) during the romidepsin infusion period. Median time from interrupting ART to plasma HIV RNA >50 copies/ml during the MAP was 14 days. Three SAEs were observed, none related to study therapy. A total of 141 AEs in 20 participants were recorded, 133 grade 1, five grade 2 and three grade 3. None of the grade 3 AEs were related to study medications.

**Conclusions:** This is the first reported dual intervention designed to target the HIV reservoir. We used Vacc-4x/GM-CSF therapeutic HIV immunization prior to romidepsin treatment and found a significant reduction in the latent HIV reservoir. These results may serve as a benchmark for further optimization of this strategy.

**27 A Patient Navigation/Contingency Management RCT for Hospitalized HIV+ Substance Users**

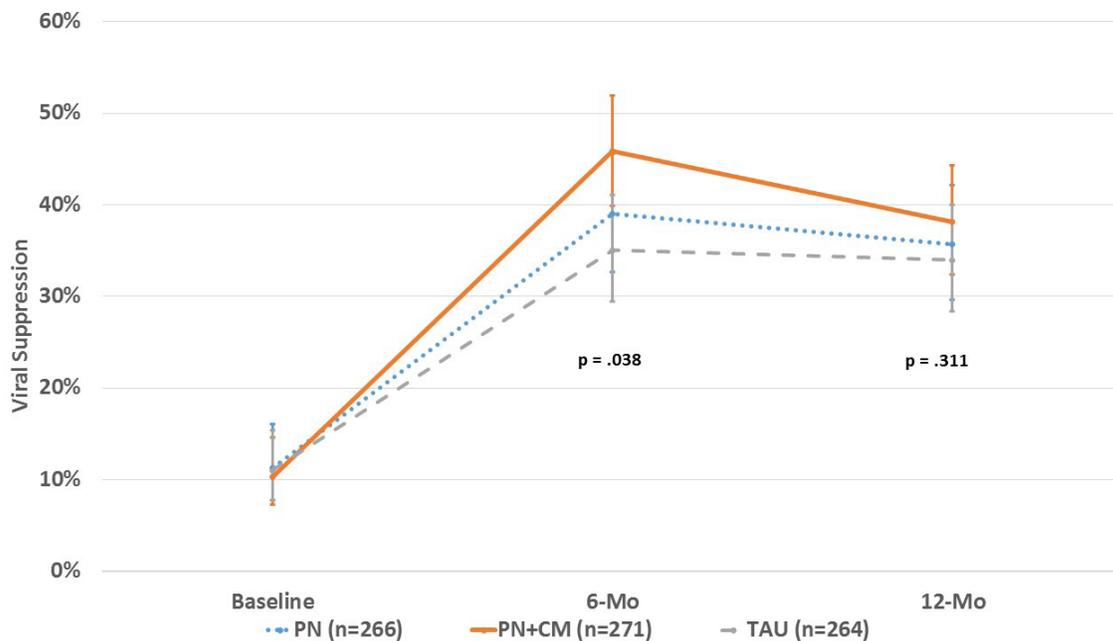
Lisa R. Metsch<sup>1</sup>; Daniel J. Feaster<sup>2</sup>; Lauren Gooden<sup>3</sup>; Moupali Das<sup>4</sup>; Tim Matheson<sup>5</sup>; Maxine L. Stitzer<sup>6</sup>; Mamta K. Jain<sup>7</sup>; Allan Rodriguez<sup>2</sup>; Raul Mandler<sup>8</sup>; Carlos del Rio<sup>9</sup>  
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**Background:** HIV-infected substance users are less likely to be virally suppressed due to lower rates of adherence to treatment and engagement in care. We tested the impact of patient navigation (PN) and contingency management (CM) on increasing viral suppression (<200 copies/μL) in HIV-infected substance users who were poorly engaged in care

**Methods:** Project Hope (CTN0049) is a completed 3-arm RCT to test 6 months of PN and PN+CM versus treatment as usual (TAU) on increasing 12-month viral suppression rates. The PN arm included up to 11 sessions in which navigators motivated participants to attend HIV care and substance use treatment and provided instrumental support including making and attending appointments with participants. PN+CM added contingency management; participants could earn up to \$1,160 for target behaviors including attending HIV care visits, picking up medications, attending substance use treatment, providing drug-free urines, and achieving viral suppression. Differences in viral suppression at 12 months, the primary outcome, were tested by treatment arm using intention-to-treat analysis with generalized estimating equations for binary outcomes. Results are final.

**Results:** 801 HIV-infected out of care substance users were recruited from 11 hospitals in the US between July 2012 and January 2014. The sample was predominately male (68%), black (78%) and middle aged (m=44, SD=10). Participants reported use of stimulants (81%), marijuana (55%), high levels of alcohol (49%) and opioids (26%). Median CD4 count was 109. Viral suppression at baseline was similar across arms (PN=11%, PN+CM=10%, TAU=11%, p=.930). The percentage of patients attending at least 6 PN sessions was 74% in PN and 90% in the PN+CM arms. Average payment to an incentivized PN+CM participant was \$668. Viral suppression at 12 months, available for 752 (94%), was not different across the 3 arms (PN=36%, PN+CM=38%, TAU=34%, p=.311). In secondary analyses, viral suppression (PN=39%, PN+CM=46%, TAU=35%, p(PN+CM vs TAU)=.038) and attendance at an HIV care visit (PN=79%, PN+CM=87%, TAU=69%, p(PN vs TAU)=.003); p(PN+CM vs PN)=.014) at 6 months were significantly different between arms.

**Conclusions:** PN and PN+CM had improved indicators of HIV care in the short run, but these improvements are transitory and did not persist. At the primary 12 month assessment, neither PN nor PN+CM had any impact on viral suppression in this sample of difficult to treat, substance using, unsuppressed HIV positive individuals recruited in the hospital.



**28 Initiating ART at a Patient's First Clinic Visit: The RapIT Randomized Trial**

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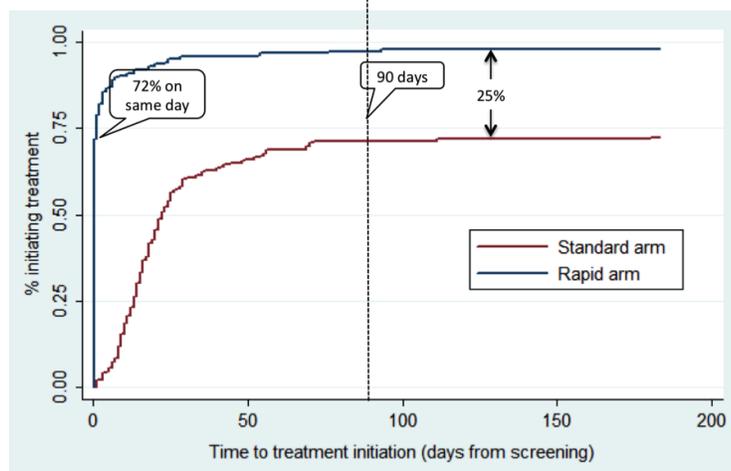
**Background:** Very high rates of patient attrition from HIV care between HIV testing and ART initiation have been documented in sub-Saharan Africa. Our objective was to estimate the effect of accelerated initiation procedures on uptake of ART and viral suppression.

**Methods:** We conducted a randomized controlled trial (RapIT Trial, NCT01710397) of immediate ART initiation in two public sector clinics in South Africa (a primary health clinic (PHC) and a hospital-based HIV clinic). Adult (≥18), non-pregnant patients receiving a positive HIV test or first CD4 count were randomized to standard or rapid initiation. On the day of HIV test or first CD4 count, rapid arm patients received a point-of-care (POC) CD4 count (Alere Pima) if needed; those ART eligible received a POC TB test (Xpert MTB/RIF) if symptomatic, rapid POC blood tests (Roche Refflotron), physical exam, education, counseling, and ARV dispensing. Patients in the control arm followed standard clinic procedures (3-5 additional clinic visits over 2-6 weeks prior to ARV dispensing). Follow up was by passive medical record review. Primary outcomes were initiation of ART ≤ 90 days and viral suppression, defined as initiated, retained in care, and suppressed (≤400 copies/ml), ≤ 10 months of study enrollment.

**Results:** Of 600 patients screened, 377 were eligible for ART and for the study (56% female, median age 35, median CD4 count 210 cells/mm<sup>3</sup>). In the rapid arm 182/187 (97%) initiated ART ≤ 90 days, compared to 136/190 (72%) in the standard arm (RR 1.36; 1.24-1.49). In the rapid arm, 119/187 (64%) initiated and were suppressed at 10 months, compared to 96/190 (51%) in the standard arm (RR 1.26; 1.05-1.50). Adjustment for sex and baseline CD4 count did not affect results. Effects were larger for the PHC than for the hospital-based HIV clinic, for unemployed than for employed patients, and for patients under age 35 than for patients over 35. 72% of rapid arm patients initiated on the same day as HIV test or first CD4 count (Figure 1). All rapid arm patients in the rapid arm who did not start ART ≤ 180 days were delayed due to TB treatment. Time used for treatment initiation in the rapid arm averaged 2.8 hours.

**Conclusions:** Offering same-day ART initiation to adult patients in South Africa increased uptake of ART by 36% and viral suppression by 26%. It is feasible and acceptable in public sector clinics, and not all POC instruments will be essential in the future. It should be considered for adoption in high-volume clinics in the public sector in Africa.

Figure 1. Cumulative incidence of ART initiation



**29 Switching Tenofovir DF to Tenofovir Alafenamide in Virologically Suppressed Adults**

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**Background:** Emtricitabine/tenofovir DF (F/TDF) is a standard-of-care nucleoside reverse transcriptase inhibitor (NRTI) backbone due to its favorable efficacy and safety profile. However, TDF can be associated with renal and bone toxicities. Tenofovir alafenamide (TAF) is a novel tenofovir prodrug that achieves 91% lower plasma tenofovir levels than TDF. In elvitegravir/cobicistat/F/TAF phase 3 studies, TAF had less adverse effect on kidneys and bone than TDF.

**Methods:** We conducted a 96-week (wk) randomized, double blind, active controlled study in virologically suppressed HIV-1 infected patients receiving F/TDF-containing regimens to evaluate the efficacy and safety of switching from F/TDF to F/TAF vs continuing F/TDF while remaining on the same third agent. Primary endpoint was virologic success at Wk 48 by ITT FDA snapshot algorithm with a pre-specified noninferiority margin of 10%. We describe the Wk 48 data.

**Results:** 663 patients were randomized and treated (F/TAF 333 vs F/TDF 330); median age 49 years, 15% women, median estimated glomerular filtration rate (eGFR) 100 mL/min. 46% were on a boosted protease inhibitor, 28% on an integrase inhibitor, 25% on a non-NRTI. Through Wk 48, virologic success (HIV-1 RNA <50 c/mL) was maintained in most patients in both treatment groups: F/TAF 94.3% vs F/TDF 93.0% (difference +1.3%, 95% CI: -2.5% to +5.1%), demonstrating noninferiority of F/TAF to F/TDF (Table). Emergent resistance was rare (0.3% vs 0). Drug related serious adverse events were rare (0 vs 0.3%). Drug discontinuation due to adverse events (AEs) was low (2.1% vs 0.9%). There were no cases of proximal renal tubulopathy in either group. Median eGFR improved by +8.4 mL/min in the F/TAF group vs by +2.8 mL/min in the F/TDF group (P <0.001). Quantitative measures of proteinuria improved in the F/TAF group but not in the F/TDF group (Table). Bone mineral density (BMD) increased in the F/TAF group but declined in the F/TDF group: hip (mean) +1.14% vs -0.15% (P <0.001) and spine +1.53% vs -0.21% (P <0.001), respectively. More patients in the F/TAF group had ≥ 3% improvement in BMD at Wk 48: hip 17% vs 9% and spine 30% vs 14%.

**Conclusions:** In virologically suppressed patients switching from F/TDF to F/TAF, high rates of virologic suppression were maintained, while renal and bone safety parameters improved. With its safety benefits relative to F/TDF, F/TAF has the potential to become an important NRTI backbone for antiretroviral treatment.

Table: Virologic Outcome and Percentage Changes in Renal Biomarkers

Virologic Outcome (ITT, FDA snapshot algorithm)	F/TAF n=333	F/TDF n=330
Virologic Success (HIV-1 RNA <50 c/mL)	314 (94.3%)	307 (93.0%)
	Diff: +1.3% (95% CI: -2.5% to +5.1%)	
Virologic Failure	1 (0.3%)	5 (1.5%)
No Virologic Data in Window	18 (5.4%)	18 (5.5%)
<b>% Changes in Renal Biomarkers (median)</b>		
Urine Protein: Creatinine Ratio*	-14.6%	+7.7%
Urine Albumin: Creatinine Ratio*	-7.7%	+12.3%
Urine Retinol Binding Protein: Creatinine Ratio*	-16.3%	+18.2%
Urine Beta-2-Microglobulin: Creatinine Ratio*	-39.6%	+22.0%

\*P < 0.001 for between-group differences

**30 ACTG 5273 Randomized Trial of Second-Line ART Supports WHO Guidance**

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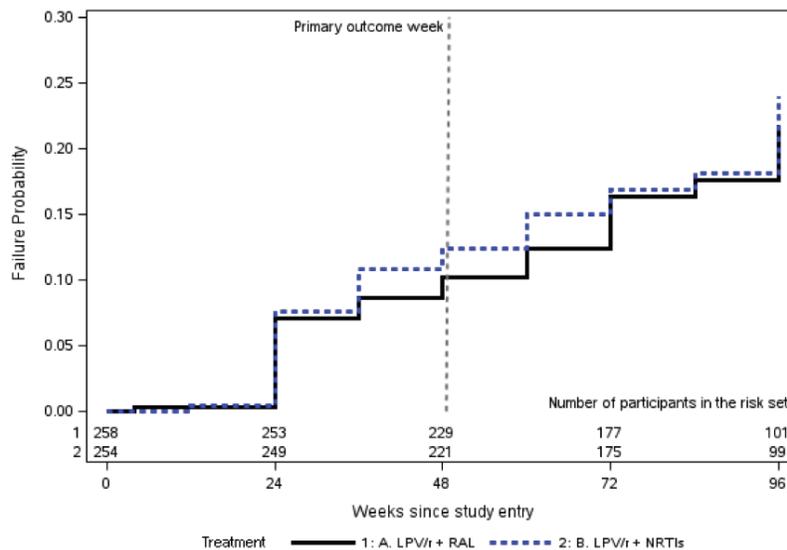
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**Background:** The WHO recommends boosted protease inhibitor + nucleos(t)ide reverse transcriptase inhibitors (NRTI) for second-line ART, but concerns about NRTI toxicity and cross-resistance have motivated the search for NRTI-free regimens. We hypothesized that boosted lopinavir (LPV/r) + raltegravir (RAL) would be virologically non-inferior to LPV/r + NRTIs as second-line ART in resource-limited settings.

**Methods:** ACTG A5273 (SELECT) was a phase III, open-label, randomized, non-inferiority study conducted at 15 sites in 9 resource-limited countries. HIV-1-infected adults with viral load (VL) ≥1000 copies/mL (cpm) after 24 weeks (wks) of a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen received LPV/r + RAL or LPV/r + NRTIs selected from an algorithm that included zidovudine for those failing tenofovir and vice versa. Non-inferiority was defined as the upper 2-sided 95% CI for the difference in cumulative probability of virologic failure (VF, confirmed VL >400 cpm at or after wk 24) estimated by Kaplan-Meier methods by wk 48 excluding 10%. Secondly, superiority was assessed by exclusion of 0. Genotypic drug resistance testing was performed retrospectively at study entry and VF.

**Results:** 512 eligible participants were randomized; the majority were female (52%), black (64%) and infected with subtype C (81%). At study entry, median CD4 count was 135 cells/mm<sup>3</sup>; VL 4.5 log<sub>10</sub> cpm, 96% had ≥1 NRTI IAS mutation and 52% ≥3. By wk 48, the cumulative probability of VF was 10.3% (95% CI: 6.5%, 14.0%) in the RAL arm and 12.4% (8.3%, 16.5%) in the NRTI arm (See figure) with a weighted (by randomization stratification factors) difference (95% CI) of -3.4% (-8.4%, 1.5%) indicating LPV/r + RAL was non-inferior and not superior to LPV/r + NRTIs. Participants in the NRTI arm with a NRTI genotypic susceptibility score (GSS) ≥1 had a higher probability of VF versus those with GSS <1 (difference -8.4% [-16.6%, -0.3%]; P=0.04). In addition, having ≥3 NRTI mutations at study entry was associated with a lower probability of VF in both arms (HR 0.45 [0.30, 0.70], P<0.001).

**Conclusions:** In the setting of extensive NRTI resistance without resistance testing the WHO recommendation of LPV/r + NRTIs for second-line ART is supported by the current study. LPV/r + RAL should be considered an alternative if NRTI toxicity is limiting. The strong association between NRTI resistance at study entry and improved response to second-line ART deserves further evaluation of mechanisms other than better adherence.



**31LB Cabotegravir+Rilpivirine as Long-Acting Maintenance Therapy: LATTE-2 Week 32 Results**

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**Background:** Cabotegravir (CAB), an HIV INSTI and rilpivirine (RPV), an HIV NNRTI are both under development as long-acting (LA) injectable nanosuspensions. LATTE-2 was designed to select an intramuscular (IM) regimen of CAB LA + RPV LA and to evaluate the safety and efficacy of 2-drug IM ART, relative to 3-drug oral ART (CAB + ABC/3TC) when used to maintain viral suppression in HIV-1 infected adults.

**Methods:** Phase 2b, multicentre, parallel group, open-label study in ART-naïve HIV infected adults. Enrolled patients that had a plasma HIV-1 RNA <50 c/mL during the 20-week Induction Period (IP) with daily oral CAB 30 mg + ABC/3TC were randomized 2:2:1 to IM CAB LA + RPV LA every 4 weeks (Q4W), every 8 weeks (Q8W), or remained on oral CAB + ABC/3TC (PO) in the Maintenance Period (MP). The primary endpoints evaluated antiviral activity by FDA snapshot algorithm, protocol defined virologic failure, and safety at a pre-specified 32 wks in MP (ITT Maintenance Exposed (ME)).

**Results:** 309 pts were enrolled and treated (ITT-E): 91% male, 20% non-white, and 19% >100,000 c/mL HIV-1 RNA. After 20 wks of IP, 91% of pts achieved HIV-1 RNA <50 c/mL by snapshot and 286 pts were randomized to maintenance therapy. In the MP, 95% (Q8W) and 94% (Q4W) of pts maintained HIV-1 RNA <50 c/mL at W32 compared to 91% on PO (ITT-ME). Two pts had consecutive HIV-1 RNA >200 c/ml during MP (PO [1 at W8]; Q8W [1 at W4]), both without NRTI, NNRTI, or INSTI resistance. The most common drug-related AE was injection site pain (92% on IM arms) with 99% of injection site reactions (ISRs) being mild (82%) or moderate (17%). ISRs lasted a median of 3 days, decreased in frequency following the first injection, and led to 2 pt (<1%) withdrawals. Most common non-ISR AEs during MP were nasopharyngitis (20%), diarrhea (12%) and headache (14%) on IM arms and nasopharyngitis (25%), headache (7%), and diarrhea (5%) on PO CAB. SAEs during MP occurred in 6% of IM pts and 5% PO pts, none drug related, with 1 unrelated death (seizure [Q4W]). Nine pts withdrew from MP due to AEs; Q8W (2%), Q4W (5%) and PO (2%). Treatment emergent lab abnormalities ≥ Grade 3 occurred in 16% of IM pts and 14% of PO pts during MP.

**Conclusions:** Q8W and Q4W CAB LA + RPV LA as 2-drug injectable maintenance therapy demonstrated comparable antiviral activity to daily oral CAB + ABC/3TC through 32 weeks in virologically suppressed pts. Injectable CAB LA + RPV LA were generally well tolerated. LATTE-2 results support continued development of this novel treatment regimen.

	CAB LA + RPV LA Q8W (n=115)	CAB LA + RPV LA Q4W (n=115)	Oral CAB 30 mg + ABC/3TC (n=56)
<b>Week 32 Snapshot Study Outcomes (ITT-ME)</b>			
%HIV-1 RNA <50 c/mL at W32: Diff in Proportions (95%CI)**	95% (3.7; -4.8, 12.2)	94% (2.8; -5.8, 11.5)	91%
Median CD4+ cells/mm <sup>3</sup> Baseline Change from Baseline at W32 (IQR) †	449 +226 (108, 330)	499 +242 (138, 348)	518 +304 (176, 515)
<b>Snapshot Virologic Non-response</b>			
Data in window not <50 c/mL	3 (3%)	1 (<1%)	1 (2%)
Discontinued due to lack of efficacy (PDVF)	1 (<1%)	0	1 (2%)
Discontinued due to Other* Reasons while Not Suppressed	1 (<1%)*	0	0
<b>Snapshot No Virologic Data</b>			
Discontinued due to AE or Death	0	4 (3%)*	1 (2%)*
Discontinued due to Other Reasons while Suppressed	1 (<1%)*	2 (2%)	2 (4%)
<b>Other Results</b>			
Number of ISR events	1054	1228	NA
Grade 1 – mild (%)	839 (80%)	1021 (83%)	
Grade 2 – moderate (%)	202 (19%)	197 (16%)	
ISR Duration ≤7 days	943 (89%)	1121 (91%)	
<b>Intent to Treat- Maintenance Exposed (ITT-ME)</b>			
BL = baseline (last value prior to first Induction Period dose at Week-20)			
IQR = Interquartile range			
**W32 represents 52 weeks on study (20 Week Induction Period followed by a 32 Week two drug Maintenance Period)			
† Based on observed case at Week 32 (Q8W: n=112, Q4W: n=108, Oral: n=50)			
*Withdrew consent due to intolerability of injections			
*Acute HCV (n=2), rash (n=1), depressive reaction (n=1), psychotic state (n=1)			

**32LB ACTG 5340: The Effect of VRC01 on Viral Kinetics After Analytic Treatment Interruption**

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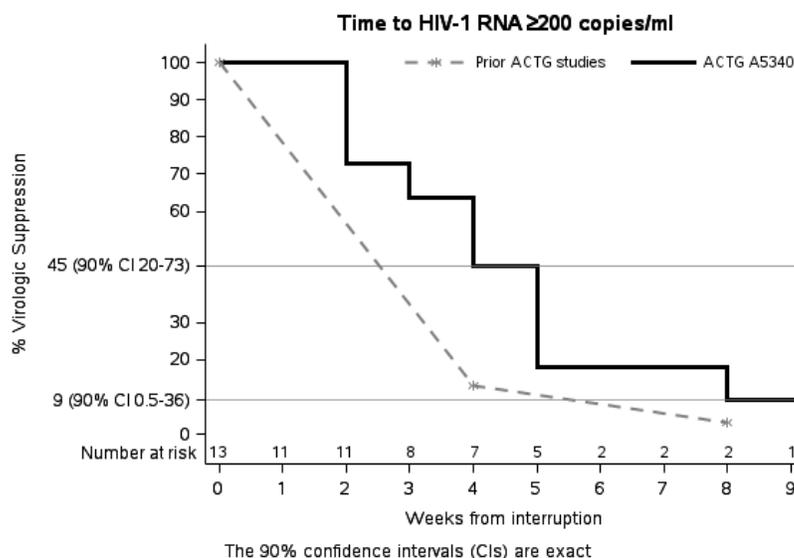
**Background:** Broadly neutralizing anti-HIV monoclonal antibodies (bNAbs) have shown promising results for the prevention and treatment of HIV. We present data evaluating whether high dose passive administration of VRC01, a bNAb targeting the CD4 binding site, can prevent or delay the return of viremia after ART interruption.

**Methods:** ACTG A5340 is an open-label study of the safety, tolerability, pharmacokinetics (PK) and antiviral activity of VRC01 in suppressed HIV-1 infected individuals undergoing a monitored analytical treatment interruption (ATI). We administered VRC01 40 mg/kg by intravenous infusion every 3 weeks for a total of 3 doses starting 1 week before the ATI. Thirteen evaluable participants provided 95% power to detect a 40% difference a rebound probability of 90% at 8 weeks without intervention using a one-sided test and 5% significance level. All participants were on protease (PI) or integrase (INSTI) based ART that was restarted after confirmed HIV RNA ≥200 c/ml.

**Results:** We enrolled 14 participants; 100% male, 50% African American. Median CD4 count was 896 c/mm<sup>3</sup> (IQR 579-1053) and nadir >200 c/mm<sup>3</sup> for all participants. Median time on ART was 4.7 years (IQR 3.8-6.0); 71% were on INSTI and 29% on PI regimens at entry. VRC01 was safe and well tolerated. No grade 3 or higher, or grade 2 VRC01-related, adverse events were observed.

One participant was excluded from antiviral activity analysis because he stopped ART before the first VRC01 dose. Despite median trough VRC01 concentrations of 90.4 mcg/ml (range 71.5-135.5) 3 weeks after the first dose, viral rebound occurred before week 8 of the ATI for the majority of participants (Figure). Notably, we detected a delay in rebound when compared to non-NNRTI historical controls from prior ACTG studies; 45% vs 13% were virally suppressed to week 4 (Fisher's exact p=0.02) and 9% vs 3% to week 8 (p=0.40). Two participants maintained suppression for 7 and 10 weeks in the absence of any other ART. Participants were off ART for a median of 5 weeks and 10/11 (2 participants are still in the ATI phase) resuppressed <50 c/ml within 4 weeks.

**Conclusions:** Passive immunization with high doses of a single bNAb (VRC01) failed to prevent rebound viremia in the majority of participants, although rebound was delayed when compared to historical controls. We are evaluating the baseline and rebound virus sensitivity to VRC01, PK, immunological and virological parameters. These data will inform the next strategies to utilize bNAbs, alone or in combination.



Oral Abstracts

### 33 Breast Milk and In Utero HIV-1 Transmission Select for Unique Envelope Signatures

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**Background:** Despite improved prevention of mother-to-child transmission (MTCT) of human immunodeficiency virus-type 1 (HIV-1) in developing countries, 240,000 children acquired the virus in 2013. HIV-1 MTCT can occur by three distinct routes: *in utero* (transplacental passage), intrapartum, and postpartum through breastfeeding. Disease progression can vary dramatically among these transmission modes, with infants infected *in utero* showing the shortest survival times.

**Methods:** Twenty-two maternal-infant transmission pairs were selected from the Zambia Exclusive Breastfeeding Study (ZEBs). Viral transmission route (*in utero* or through breastfeeding) was determined by the timing of the first HIV-1 polymerase chain reaction (PCR)-positive test in the infant. Full-length gp160 sequences were cloned from either reverse-transcribed plasma RNA or from cellular DNA. Neutralization assays were performed using TZM-bl cells and pseudotyped virus bearing envelope sequences from maternal or infant isolates. Affinofile cells were used to assess CD4 surface density requirements for a given isolate to gain viral entry. Inhibitor slope values were determined by fitting the median effect model to experimental dose response curves.

**Results:** Analysis of 647 viral envelopes from 22 maternal-infant pairs revealed unique genotypic and phenotypic signatures that depend upon transmission route. Relative to maternal strains, intrauterine HIV transmission selects infant isolates that are more resistant to soluble CD4 (sCD4) neutralization ( $p < 0.001$ ), have shorter V1 loops with fewer potential N-glycosylation sites (PNGs) ( $p \leq 0.017$ ), and have fewer V5 PNGs ( $p = 0.017$ ). Breast milk transmission selects infant isolates with fewer gp41 PNGs than their maternal counterparts ( $p = 0.017$ ) and that are more sensitive to the glycan-dependent broadly neutralizing antibodies PG9 and PG16 ( $p \leq 0.014$ ). In addition, median effect analysis suggests that HIV-1 envelope trimers engage target CD4 receptors with negative cooperativity. Finally, experiments with Affinofile cells indicate that compared to maternal viral strains, virus from infants require increased levels of surface CD4 receptor for productive infection.

**Conclusions:** These data provide the first evidence for transmission route-specific selection of HIV-1 variants, and suggest a role for envelope-CD4 avidity in viral entry. These findings potentially inform therapeutic strategies or vaccine designs that can be tailored over the course of HIV-1 exposure.

### 34 Impact of Option B+ on ART Uptake and Retention in Swaziland: A Stepped-Wedge Trial

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**Background:** Retaining HIV-infected pregnant and postpartum women in care is critical to prevent mother-to-child HIV transmission (PMTCT) and promote maternal health. New PMTCT approaches call for lifelong antiretroviral therapy (ART) for all HIV+ pregnant women (Option B+), a departure from strategies that used CD4 count to determine ART eligibility (Option A). Yet there are few implementation data on the impact of Option B+ on maternal retention in antenatal and postnatal care.

**Methods:** Using a stepped-wedge design the 'Sitkulwane Lesiphephile-Safe Generations' study compared maternal retention under Option A (based on  $\leq 350$  cells/ $\mu$ L) versus Option B+ in 10 primary care facilities across Swaziland. Pregnant HIV+ women not on ART making a first antenatal visit formed monthly facility-level cohorts that were followed through 6 months postpartum using routine health records. In analysis, the month of transition from A to B+ was excluded. Retention was defined as engagement in care within 56 days of delivery during the antenatal period and during a 3-month window before 6 months postpartum. Generalized estimating equations with a probit link were used to generate adjusted risk ratios (aRR) comparing outcomes under Options A versus B+ after accounting for age, CD4, gestation at 1<sup>st</sup> antenatal visit, and known HIV status.

**Results:** 2315 women were included: 45% ( $n=1043$ ) under B+ and 55% ( $n=1272$ ) under A. Patient characteristics were similar under B+ and A: mean age, 26 years; median gestational age at first antenatal visit, 20 weeks; median CD4, 404 cells/ $\mu$ L; CD4  $\leq 350$ , 33%. After transitioning to B+, the proportion of women receiving ART antenatally was higher (93%) vs A (30%;  $p < 0.001$ ). The proportion of women with CD4  $\leq 350$  initiating ART antenatally also increased under B+ (94% vs 63%;  $p < 0.001$ ). Among all HIV+ women, 61% attended at least one visit prior to delivery: 68% under B+ vs 54% under A (aRR, 1.30;  $p < 0.001$ ). Overall, postpartum retention was low (37%). In the analysis of all HIV+ women, postpartum retention was significantly higher under B+ (50%) vs A (26%) (aRR, 1.54;  $p < 0.001$ ). However, when the analysis was restricted to women on ART, postnatal retention was somewhat lower under B+ vs A (53% vs 65%; aRR, 0.73;  $p < 0.001$ ).

**Conclusions:** Implementation of Option B+ greatly increased ART initiation antenatally and improved ART coverage among women with advanced HIV disease in Swaziland, but postpartum retention remains an important challenge requiring urgent intervention.

### 35LB National HIV Transmission in 4-12 Week Olds in Malawi's PMTCT Option B+ Program

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**Background:** Option B+ was conceptualized and implemented in Malawi in 2011. Recognizing that additional data would be needed to validate program results documented by the Ministry of Health, and to provide further detail on variation in transmission by ART coverage and timing, a two year cohort study called the National Evaluation of Malawi's PMTCT Program (NEMAPP) was implemented in November 2014. A primary objective of NEMAPP is to measure mother-to-child-transmission (MTCT) in the era of Option B+.

**Methods:** NEMAPP is ongoing at 54 randomly selected health facilities in 10 districts. A stratified cluster sampling design was used to identify a nationally representative sample of 4-12 week old infants. Mothers were consecutively consented and screened for HIV while attending an under-5 clinic, and all identified HIV-exposed infants underwent HIV-1 DNA testing. This paper presents unweighted results of early infant transmission at the time of enrollment into NEMAPP.

**Results:** Amongst 1,851 HIV-positive mothers of 4-12 week old infants, 98.2% reported knowing their HIV status in pregnancy. Overall MTCT was 3.9%. Among those on ART in pregnancy (coverage 93.5%) MTCT was 2.8%. Of the 6.5% of women who either never start ART, or chose to stop ART at any time during or immediately after pregnancy, MTCT was 20%. MTCT varied by timing of ART initiation: from 1.4% in the 46.5% of women on ART prior to pregnancy, to 21.3% in the 5.8% of women who had never started ART.

**Conclusions:** Overall, vertical transmission of HIV is very low under Option B+. In women who are entering pregnancy already on ART, transmission is now on a similar scale to that observed in developed nations. Any ART coverage, even when started postpartum or taken temporarily, as in the case of defaulters, results in significant reduction in transmission.

	Child's HIV-1 DNA Result		p-value
	Positive	Negative	
Overall Transmission	73 (3.9%)	1,778 (96.1%)	
MTCT by Option B+ Coverage			p<0.000
No ART / Stopped ART	24 (20.0%)	96 (80.0%)	
On ART	49 (2.8%)	1,682 (97.2%)	
MTCT by timing of Mother's ART Initiation			p<0.000
Before this pregnancy	12 (1.4%)	849 (98.6%)	
1st or 2nd trimester	27 (3.9%)	674 (96.1%)	
3rd trimester	6 (4.3%)	133 (95.7%)	
Postpartum	4 (13.3%)	26 (86.7%)	
Stopped ART	1 (8.3%)	11 (91.7%)	
No ART	23 (21.3%)	85 (78.7%)	

**36 Impact of Maternal Tenofovir Use on HIV-Exposed Newborn Bone Mineral**

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**Background:** A US cohort study (CID 2015;61:996) reported 12% lower newborn mean bone mineral content (BMC) after maternal tenofovir disoproxil fumarate (TDF) use.

**Methods:** The P1084s substudy sought to compare newborn BMC by exposure to maternal antiretroviral (ARV) regimens at >14 weeks gestational age (GA) randomly assigned in the IMPAACT PROMISE trial: Arm 1: zidovudine[ZDV] (+ single-dose nevirapine+ TDF/emtricitabine[FTC] tail); Arm 2: ZDV/lamivudine/lopinavir-ritonavir[LPVr]; Arm 3: TDF/FTC/LPVr. Infants underwent whole-body (WB) and lumbar spine (LS) dual-energy X-ray absorptiometry (DXA) BMC measurements by age 28 days at 8 African sites equipped and trained for infant DXA scans. Standardized scan analysis was performed centrally. The accrual target of 150 infants per Arm was based on 80% power to detect a pair-wise difference of 4-5% in mean WB-BMC and 6-7% in mean LS-BMC. Maternal and infant characteristics were compared with Fisher's exact, Chi-square or Kruskal-Wallis tests, as appropriate. Mean BMC differences were compared with Student's t-test. Because mothers enrolled in the substudy after randomization, we used multivariable linear regression to adjust for baseline maternal factors and infant factors at time of DXA scan.

**Results:** Of 452 eligible mothers, data from 425 infants remained for analysis after accounting for twins(6), fetal (8) or neonatal death(10) and drop-outs(15). Mothers differed across Arms on age (median 25 vs 27 vs 27 years, p=.008). Newborns differed in birth weight (median 3090 vs 2900 vs 2900g, p<.001) and weight-for-length Z-score (mean -0.4 vs -0.8 vs -0.8, p=.032) but not GA (median 39 weeks for all Arms, p=.264) or birth length (median 49cm for all Arms, p=.327). By Arm, mean LS-BMC were 1.73 vs. 1.64 vs 1.72g and WB-BMC were 73.1 vs. 65.1 vs. 63.3g. Pairwise comparisons revealed no significant differences between Arms 2 and 3 (primary objective) for LS- and WB-BMC but significantly lower mean WB-BMC in Arms 2 and 3 compared to Arm 1, which persisted after adjustment (Table). Differences of borderline significance emerged in some pairwise comparisons of LS-BMC when adjusted for maternal or maternal and infant factors.

**Conclusions:** No adverse infant BMC effect was linked to maternal TDF use. Initiation of a triple-ARV, LPVr-containing regimen during pregnancy may lead to lower newborn bone mineralization.

**TABLE: Comparison of unadjusted and adjusted newborn (up to 28 days old) mean BMC of lumbar spine and whole-body, by exposure to randomized maternal antiretroviral regimen Arm during pregnancy.**

DXA scan region	Regimen	N	Mean BMC (g)	Comparison	Mean difference(g) [95% CI] (% shift <sup>2</sup> )	P value	Mean difference(g) [95% CI] adjusted for baseline factors <sup>1</sup>	P value	Mean difference(g) [95% CI] adjusted for factors at baseline & DXA <sup>1</sup>	P value
Lumbar spine (LS)	Arm 1 ZDV(+sdNVP+TDF-FTC tail)	117	1.73	Arm 1 minus Arm 2	0.09 [0.00, 0.17] (-5.2%)	.05	0.10 [0.02,0.18]	.02	0.07 [-0.01, 0.15]	.09
	Arm 2 ZDV-3TC-LPVr	127	1.64	Arm 2 minus Arm 3 <sup>2</sup>	-0.08 [-0.16,0.01] (-4.9%)	.09	-0.07 [-0.15, 0.01]	.10	-0.08 [-0.16, 0.00]	.04
	Arm 3 TDF-FTC-LPVr	113	1.72	Arm 1 minus Arm 3	0.01 [-0.08,0.1] (-0.6%)	.82	0.03 [-0.05, 0.11]	.43	0.01 [-0.07, 0.09]	.79
Whole body (WB)	Arm 1 ZDV(+sdNVP+TDF-FTC tail)	99	73.06	Arm 1 minus Arm 2	7.97 [3.97,11.96] (-10.9%)	<.001	7.51 [3.83, 11.19]	<.001	5.82 [2.10, 9.54]	.002
	Arm 2 ZDV-3TC-LPVr	104	65.09	Arm 2 minus Arm 3 <sup>2</sup>	1.76 [-2.43,5.95] (-2.7%)	.41	2.05 [-1.82,5.91]	.30	1.22 [-2.31, 4.75]	.50
	Arm 3 TDF-FTC-LPVr	96	63.33	Arm 1 minus Arm 3	9.73 [5.49,13.96] (-13.3%)	<.001	10.10 [6.26, 13.93]	<.001	8.69 [4.78, 12.60]	<.001

<sup>1</sup>Baseline: country, maternal age and height; DXA: infant age and length at time of DXA scan. <sup>2</sup>Primary substudy objective was comparison of Arm 2 to Arm 3. <sup>3</sup>% shift calculated as (Arm X-Arm Y)\*100/Arm X for comparison of Arm X minus Arm Y. Abbreviations: DXA= dual-energy X-ray absorptiometry; BMC = bone mineral content; g=grams ;CI = confidence interval; ZDV = zidovudine; sdNVP = single-dose nevirapine; TDF = tenofovir disoproxil fumarate; FTC = emtricitabine; 3TC = lamivudine; LPVr = lopinavir-ritonavir.

### 37 Similar Mortality With Cotrimoxazole vs Placebo in HIV-Exposed Uninfected Children

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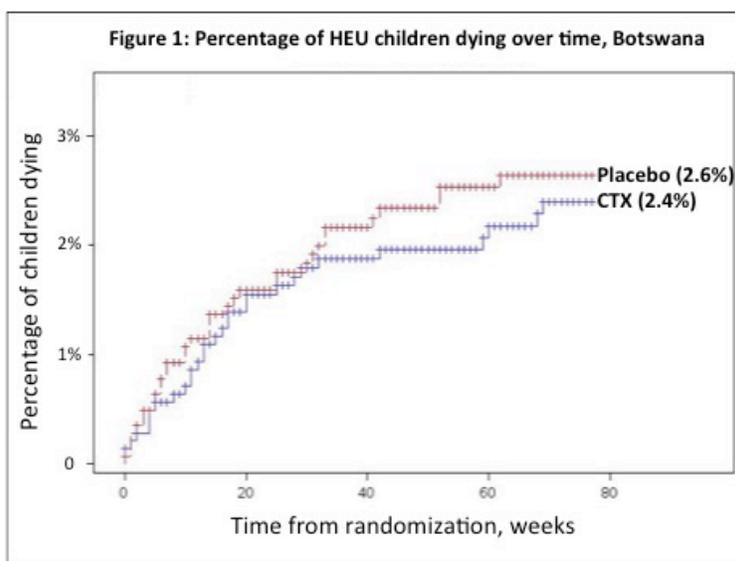
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**Background:** HIV-exposed uninfected (HEU) children experience higher mortality than HIV-unexposed children. Cotrimoxazole (CTX) reduces mortality among HIV-infected children, but no randomized clinical trials have evaluated its efficacy among HEUs.

**Methods:** We randomized HEU children in a non-malarial region of Botswana to receive either double-blinded CTX or placebo from 14-34 days through 15 months of life. Children were followed every 1-3 months through 18 months; infants diagnosed with HIV after randomization transitioned to open-label CTX. Feeding method was chosen by mothers, with either formula (provided by the Botswana government) or breastfeeding supported with maternal or infant antiretroviral prophylaxis. Breastfed infants were randomized to either 6 or 12 months duration. Primary analysis was intent-to-treat (restricted to infants HIV negative at randomization) and compared mortality at age 18 months using Kaplan-Meier estimates.

**Results:** From May 2011-April 2015, 2866 HEU children were randomized (1432 CTX, 1434 placebo); the study was then stopped at the recommendation of the Data and Safety Monitoring Board because it was unlikely to show a benefit of CTX. Loss-to-follow-up was low: 95% of infants completed study follow-up; 72% received continuous study drug through 15 months of age, death or study closure. In primary analysis, mortality was similar by randomized arm: 30 deaths in the CTX arm vs. 34 deaths in the placebo arm; estimated mortality at age 18 months was 2.4% vs. 2.6% (difference: 0.2%; 95% CI: -1.0% to 1.5%; Figure 1). The following outcomes did not differ by CTX and placebo arms: hospitalization (10.8% vs. 12.4%,  $p=0.24$ ), grade 3/4 diagnosis (16.1% vs. 17.8%,  $p=0.36$ ), or grade 3/4 anemia (8.0% vs. 8.2%,  $p=0.84$ ). More infants in the CTX vs. placebo arm experienced grade 3/4 neutropenia (7.9% vs. 5.8%,  $p=0.05$ ). Fewer infants breastfed than expected (20% overall), and few deaths (11, 2.3%) occurred in breastfed children by age 18 months: 8 vs. 3 among those randomized to breastfeed for 6 vs. 12 months, and only 3 vs. 2 deaths after 6 months.

**Conclusions:** Prophylactic CTX did not improve 18-month survival or other clinical outcomes among HEU children in southern Botswana. In low-mortality, non-malarial settings with low risk for late MTCT, prolonged CTX for HIV-exposed children may not be required.



### 38 Urgent Versus Post-Stabilization ART in Hospitalized Children: A Randomized Trial

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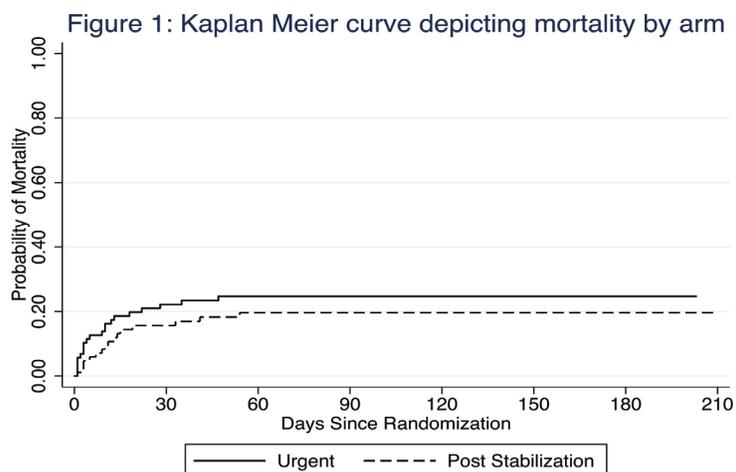
**Background:** Many HIV-infected children are diagnosed with HIV at hospitalization and are at high risk of mortality. Urgent ART may accelerate recovery or worsen outcomes with immune reconstitution. We conducted a randomized trial comparing urgent versus post-stabilization ART among hospitalized ART-naïve HIV-infected children.

**Methods:** HIV-infected children 0-12 years were enrolled at 4 hospitals in Nairobi and Kisumu, Kenya, and randomized to receive ART within 48 hours (urgent arm) or 7-14 days (post-stabilization arm) (NCT02063880). Children were followed at 1, 2, 4, 8, 12, 16, 20 and 24 weeks and monitored for mortality, drug toxicity and immune reconstitution inflammatory syndrome (IRIS). Adverse events were graded using Division of AIDS severity grading. Interim efficacy and futility analyses were performed using O'Brien-Fleming boundaries when ~50% of the expected primary endpoints (65 deaths) accrued. An alternative hypothesis of a hazard ratio (HR) of 0.5 and null hypothesis of a HR of 1.0 were used to determine efficacy and futility, respectively.

**Results:** Of 185 enrolled, 177 were randomized; median age was 1.9 years (IQR 0.8-4.7), 45% were female, 57% had weight for age Z-scores of <-2, and were comparable between arms. CD4 counts were lower in the urgent compared to the post-stabilization arm (12.5% versus 17%,  $p=0.02$ ). Pneumonia, malnutrition and anemia contributed to 61%, 32% and 24% of admission diagnoses, respectively. Among 162 (92%) children who initiated ART, 85 (52%) were in urgent arm. Post-randomization, there were 94 severe adverse events including 37 deaths.

At interim analysis, 177 subjects contributed 630.4 person-months follow-up time post-randomization; 308.7 in urgent and 321.7 in the post stabilization arm. Median time to ART was 1 day (IQR 1, 1) in urgent arm and 8 days (IQR 7, 11) in post-stabilization arm. Incidence of mortality was 82.8 per 100 person-years in urgent arm and 60.6 per 100 person-years in post-stabilization arm [HR 1.36 95% CI (0.71, 2.60)] (Figure 1). Adjusting for baseline CD4 count, aHR was 1.25 (95% CI 0.65, 2.41). During interim analysis the Data Safety and Monitoring Board stopped randomization as the futility boundary was crossed. Independent adjudication on IRIS classification is ongoing.

**Conclusions:** Urgent ART for hospitalized HIV-infected children did not decrease mortality. HIV diagnosis and initiation of ART before symptomatic disease remains critical for survival of HIV-infected children.



**39 Three-Year Outcomes in PMTCT-Exposed Children Switched to EFV Once Suppressed on LPVr**

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**Background:** We have previously demonstrated the non-inferiority in virological control through 48 weeks of switching to efavirenz (EFV) vs. remaining on lopinavir/ritonavir (LPV/r) in PMTCT-exposed children initially suppressed on LPV/r. Here we describe the sustainability of outcomes over 3 years.

**Methods:** From 2010-2013, 298 HIV-infected children exposed to PMTCT containing nevirapine who initiated LPV/r-based treatment <3 years of age and who had HIV RNA <50 copies/mL were recruited into a randomized clinical trial in Johannesburg, South Africa. Children were randomized to remain on LPV/r or to switch to EFV and were followed for 48 weeks. Primary outcomes were viral rebound (HIV RNA >50 copies/mL) and viral failure (HIV RNA >1000 copies/mL confirmed). After trial completion, participants were invited to enroll into an observational cohort study including 6-monthly follow-up visits. We examined differences in viral rebound, viral failure, CD4 percent and lipids across arms over 3 years of follow-up. All analyses were intent-to-treat.

**Results:** Among 298 children enrolled in the original trial, 237 also enrolled in the observational study. Children were followed a median of 37 months (IQR 29.2-48.2) from randomization. Among those in follow-up at 3 years, 94% of those in the EFV arm remained on EFV and 87% in the LPV/r arm remained on LPV/r. Over 3 years of follow-up, the risk of viral rebound was lower in the EFV arm vs. the LPV/r arm (OR 0.64, p=0.005), as was the risk of viral failure (OR 0.50, p=0.05); the cumulative probability of rebound was lower in the EFV arm at 1, 2 and 3 years (Table). The risk of CD4% <35 was reduced in the EFV arm vs. the LPV/r arm (OR 0.56, p=0.007). Additionally the risk of elevated or abnormal lipids was lower among those randomized to EFV, including total cholesterol ≥5.2 mmol/L (OR 0.40, p=0.002), LDL ≥3.4 mmol/L (OR 0.45, p=0.002), and triglycerides >1.69 mmol/L (OR 0.42, p<0.0001). HDL did not significantly differ by arm.

**Conclusions:** Three years after randomization, we found no evidence that children randomized to EFV had compromised virological outcomes relative to remaining on LPV/r. Children in the EFV arm had a lower risk of viral rebound, higher CD4 percentages, and improved lipid profiles compared to children randomized to LPV/r. Our results support the utility of this strategy, which has several advantages, including simplification of tuberculosis treatment, palatability, potential for once-daily dosing, lower cost and preservation of second line options.

Table. Number (n) and Kaplan-Meier (KM) probability of viral rebound (HIV RNA >50 copies/mL) and viral failure (HIV RNA >1000 copies/mL confirmed) by arm and years since randomization.

	Year	LPV/r		EFV	
		n	KM probability (95% CI)	n	KM probability (95% CI)
Rebound	1	42	0.28 (95% CI 0.22-0.36)	26	0.17 (95% CI 0.12-0.25)
	2	66	0.48 (95% CI 0.40-0.57)	49	0.37 (95% CI 0.29-0.46)
	3	91	0.70 (95% CI 0.62-0.78)	70	0.59 (95% CI 0.49-0.68)
<i>Log-rank test p-value = 0.04</i>					
Failure	1	3	0.02 (95% CI 0.01-0.07)	4	0.03 (95% CI 0.01-0.07)
	2	7	0.06 (95% CI 0.03-0.11)	4	0.03 (95% CI 0.01-0.07)
	3	11	0.09 (95% CI 0.05-0.17)	7	0.06 (95% CI 0.03-0.12)
<i>Log-rank test p-value = 0.20</i>					

**40 Efavirenz Is Associated With Higher Bone Mass in South African Children With HIV**

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**Background:** Ritonavir-boosted lopinavir (LPV/r) is recommended as the first line regimen for HIV-infected children but has limitations for long term use. A randomized clinical trial was undertaken in Johannesburg, South Africa to evaluate the safety and efficacy of pre-emptive switching to efavirenz (EFV) vs. remaining on LPV/r in children initially suppressed on LPV/r. The primary results of the trial have been previously reported but here we investigate whether the switch to EFV is associated with beneficial outcomes in terms of bone development. HIV infection affects bone accrual but there are limited data on how to optimize antiretrovirals to improve bone outcomes in children.

**Methods:** 220 HIV-infected children aged 5-10 years (mean 6.4) were enrolled 1-4 years (mean 2.1) after randomization in the trial. 180 similarly-aged HIV-uninfected children were recruited at the same site in Johannesburg for comparison. Physical activity (PA) and dietary intake were assessed by questionnaire. Bone mineral content (BMC), fat mass, and lean body mass of the whole body (WB) were assessed by DXA (Hologic Discovery W). Sex-specific BMC-for-height Z-scores for the infected children were generated using the BMC-for-height distribution of the uninfected controls. Children with HIV currently receiving EFV were compared to those on LPV/r. Results were adjusted for age, fat mass, lean mass, vigorous PA, and dietary vitamin D and calcium, CD4, and viral load. Analyses were also stratified by sex. Intent-to-treat analyses based on the original assigned regimen were also done. **Results:** Among HIV-infected children 110 were on EFV and 110 LPV/r at the time of assessment. All children were also on 2 NRTIs, including 3TC and ABC, AZT, or d4T. None were on TDF. The BMC Z score was -0.49 in the EFV group and -1.07 in the LPV/r group. This association remained significant (p<0.001) and of a similar magnitude (Z-score difference 0.58) after adjustment for age, fat mass, lean mass, vigorous PA, dietary vitamin D and calcium, CD4, and viral load. Sex-stratified analysis showed similar size effects in both boys and girls. In intent-to-treat analyses based on the originally assigned regimen, results were similar. Higher fat and lean body mass were also independently associated with better bone mass outcomes.

**Conclusions:** Accrued bone mass is positively associated with switching to EFV-based ART (compared to remaining on LPV/r). Use of bone friendly drugs may be beneficial for bone health in children with HIV.

**41 Early Antiretroviral Therapy Does Not Improve Vascular Function: A START Substudy**

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**Background:** Both HIV infection and antiretroviral therapy (ART) may increase cardiovascular disease (CVD) risk. Assessments of vascular function can be used to study the pathogenesis and progression of CVD. We compared the effect of early ART initiation (CD4 counts >500 cells/mm<sup>3</sup>) with untreated HIV/deferred ART (until CD4 < 350 cells/mm<sup>3</sup>) on small and large arterial elasticity (SAE and LAE) among a subset of ART-naïve adults in the START trial.

**Methods:** Radial artery waveforms were recorded non-invasively using a tonometer at baseline, months 4, 8, 12, and then annually. SAE and LAE were derived from analysis of the diastolic pulse waveform (CR2000, HDI). Randomized treatment groups (early vs. deferred ART) were compared with linear models at each visit and longitudinal mixed models overall. Additional analyses included censoring deferred group participants who started ART and for protocol-specified subgroups.

**Results:** Among 332 participants at 21 sites in 8 countries on 6 continents: mean (SD) age was 35 (10), 70% male, 66% non-white, 30% smokers, 5% taking BP-lowering therapy, 3% taking lipid lowering therapy, and median [IQR] duration of HIV diagnosis 1.3 years [0.4, 3.1], CD4 count 625 cells/mm<sup>3</sup> [562, 729], HIV RNA 4.2 log<sub>10</sub> copies/mL [3.7, 4.7], and 10-year Framingham risk score for coronary heart disease 1.2% [0.3, 4.2]. Mean (SD) SAE and LAE values at baseline were 7.3 (2.9) mL/mmHg x100 and 16.6 (4.1) mL/mmHg x10, respectively. Median follow-up was 40 months. Median time on ART was 30 and 7 months in the early and deferred ART groups, respectively. Neither treatment group demonstrated significant within-person changes in SAE or LAE over follow-up. Differences in SAE and LAE between early and deferred ART over time are shown in the table. The lack of significant differences persisted in comparisons restricted to ≤1 (or >1) year of follow-up, after censoring participants in deferred group who started ART, and among subgroups defined by CVD and HIV risk factors.

**Conclusions:** Among a diverse global population of HIV+ persons with high CD4 counts, early ART initiation did not improve arterial elasticity. These randomized data suggest that early ART treatment may not have a substantial influence vascular function among younger HIV+ individuals with preserved immunity.

**TABLE: Linear Regression Estimates of Change in Small and Larger Arterial Elasticity at Substudy Visits and Longitudinal Mixed Model Estimate of Overall Change Using All Available Data**

	Small Artery Elasticity Difference* (95%CI)	p-value	Large Artery Elasticity Difference* (95%CI)	p-value
At 4 months	0.29 (-0.24, 0.82)	0.28	-0.38 (-1.14, 0.38)	0.33
At 8 months	0.22 (-0.27, 0.71)	0.38	0.10 (-0.65, 0.84)	0.79
At 12 months	0.30 (-0.27, 0.87)	0.30	-0.15 (-0.91, 0.61)	0.70
At 24 months	0.35 (-0.20, 0.90)	0.20	-0.10 (-0.68, 0.88)	0.80
At 36 months	0.22 (-0.39, 0.83)	0.48	0.29 (-0.71, 1.29)	0.57
All available data	0.29 (-0.09, 0.68)	0.14	-0.07 (-0.61, 0.48)	0.81

\*Treatment group differences (in SAE or LAE units) adjusted for baseline value. Positive values reflect an improvement in arterial elasticity in the early (compared to deferred) ART group.

**42 Comparing Cardiovascular Disease Risk Scores for Use in HIV-Infected Individuals**

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**Background:** While cardiovascular disease (CVD) risk stratification tools exist for use in the general population, they may not accurately estimate risk in persons living with HIV (PLWH). Examining the performance of CVD risk scores in PLWH requires large studies with comprehensive clinical data and well-validated outcomes.

**Methods:** We developed a state-of-the-art screening algorithm and central adjudication protocol for the validation of incident myocardial infarction (MI) in the CFAR Network of Integrated Clinical Systems (CNICS), which harmonizes comprehensive clinical data on PLWH in routine care at multiple US sites. Among PLWH enrolled between 1996-2014, we compared the performance of 3 CVD risk scores developed in the general population (Framingham, ATP-3, and 2013 ACC/AHA ASCVD) and one developed for use in PLWH (D:A:D) using area under the curve (AUC). The Universal Definition of MI classifies MI by type. Type 1 MI (T1MI) result spontaneously from atherosclerotic plaque instability, whereas type 2 MI (T2MI) occur secondary to oxygen demand/supply mismatch of any cause such as sepsis. We compared the AUC for risk scores for T1MI, T2MI, and all MIs combined. Beginning in 2007, CNICS patients completed clinical assessments every 4-6 months that included tobacco use. We repeated analyses among this subset to ensure smoking status was updated for those who quit or started smoking.

**Results:** There were 243 incident MIs among 11,338 PLWH during a mean follow-up of 4.3 years. ASCVD had a significantly better AUC than other scores for all MI and for T2MI (Table) including the DAD AUC ( $p < 0.001$ ), and was not inferior to the other AUCs for T1MI. Our results were similar in the subset of PLWH with time-updated smoking status.

**Conclusions:** The large size, comprehensive clinical data and central adjudication of MI by type in CNICS allows for direct comparison of clinical risk scores in PLWH. Some variations across risk scores are to be expected given differences in the outcome (i.e. predicting CVD vs. MI). The addition of HIV-specific variables as in the DAD score did not improve discrimination compared with ASCVD, however inclusion of different HIV-specific measures may lead to improved discrimination and is planned in future analyses. ASCVD performed as well or better than other risk scores across all MI events and the superior performance in detecting T2MI is worthy of additional investigation.

**Table. Comparison of AUC values for type 1 MI events, type 2 MI events and all MI events combined for 4 risk scores in HIV**

	Framingham		DAD		ATP3		ASCVD	
	AUC	CI	AUC	CI	AUC	CI	AUC	CI
Type 1 MI	0.725	0.69,0.77	0.741	0.70,0.78	0.735	0.70,0.77	0.750	0.71,0.79
Type 2 MI	0.634*	0.58,0.69	0.626*	0.57,0.68	0.627*	0.57,0.68	0.720	0.67,0.77
All MI	0.687*	0.65,0.72	0.693*	0.66,0.73	0.690*	0.66,0.72	0.740	0.71,0.77

\* AUC significantly different from ASCVD AUC

**43 Stroke Incidence Highest in Women and Black HIV-Infected Participants in ALLRT Cohort**

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**Background:** While rates of stroke are higher in HIV-infected compared with age-matched HIV-uninfected individuals, many questions persist regarding the nature of cerebrovascular disease in HIV. We leveraged the large ACTG Longitudinal Linked Randomized Trials (ALLRT) cohort and its parent studies to investigate stroke incidence and associated risk factors in HIV-infected individuals.

**Methods:** We conducted a prospective observational cohort study of ART-naïve participants without a history of stroke who initiated ART from June 1998 to June 2011. The primary outcome was first-ever stroke or transient ischemic attack (TIA) documented at study follow up visits through centralized reporting. Age-adjusted Poisson regression models with time-updated covariates were used to identify traditional and HIV-specific risk factors for incident stroke/TIA.

**Results:** Of 6,933 participants included in the analysis, 20% were women, 37% were non-Hispanic Blacks and 21% were Hispanic. Median pre-ART age was 37 years, pre-ART CD4 count was 243 cells/uL and pre-ART HIV RNA was 57,624 copies/mL. Fifty-four stroke/TIAs occurred over 32,023 person-years (PY). The incidence rate of stroke/TIA in women was 0.29 per 100 PY versus 0.14 per 100 PY in men [age-adjusted relative risk (RR) for female sex 1.72, 95% CI 0.96-3.08]. Incidence of stroke/TIA in non-Hispanic Blacks was 0.25 per 100 PY compared with 0.08 per 100 PY in Hispanics/Other (age-adjusted RR 2.94, 95% CI 1.22-7.14) and 0.16 per 100 PY in Whites (RR 1.67, 95% CI 0.95-2.94). In a multivariable model (Table), traditional risk factors that conferred greater risk of stroke/TIA were older age, LDL  $\geq$  160 mg/dL and hypertension. Of HIV-related factors, time-updated CD4 count  $\leq$  200 cells/uL and HIV RNA  $>$  200 copies/mL were associated with increased risk of stroke/TIA. We found no statistically significant association of injection drug use, hepatitis C infection or recent use of any class of ART with stroke/TIA.

**Conclusions:** Age-adjusted incidence of stroke/TIA was highest in women and non-Hispanic Blacks in this cohort of HIV-infected participants. Investigation into the association between female sex and non-Hispanic Black race with stroke/TIA in HIV is merited. In addition to an association with several modifiable traditional risk factors, stroke/TIA was also associated with immunodeficiency and poor virologic control. This raises the possibility that immunologic sequelae of uncontrolled viremia may also contribute to stroke risk in HIV infection.

**Table: Unadjusted and adjusted relative risk of incident stroke/TIA associated with demographic, vascular and HIV-specific factors**

		Unadjusted		Age-adjusted		Final*	
		Relative Risk (95% CI)	P-value	Relative Risk (95% CI)	P-value	Relative Risk (95% CI)	P-value
Sex (vs Male)	Female	2.06 [1.17, 3.63]	0.01	1.72 [0.96, 3.08]	0.07		
Race/Ethnicity (vs Black)	Hispanic+Other	0.31 [0.13, 0.74]	0.009	0.34 [0.14, 0.82]	0.02	0.39 [0.16, 0.96]	0.04
	White	0.62 [0.35, 1.09]	0.1	0.60 [0.34, 1.05]	0.07	0.74 [0.41, 1.35]	0.3
Time-updated age	Every 10 year increase	2.43 [2.02, 2.91]	<0.001			2.39 [1.96, 2.91]	<0.001
Time-updated LDL (vs <160)	$\geq$ 160 mg/dL	2.88 [1.40, 5.92]	0.004	2.41 [1.16, 5.00]	0.02	2.95 [1.40, 6.20]	0.004
Time-updated hypertension	Yes	3.37 [1.93, 5.87]	<0.001	2.92 [1.65, 5.17]	<0.001	3.05 [1.74, 5.35]	<0.001
Time-updated BMI (vs Underweight/Normal)	Overweight	0.50 [0.26, 0.96]	0.04	0.45 [0.24, 0.87]	0.02	0.45 [0.23, 0.86]	0.02
	Obesity	0.75 [0.38, 1.47]	0.4	0.66 [0.33, 1.30]	0.2	0.57 [0.28, 1.13]	0.1
Time-updated CD4 (vs >500)	$\leq$ 200 cells/uL	2.59 [1.24, 5.41]	0.01	2.85 [1.36, 6.01]	0.006	1.89 [0.92, 3.90]	0.08
	201-500	1.38 [0.76, 2.51]	0.3	1.41 [0.77, 2.56]	0.3	1.24 [0.67, 2.27]	0.5
Time-updated HIV RNA (vs $\leq$ 200)	$>$ 200 copies/mL	2.34 [1.31, 4.19]	0.004	3.12 [1.71, 5.66]	<0.001	2.57 [1.41, 4.66]	0.002

\*Final includes all  $p \leq 0.05$  variables from the age-adjusted analysis.

**441B Aspirin Fails to Impact Immune Activation or Endothelial Function in Treated HIV**

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**Background:** Immune activation persists despite optimally treated HIV infection and predicts non-AIDS co-morbidities including cardiovascular disease and certain cancers.

Activated platelets play a key role in thrombosis and inflammation, and HIV induces platelet activation by direct and indirect mechanisms. Aspirin is a potent inhibitor of platelet activation. We hypothesized that aspirin would reduce immune activation and improve endothelial function in antiretroviral therapy (ART)-suppressed HIV-infected individuals.

**Methods:** ACTG A5331 was a prospective, double-blind, randomized, placebo-controlled 3-arm trial of HIV-infected participants on suppressive ART for  $>$ 48 weeks randomized to daily aspirin 100mg, aspirin 300mg or placebo for 12 weeks followed by a 4 week washout. The primary outcome was sCD14 and secondary outcomes included FMD, D-dimer, other soluble and cellular immune activation markers, and thromboxane (a direct readout of cyclooxygenase inhibition). Multivariable linear regression was used to assess differences and differential effects between treatment arms at a 5% a level.

**Results:** 121 participants enrolled; 8 did not complete treatment, were non-adherent, or had a serious bacterial infection and were excluded from analyses. Of 113 per-protocol participants, all had HIV VL<50 copies/mL, 81% were male; median age 49 years; median CD4+ T cell count 616/mm<sup>3</sup>. Aspirin was well tolerated. There was one protocol-defined toxicity of bleeding in stool in the placebo arm. Key results are shown in the table. Serum thromboxane was significantly inhibited by aspirin, suggesting high study drug adherence and aspirin efficacy to inhibit cyclooxygenase. There were no consistent differences between the 300mg or 100mg aspirin arms vs. placebo for sCD14, FMD, or any of the other immunologic endpoints, though the 300mg aspirin arm experienced a greater increase in sCD163 than the placebo arm. Interactions by current smoking, sex, age, ART regimen, and baseline marker tertile were assessed with aspirin showing less of an increase in sCD163 among smokers (p=0.047) and women (p=0.031) and greater reductions in D-dimer among smokers (p=0.03).

**Conclusions:** Aspirin for 12 weeks does not appear to have a major impact on immune activation or endothelial function in ART-suppressed HIV-infected individuals. While this study does not support the use of aspirin as an anti-inflammatory in patients with HIV infection, there may be some subgroups that derive benefit, which could be explored in future studies.

Biomarker	Fold Change from Baseline to Week 12 (95 % CI)				
	Placebo (N=37)	Aspirin 100mg (N=38)	P Value <sup>1</sup>	Aspirin 300mg (N=38)	P value <sup>1</sup>
Serum thromboxane(ng/mL)	1.21 (0.86, 1.70)	0.21 (0.16, 0.30)	<0.001	0.28 (0.20, 0.39)	<0.001
sCD14 (ng/mL)	0.97 (0.93, 1.02)	1.03 (0.98, 1.08)	0.70	0.99 (0.94, 1.04)	0.70
sCD163(ng/mL)	0.98 (0.89, 1.07)	1.03 (0.94, 1.13)	0.44	1.12 (1.03, 1.23)	0.037
IL-6(pg/mL)	1.03 (0.87, 1.21)	1.13 (0.96, 1.33)	0.096	1.03 (0.87, 1.21)	0.38
D-dimer (ng/mL)	1.02 (0.91, 1.13)	0.99 (0.89, 1.10)	0.69	1.08 (0.97, 1.19)	0.46
KT ratio (nM/uM) <sup>2</sup>	-1.3 (-3.6, 1.1)	-3.0 (-5.3, -0.7)	0.30	0.5 (-1.8, 2.7)	0.30
%CD38+HLA-DR+ CD8+ T cells <sup>2</sup>	-0.4 (-2.2, 1.3)	-1.5 (-3.2, 0.2)	0.40	-1.2 (-2.9, 0.5)	0.56
FMD (%) <sup>2</sup>	-0.5 (-1.3, 0.4)	-1.2 (-2.1, -0.4)	0.091	-0.5 (-1.3, 0.4)	0.61

<sup>1</sup>P value tests difference between Aspirin arm and placebo arm

<sup>2</sup>Changes in KT ratio, CD8 activation, and FMD were modeled as absolute changes (not relative changes).

**45 Body Composition Changes on DRV/r + Either RAL or TDF/FTC As First-Line ART**

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**Background:** Data on body composition, adipokines and inflammatory markers changes after initial therapy with a nucleos(t)ide reverse transcriptase inhibitor (NtRTI)- sparing or containing regimen are scarce. The effect of tenofovir/emtricitabine (TDF/FTC) vs raltegravir (RAL), both in association with ritonavir-boosted darunavir (DRV/r) could be relevant for treatment choice.

**Methods:** NEAT001/ANRS143 is a randomised 1:1, open-label, non-inferiority trial comparing DRV/r + RAL or TDF/FTC in 805 ART naïve HIV-infected adults. We compared

percentage change in lean mass, limb fat mass, trunk fat mass and total body fat mass assessed by dual energy X-ray absorptiometry scans (DXA) in a substudy of 146 subjects (random sample). Main endpoint was mean % change of limb fat at W96. Secondary endpoints: relationship between changes in body composition variables, and inflammatory/metabolic markers (IL-6, insulin, leptin, adiponectin, FGF-23).

**Results:** 126 (61 DRV/r + RAL and 65 DRV/r + TDF/FTC) had at least one follow-up DXA available. At baseline: 91% male, 14% black, median age 40 years, median BMI 23.2 Kg/m<sup>2</sup>, HIV-1 RNA load 4.7 log<sub>10</sub> copies/mL, CD4 count 338 cells/μL. There was no difference in limb fat mass at W96 between arms (Table). At W96 there was a greater increase in the DRV/r + RAL arm in mean trunk fat mass (15.5% in DRV/r + RAL vs. 8.7% in DRV/r + TDF/FTC; p = 0.026) and total body fat mass (4.1% in DRV/r + RAL vs. 0% in DRV/r + TDF/FTC; p = 0.032). These differences remained robust to adjustment for baseline CD4 count and viral load. Baseline insulin and leptin levels were correlated with baseline limb fat/trunk fat mass [r=0.31 (p=0.0043)/r=0.28 (p=0.0011); r=0.63 (p<0.0001)/r=0.50(p<0.0001), respectively]. Adiponectin was correlated with baseline limb fat mass only [r=0.40 (p<0.0001)]. After adjustment, persons with a 10% increase in leptin between baseline and W48 had a 0.5% (95% CI 0.3-0.7; p<0.0001) and 0.3% (95% CI 0.1-0.6; p=0.013) increase in limb fat mass at W48 and W96 respectively and a 0.4% increase in trunk fat at W96 (95% CI 0.1-0.6; p=0.014). There was no association between baseline IL-6 or insulin and changes in limb or trunk fat mass.

**Conclusions:** The NtRTI sparing regimen produced a higher increase in total and trunk fat mass than the TDF/FTC containing regimen. These changes were correlated with changes in leptin levels.

	Mean % Change (95%CI)		% Change (95%CI)	p-value
	DRV/r + RAL	TDF/FTC + DRV/r		
<b>Week 48</b>				
Limb fat mass	N= 53 22.7 (9.6, 35.8)	N= 62 9.7 (1.7, 17.7)	-13 (-27.9, 1.9)	0.09
Trunk fat mass	N= 49 14.4 (7.2, 21.7)	N= 62 12 (6.3, 17.7)	-2.4 (-11.6, 6.8)	0.61
Total body lean mass	N= 53 10.8 (-2.1, 23.7)	N= 62 0.9 (-0.7, 2.5)	-9.3 (-22, 2.4)	0.11
Total body fat mass	N=53 12 (2.4, 21.6)	N=62 2.8 (1.4, 4.2)	-9.1 (-18.1, -0.1)	0.05
<b>Week 96</b>				
Limb fat mass	N= 49 10.9 (0.9, 20.9)	N= 55 3.8 (-4.4, 12)	-7.1 (-20, 5.8)	0.29
Trunk fat mass	N= 50 15.5 (-2.9, 35.7)	N= 55 8.7 (0.5, 16.9)	-16.9 (-31, 2.8)	0.026
Total body lean mass	N= 49 1.4 (-1, 3.8)	N= 55 -1.1 (-4.0, 1.8)	-2.4 (-6.1, 1.3)	0.22
Total body fat mass	N=49 4.1 (1.6, 6.7)	N=55 0 (-2.6, 2.6)	-4.1 (-7.8, -0.4)	0.032

**46 Antiretrovirals, Fractures, and Osteonecrosis in a Large European HIV Cohort**

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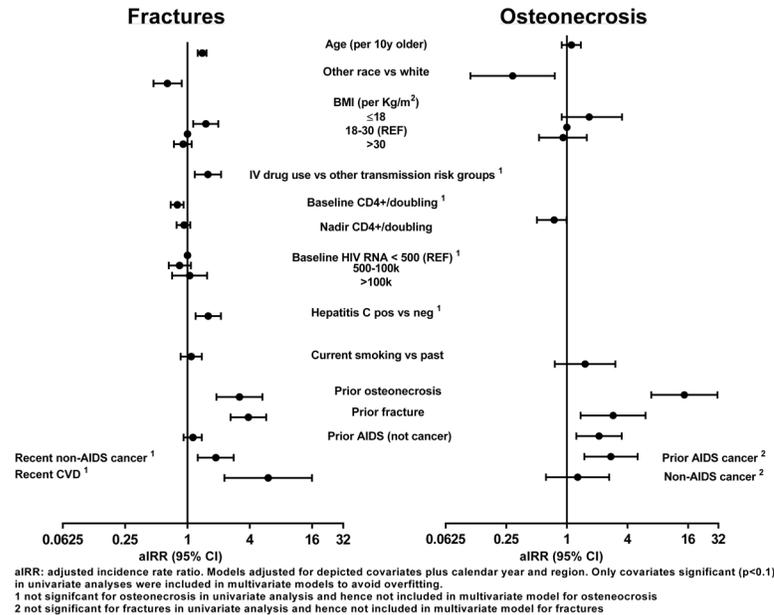
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**Background:** It is well established that antiretrovirals (ARVs) affect markers of bone turnover, but less is known about their effect on risk of fractures and femoral osteonecrosis. We hypothesized exposure to ARVs including tenofovir (TDF) would increase the risk of both outcomes.

**Methods:** EuroSIDA participants were prospectively followed from baseline (Jan 2004) until last visit or death to assess fractures and femoral osteonecrosis. Poisson regression was used to identify clinical, laboratory and demographic factors associated with either bone endpoint. Ever, current and cumulative exposures to each ARV were added to the multivariate model.

**Results:** During 86118 person-years of follow up (PYFU) among 11820 eligible persons (median age 41y, 75% male, 86% white, median baseline CD4 440/mm<sup>3</sup> and 70.4% virologically suppressed), there were 618 incident fractures (incidence/1000PYFU 7.2; 95%CI 6.6-7.7) and 89 incident cases of osteonecrosis (1.0;0.8-1.3). After adjustment, higher risk of fractures was associated with older age, white race, lower BMI, IV drug use, lower baseline CD4, HCV-coinfection, prior osteonecrosis, prior fracture, recent non-AIDS cancer and recent cardiovascular disease (last 12 months) (Figure). The crude incidence of fracture was 8.1/1000PYFU (7.3-8.9) in those ever exposed to TDF compared to 4.7 (4.1-5.4) in those never exposed; corresponding figures for persons currently on and off TDF were 7.8 (6.8-8.7) and 5.6 (5.0-6.3). After adjustment, persons who had ever used TDF (1.40; 1.15-1.70) or who were currently on TDF (1.25; 1.05-1.49) had a significantly higher incidence of fractures. There was no association between longer exposure to TDF and fractures (1.02/5y exposure; 0.94-1.25). No other ARV was associated with fractures (all p>0.1). Risk of osteonecrosis was associated with white race, lower nadir CD4, prior osteonecrosis, prior fracture and prior AIDS (Figure). Persons who had ever used didanosine, indinavir, saquinavir, lopinavir/r, or TDF had higher risk of osteonecrosis, but this association was no longer observed in models adjusted for confounders (not shown).

**Conclusions:** In HIV infection, host factors, HIV-specific variables and co-morbidities contribute to risk of fractures and osteonecrosis. TDF but not other ARVs was an independent risk factor for fractures. There was no association between the use of any of the ARVs and risk of osteonecrosis.



**47 A Single Dose Zoledronic Acid Prevents Antiretroviral-Induced Bone Loss**

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**Background:** HIV enhances bone loss, and close to 2/3 of HIV patients are osteopenic and 15% osteoporotic, leading to 2-9 fold higher fracture prevalence in the aging HIV population. Antiretroviral therapy (ART) further worsens this loss, inducing an additional 2-6% loss in bone mineral density (BMD), mostly within the first 48 weeks, providing a window for prophylaxis with long-acting antiresorptives such as zoledronic acid (ZA).

**Methods:** We randomized non-osteoporotic, viremic ART-naïve adult HIV-patients initiating ART with atazanavir/ritonavir+tenofovir/emtricitabine to a single ZA (5mg) vs. placebo (PL) infusion in a double-blinded placebo controlled phase 2 trial. Laboratory and safety measures, plasma bone turnover markers including C-terminal telopeptide of collagen (CtX), a sensitive marker of bone resorption, and BMD were performed at weeks 0, 12, 24, and 48. Repeated-measures analyses using mixed linear models were used to estimate and compare study endpoints.

**Results:** Of the 63 subjects enrolled, 84% were black, 16% white, and 21% women with a mean age of 39.6 years. Treatment with ZA was associated with a 74% reduction in bone resorption relative to PL at 12 weeks [CtX: 0.08 ng/ml (ZA) vs. 0.31 ng/ml (PL), p<0.001; mean difference=-0.23 ng/ml (95% CI: -0.31, -0.14)] with 65% and 56% relative reduction at 24 and 48 weeks respectively. ZA led to an 8% increase in lumbar spine BMD at 12 weeks relative to PL [1.305 g/cm<sup>2</sup> vs. 1.204 g/cm<sup>2</sup>, p=0.003; mean difference=0.101 g/cm<sup>2</sup> (95% CI: 0.035, 0.168)], with a greater increase of 11% at 24 and 48 weeks. Of note, BMD at the lumbar spine increased 1.9% (95% CI: 0.39, 4.22) from baseline to 48 weeks in the ZA arm but decreased 4.4% (95% CI: 2.63, 6.24) in the PL arm. Lumbar spine T- and Z-scores were significantly higher in patients receiving ZA vs. PL at weeks 12, 24, and 48 (all p<0.05). Significant trends were also observed at the hip and femoral neck. The rate of virologic suppression and mean CD4 T cell increase over 48 weeks were similar between the arms. ZA was well tolerated without major side effects.

**Conclusions:** In this single center proof-of-concept study, a single infusion of ZA at the time of ART initiation prevented ART-induced bone resorption and bone loss at key fracture-prone anatomical sites. These effects were observed as early as 12 weeks and persisted through 48 weeks, the period when ART-induced bone loss is most intense. Replication of these results in a confirmatory multicenter randomized clinical trial is warranted.

**48LB Recovery of Bone Mineral Density After Stopping Oral HIV Preexposure Prophylaxis**

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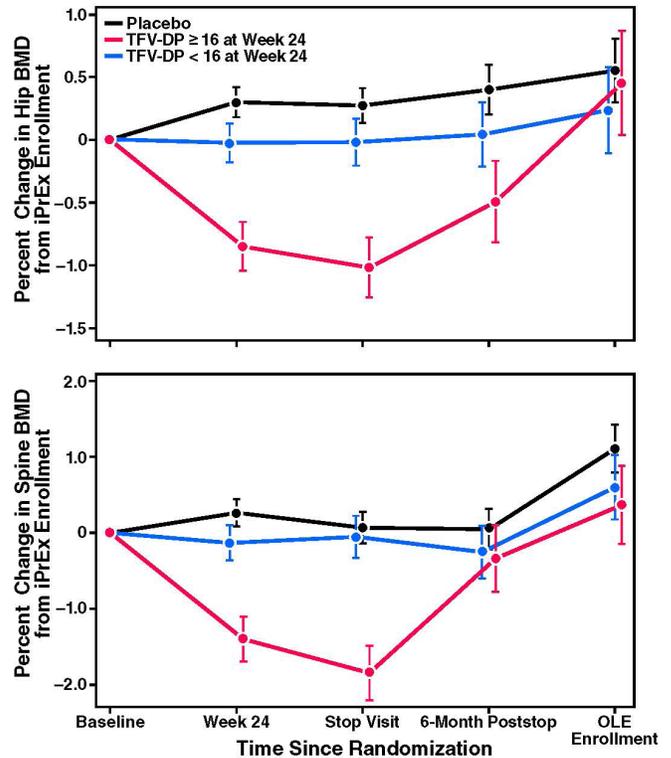
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**Background:** Oral pre-exposure prophylaxis (PrEP) containing emtricitabine/tenofovir disoproxil fumarate (FTC/TDF) is associated with small decreases in bone mineral density (BMD) compared with placebo. Whether BMD recovers completely after stopping PrEP is not known.

**Methods:** The iPrEx trial was a blinded randomized trial of daily oral FTC/TDF PrEP versus placebo among men and transgender women who have sex with men. The randomized phase was followed by an open label extension (iPrEx OLE) that started after a variable gap in PrEP use. An optional substudy measured BMD by dual-energy X-ray absorptiometry (DXA) every 24 weeks during PrEP use, 24 weeks after stopping PrEP, and at the beginning of iPrEx OLE. A concentration of tenofovir-diphosphate (TFV-DP) of 16 fmol per million (fmol/m) viably cryopreserved peripheral blood mononuclear cells was associated with a 90% reduction in HIV incidence and indicated use of 2 to 3 tablets per week. BMD in participants with week 24 TFV-DP levels above 16 fmol/m were compared with those randomized to receive FTC/TDF who had lower drug concentrations and to those in the placebo group.

**Results:** 498 people were enrolled in the iPrEx DXA substudy, in which BMD decreased during the first 24 weeks of PrEP use (Figure). 352 (71%) had DXA scans 24 weeks after stopping study medication, and 289 (58%) had scans at the start of iPrEx OLE, which occurred a median of 73 weeks (interquartile range: 59 to 87) after stopping study medication. Among those with scans at the start of iPrEx OLE, the median age was 29, and 12% percent identified as trans. Average BMD in the spine and hip accumulated after PrEP use stopped: among those with TFV-DP >16 fmol/m at week 24, average annualized recovery rates after stopping PrEP were 1.81±0.36% in the spine (P=0.01 vs. placebo) and 1.13±0.27% in the hip (P=0.002 vs. placebo). In this group, average BMD recovered completely within 6 months after stopping PrEP in the spine, and by the start of iPrEx OLE in both the hip and spine (Figure). Evidence of BMD recovery persisted after adjusting for differences in study retention by age and drug concentrations and in multiple imputations of BMD values.

**Conclusions:** BMD loss is observed with levels of FTC/TDF PrEP use that are near the minimum required for providing high-level protection from rectal HIV exposure. In this predominately young adult population, there was recovery of BMD to placebo levels after stopping FTC/TDF PrEP.



**49 Male Partner Home HIV Testing vs Clinic Invitation in Pregnancy: A Randomized Trial**

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**Background:** Partner HIV testing during pregnancy has been difficult to achieve in sub-Saharan Africa as men seldom attend antenatal appointments. Home-based testing may be an effective alternative method to test partners and identify discordant couples in pregnancy. A randomized clinical trial was conducted in Kenya to determine whether home-based partner education and HIV testing (HOPE) during pregnancy results in higher uptake of testing by partners and increased detection of HIV-discordant couples compared to those who receive a clinic invitation letter.

**Methods:** Pregnant women attending a first antenatal visit at Kisumu East District Hospital from October 2012 to May 2013 were randomized to receive the HOPE intervention or a clinic invitation letter for the woman’s partner (INVITE). The HOPE intervention was a scheduled home visit by a male/female pair of community health workers within 2 weeks of enrollment with couple HIV testing and counseling. In both arms, women had follow-up visits at 6 and 14 weeks postpartum at the clinic and couples had 6 month postpartum home visits. Relative risks were calculated between the two arms.

**Results:** Among 1,101 women screened, 620 (56%) were eligible, and 601 (97%) were enrolled. At enrollment, mean age of women was 24.9 years and 19.1% were HIV positive. Retention was high at 6 months postpartum (88% of women, 86% of men). During the study period, 233 (87%) of 247 men in HOPE reported being tested for HIV during the study period compared to 108 (39%) of 240 men in INVITE (Relative Risk [RR] 2.10; 95% CI: 1.82-2.42). Furthermore, 217 (88%) of 248 women in HOPE knew their partner’s status compared to 98 (39%) of 254 women in INVITE (RR 2.27; 95% CI: 1.93-2.67), 192 (77%) of 248 women in HOPE had been tested as a couple compared to 62 (24%) of 254 women in INVITE (RR 3.17; 95% CI 2.53-3.98) and 33 (13%) of 248 women in HOPE were identified as being in a discordant partnership compared to 10 (4%) of 254 women in INVITE (RR 3.38; 95% CI: 1.70-6.71).

**Conclusions:** Home partner testing resulted in significantly more partner and couple HIV testing, disclosure, and identification of discordant couples compared to a clinic invitation letter to the male partner. This intervention has the potential for prevention of incident HIV infection in pregnancy and MTCT as well as testing harder to reach populations. There is a need to develop strategies to adapt and scale-up similar interventions in settings with high HIV prevalence and low male partner participation in PMTCT.

Home-based Partner Education and HIV Testing (HOPE) vs. Clinic Invitation (INVITE) in Pregnancy: A Randomized Clinical Trial in Kisumu, Kenya, October 2012 - June 2015 (n=601)

Male report Between enrollment during pregnancy & 6 months postpartum <sup>z</sup>	HOPE (n=247)	INVITE (n=240)	RR	95% CI
Male HIV tested	233 (87%)	108 (39%)	2.10	1.81-2.42
Female report Between enrollment during pregnancy & 6 months postpartum <sup>z</sup>	HOPE (n=248)	INVITE (n=254)	RR	95% CI
Female knows male status	217 (88%)	98 (39%)	2.27	1.93-2.67
Tested as couple	192 (77%)	62 (24%)	3.17	2.53-3.98
Discordant couples identified	33 (13%)	10 (4%)	3.38	1.70-6.71

<sup>z</sup>Of 568 women with live births and no neonatal deaths, 487 men (86%) had 6 month postpartum visits and 502 women (88%) had both a 6 week and 6 month postpartum visit which captures information for entire study period

50 Effectiveness of Partner Services for HIV in Kenya: A Cluster Randomized Trial

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**Background:** Case finding using HIV assisted partner services (aPS) is widely practiced in the United States and Europe but less so in Africa due to limited data on effectiveness in routine health settings. We report preliminary results of a cluster randomized trial to assess the effect of immediate aPS on rates of 1) HIV testing, 2) case-finding of HIV-infected individuals, and 3) linkages to HIV care for sexual partners.

**Methods:** In this cluster randomized trial, eligible HIV-infected adults were recruited from 18 HIV testing sites randomized to immediate (intervention) or delayed (control) aPS. In both intervention and control arms, index cases were asked to provide names and contact information of sexual partners in the preceding three years. Notification, testing and referral to care (if HIV-infected) of sexual partners occurred immediately in the intervention arm and 6 weeks after enrollment in the control arm. Generalized estimating equations with a Poisson link and independent correlation structure were used to evaluate the effect of the intervention on outcomes. The study was registered in ClinicalTrials.gov (NCT01616420).

**Results:** The study enrolled 1119 index cases from 18 different clusters (550 immediate arms; 569 delayed arm) who mentioned 1872 sexual partners. Among these mentioned sexual partners, 1292 (69%), [620 immediate arm; 672 delayed arm] were enrolled. Enrollment and follow-up data were available for 579 (63%) and 672 (70%) of sexual partners mentioned in the immediate and delayed arms, respectively. Among 913 partners mentioned, 388 (42.5%) tested for HIV in the immediate arm, and among 959 partners mentioned, 118 (12.3%) tested in the delayed arm. Immediate aPS increased testing rates four-fold (Incidence rate ratio (IRR) 3.78, 95% CI: 3.08-4.65). The IRR comparing rates of first-time testers between immediate and delayed arms was 11.50 (95% CI: 5.56-23.78). Immediate aPS also significantly increased the number testing positive and enrolling into HIV care (IRR 3.22 [95% CI: 2.26-4.61] and 3.95 [95% CI: 2.48-6.28] respectively).

**Conclusions:** aPS was highly effective and resulted in increased HIV case finding and linkage to care for HIV-infected sexual partners in this sub-Saharan African setting. aPS should be considered as a key strategy to improve delivery of HIV testing and counseling in Kenya and other high HIV prevalence settings where large numbers of the HIV-infected individuals do not know their status.

51 Optimal Timing of Home-Based HIV Testing in Western Kenya

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<sup>1</sup>Imperial Coll London, London, UK; <sup>2</sup>Univ of Toronto, Toronto, ON, Canada; <sup>3</sup>Academic Model Providing Access to Healthcare, Eldoret, Kenya; <sup>4</sup>Moi Univ, Eldoret, Kenya; <sup>5</sup>Brown Univ, Providence, RI, USA

**Background:** Achieving UNAIDS 90-90-90 goals for ART coverage will require new strategies for diagnosing and linking HIV positive persons to care. Home-based counselling and testing is one strategy that has been piloted and considered in sub-Saharan Africa. We used mathematical modelling to evaluate how HBCT campaigns can be improved, through the optimal-timing and enhancement of testing rounds, to bring about greater health outcomes over a 20 year period.

**Methods:** We created an individual-based mathematical model to describe the HIV epidemic and the experiences of care among HIV-infected adults in the data rich example setting of Kenya. We calibrated the model to a longitudinal dataset from the Academic Model Providing Access To Healthcare (AMPATH) programme describing the routes into care, losses, and clinical outcomes. We simulated various permutations of HBCT campaigns between 2016 and 2036, and for each assessed the impact and total cost of care cost for a further 20 years.

**Results:** We find that simulating five equally spaced rounds of population-wide HBCT between 2016 and 2036, averts 1.59m DALYs at a cost of \$1,018 per DALY averted. By altering the timing of HBCT rounds for a range of campaigns containing different numbers of rounds, we find that four testing rounds in 2016, 2017, 2020 and 2032 reduces the cost per DALY averted by 10%, and maximises the health impact by averting 2.5% more DALYs than the five-round status quo campaign. By improving linkage to care for individuals testing through HBCT to clinics, the space between optimally-timed HBCT rounds increases, and we find that four rounds, avert over two million DALYs (51% more than the status quo). However, achieving the UNAIDS 90-90-90 targets by 2020 also requires other aspects of care to be strengthened. For these targets to be met, the addition of active outreach for individuals lost from ART care is required. An HBCT campaign consisting of two rounds (2016 and 2017) and active outreach will avert 6.58m DALYs at a cost per DALY of \$220 (78% less than the status quo).

**Conclusions:** HBCT campaigns can improve health outcomes for patients when rounds are optimally timed and structurally enhanced. Countries implementing HBCT should avoid naively-spaced testing rounds, tailor campaigns to individual settings and further strengthen other aspects of the care cascade to achieve UNAIDS 90-90-90 goals.

**52 Estimating the Lifetime Risk of a Diagnosis of HIV Infection in the United States**

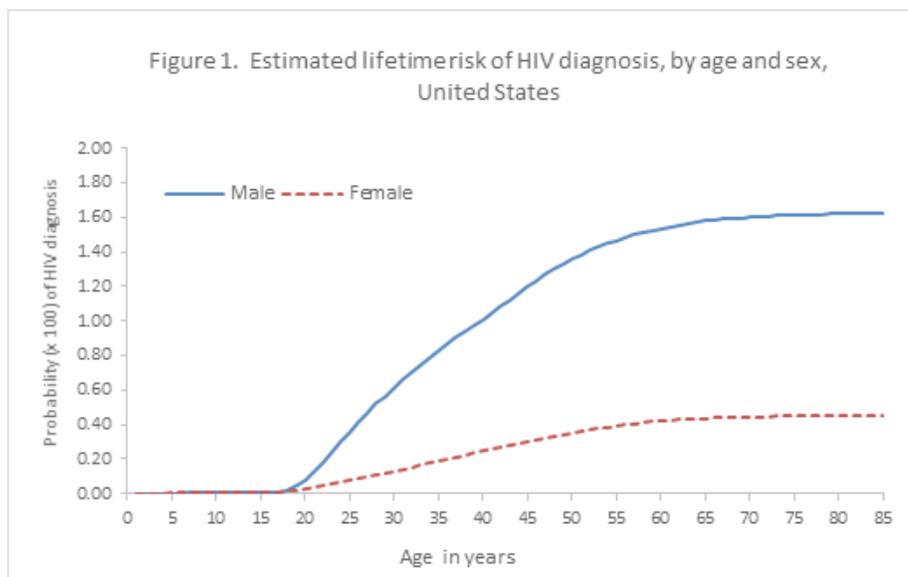
**Kristen Hess;** Xiaohong Hu; Amy Lansky; Jonathan Mermin; H. Irene Hall  
*CDC, Atlanta, GA, USA*

**Background:** Estimates of lifetime risk can be used to compare the burden of disease across populations. This method is frequently used to describe cancer risk, but has infrequently been used for HIV infection. We estimated the lifetime risk of an HIV diagnosis for sex, age, and racial/ethnic subgroups as well as by state.

**Methods:** HIV diagnosis, mortality, and census population data were used to derive lifetime and age-conditional risk estimates of being diagnosed with HIV. Data on HIV diagnoses (adjusted for reporting delays) were obtained from the National HIV Surveillance System (NHSS). The numbers of HIV diagnoses (NHSS) and non-HIV deaths (mortality data) between 2009 and 2013 were used to calculate probabilities of a diagnosis of HIV at a given age, conditional on never having developed HIV prior to that age using a competing risks method. The lifetime risk estimate is the cumulative probability of being diagnosed with HIV from birth. Age-conditional risk measures were the probabilities of an individual of a specified age being diagnosed with HIV within ten years. The lifetime and age-conditional risk estimates were calculated for the entire population and each combination of gender and race/ethnicity. Lifetime risk estimates were also calculated by state. All calculations were conducted in DevCan 6.7.3. Comparisons were made to findings from a 2004-2005 analysis.

**Results:** Overall, the estimated lifetime risk of being diagnosed with HIV was 1.05%, meaning that approximately 3 million Americans (or 1 in 96 people) will be diagnosed with HIV in their lifetime. This was a decrease from a 2004-2005 estimate (1.29%). Among males the estimated risk was 1 in 62, and among females it was 1 in 221. At every age, males had a higher estimated lifetime risk than females (Figure 1). For males and females, the highest lifetime risk was among blacks (male: 1 in 19; female: 1 in 46). The estimated lifetime risk among Hispanics/Latinos was 1 in 47 among males and 1 in 214 among females. Among white males the lifetime risk was 1 in 127 and among white females it was 1 in 851. The lifetime risk estimates varied by state from 1 in 43 in Georgia to 1 in 662 in North Dakota. The highest lifetime risk was in Washington D.C. (1 in 13), an urban district.

**Conclusions:** The overall lifetime risk has decreased. However, without improvements in prevention, millions of Americans are expected to acquire HIV infection during their lifetime, and large disparities persist by sex and race/ethnicity.



**53 Increased HIV Viral Suppression Among US Adults Receiving Medical Care, 2009-2013**

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**Background:** Persons living with HIV who achieve viral suppression have greatly improved health outcomes and decreased risk of transmitting HIV to others. Increasing the number of persons living with HIV who are virally suppressed is key to reaching national HIV prevention goals in the United States.

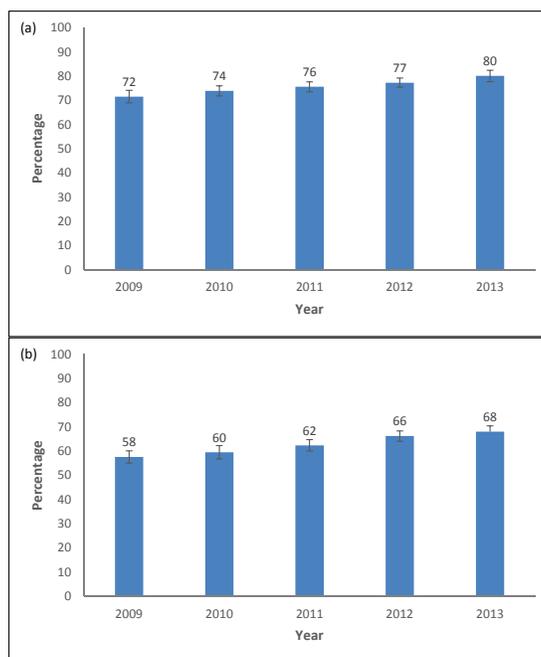
**Methods:** We used 2009 – 2013 Medical Monitoring Project (MMP) data to estimate the proportion of persons receiving HIV medical care who achieved HIV viral suppression (< 200 copies/mL) at both last test and at all tests in the previous 12 months. MMP is a surveillance system that produces nationally representative information about persons receiving HIV medical care in the United States. Data were collected from 23,125 persons using interviews and medical record abstractions. We assessed temporal trends in viral suppression overall and by gender, age, race/ethnicity, and sexual behavior/orientation.

**Results:** The proportion of persons whose HIV virus was suppressed at most recent test increased from 72% to 80% from 2009 – 2013 ( $\beta=0.02$ ,  $P$  for trend < 0.01). This positive trend was statistically significant among men and women; all age groups; non-Hispanic blacks, non-Hispanic whites and Hispanics; and men who have sex with men, men who have sex with women, and women who have sex with men. The largest increases were among 18–29 year olds (56% to 68%;  $\beta=0.03$ ,  $P$  for trend < 0.01), 30 – 39 year olds (62% to 75%;  $\beta=0.03$ ,  $P$  for trend < 0.01), and non-Hispanic blacks (64% to 76%;  $\beta=0.03$ ,  $P$  for trend < 0.01).

The proportion of persons whose HIV virus was suppressed at all tests during the previous 12 months increased from 58% to 68% ( $\beta=0.03$ ,  $P$  for trend < 0.01) from 2009 – 2013. This positive trend was statistically significant among all sub-groups by gender, age, race-ethnicity, and sexual behavior/orientation. The largest increases were among 18–29 year olds (32% to 51%;  $\beta=0.05$ ,  $P$  for trend < 0.01), 30–39 year olds (47% to 63%;  $\beta=0.04$ ,  $P$  for trend < 0.01), and non-Hispanic blacks (49% to 61%;  $\beta=0.03$ ,  $P$  for trend < 0.01).

**Conclusions:** Persons receiving HIV medical care are increasingly likely to achieve viral suppression. Young people and non-Hispanic blacks, who had the lowest levels of viral suppression in 2009, showed the most improvement over time. Recent efforts to engage persons living with HIV in medical care and promote early antiretroviral therapy use may have contributed to these increases, bringing us closer to realizing key goals of the National HIV/AIDS Strategy.

Figure 1. Percentage of persons with (a) HIV viral suppression (<200 copies/mL) at last test during the past 12 months and (b) HIV viral suppression (<200 copies/mL) at all tests during the past 12 months: Medical Monitoring Project, 2009 – 2013



54 **Narrowing the Gap in Life Expectancy for HIV+ Compared With HIV- Individuals**

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**Background:** Survival of HIV-infected individuals has dramatically improved with combination antiretroviral therapy (ART). However, it is unclear how large a gap in life expectancy remains between HIV-infected and HIV-uninfected individuals, and how much of that gap is attributable to underlying differences in demographics or risk factors.

**Methods:** We conducted a cohort study of HIV-infected adults who were members of Kaiser Permanente California during 1996-2011, and HIV-uninfected members matched 10:1 on age, gender, medical center, and year. Deaths were comprehensively ascertained through 2011 from the electronic health record, California death certificates, and Social Security Administration datasets. We used abridged life tables to estimate the average number of years of life remaining at age 20 (“life expectancy at age 20”) in HIV-infected and HIV-uninfected individuals in 1996-2006 and 2007-2011. For the recent era, we estimated life expectancy at age 20 by demographics and HIV risk group. We also estimated life expectancy in recent years among patients who initiated ART early (i.e., with CD4 ≥500 cells/μL), and in subgroups of these early-treated HIV patients and HIV-uninfected individuals without modifiable risk factors.

**Results:** Among 25,768 HIV-infected and 257,600 HIV-uninfected individuals, there were 2,229 and 4,970 deaths, with mortality rates of 1,827 and 326 per 100,000 person-years, respectively. In 1996-2006, life expectancies at age 20 among HIV-infected and HIV-uninfected individuals were 36.0 and 62.3 years, respectively, corresponding with a gap of 26.3 years (95% confidence interval: 24.8-27.8). In 2007-2011, life expectancy at age 20 for HIV-infected individuals increased to 48.5 years, narrowing the gap to 13.8 years (Table). The lowest life expectancies at age 20 for HIV patients in 2007-2011 were among blacks (45.2 years) and those with a history of injection drug use (42.6 years). In 2007-2011, HIV patients who initiated ART with ≥500 cells/μL had a life expectancy at age 20 of 53.8 years, corresponding with a gap, relative to HIV-uninfected individuals, of 8.5 years. The gap narrowed further to 6-7 years in subgroups without a history of hepatitis B or C infection, drug/alcohol abuse, or smoking.

**Conclusions:** Even with early ART initiation, an approximately nine-year gap in life expectancy remains between HIV-infected and HIV-uninfected individuals with access to care. Mitigation of risk factors among HIV-infected individuals may further reduce the survival disparity.

**Table. Expected years of life remaining at age 20 among HIV-infected and HIV-uninfected individuals during 2007-2011, overall and by modifiable risk factors**

	Expected years of life remaining at age 20 (95% confidence interval)			
	HIV-infected	HIV-uninfected	Difference	P <sup>1</sup>
Overall	48.5 (47.2-49.8)	62.3 (61.8-62.7)	13.8 (12.4-15.2)	<0.001
	HIV-infected and initiated ART with CD4 ≥500 cells/μL			
Overall	53.8 (51.8-55.8)	62.3 (61.8-62.7)	8.5 (6.4-10.5)	<0.001
No hepatitis B or C	54.7 (52.6-56.9)	61.5 (61.1-61.9)	6.8 (4.6-8.9)	<0.001
No drug/alcohol abuse	56.2 (54.0-58.3)	62.6 (62.1-63.0)	6.4 (4.2-8.6)	<0.001
No smoking	55.3 (52.8-57.8)	61.1 (60.6-61.5)	5.8 (3.2-8.3)	<0.001

<sup>1</sup>P-values were obtained from z-tests.

**55 Perception of Infectiousness in HIV-Infected Persons After Initiating ART: ACTG A5257**

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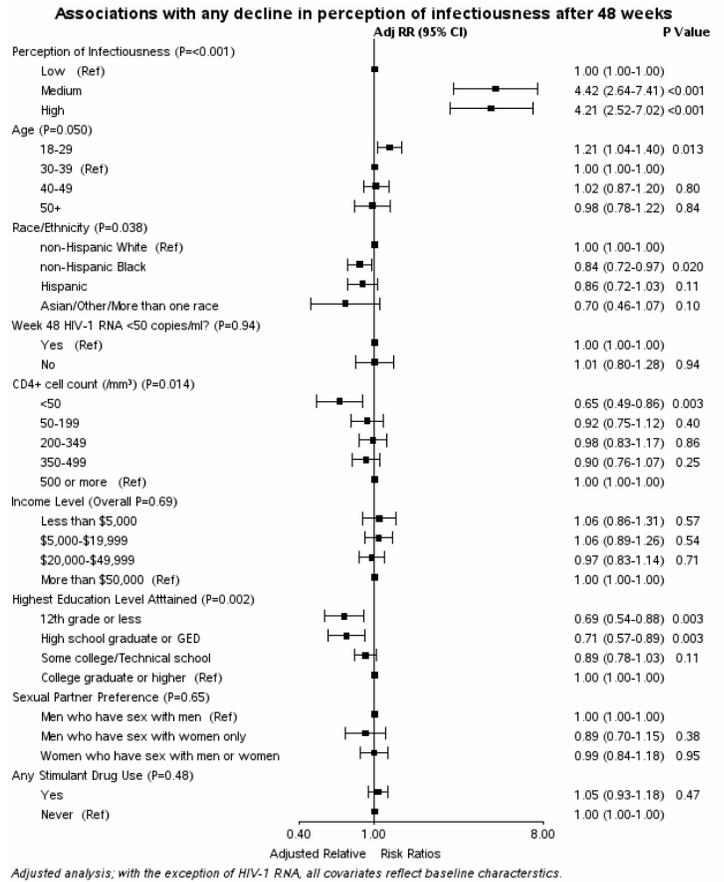
<sup>1</sup>David Geffen Sch of Med at Univ of California Los Angeles, Los Angeles, CA, USA; <sup>2</sup>Harvard Sch of PH, Boston, MA, USA; <sup>3</sup>Northwestern Univ, Chicago, IL, USA; <sup>4</sup>Emory Univ Sch of Med, Atlanta, GA, USA; <sup>5</sup>Univ of Miami, Miami, FL, USA

**Background:** Antiretroviral therapy (ART)-induced viral suppression has been shown to reduce sexual transmission of HIV. Data for prevention of HIV transmission are strongest for heterosexual transmission. ART does not produce immediate or universal viral suppression, and perception of infectiousness (POI) may impact sexual risk taking. Changes in POI as a result of ART may have implications for transmission and population incidence.

**Methods:** A5257 followed 1809 ART-naïve participants in a randomized open-label ART study for up to 192 weeks(W). Self-report of POI was assessed at baseline and annually via a visual analog scale (0-100). Pre-defined infectiousness categories were high (67-100), medium (34-66), low (1-33), *not infectious* (0). We evaluated a decline in POI defined as  $\geq 10$  unit absolute decrease with a change in POI category at W48 relative to baseline; a decline to not infectious POI was evaluated as a secondary outcome. Multivariable binary Poisson regression was used to evaluate factors associated with these outcomes.

**Results:** At baseline (pre-ART), 58%, 26%, 10%, 6% participants self-categorized as *high*, *medium*, *low*, or *not infectious*, respectively. At W48, 38%, 20%, 32%, and 10% self-categorized as *high*, *medium*, *low*, or *not-infectious*; 91% had HIV RNA <50 c/mL. 49% reported a reduction in POI from baseline to W48. In multivariable analysis, higher baseline POI, younger age and higher education were associated with greater risk of a decline in POI; non-Hispanic Black race and pre-ART CD4 <50 cells was associated with lower risk (Figure). Factors associated with a decline to *not infectious* POI (n=99) were baseline POI, female sex, and absence of stimulant drug use. Associations with RNA suppression were not apparent for either outcome (p=0.94 and 0.87, respectively). Only 8 of 99 participants who perceived themselves as not infectious on ART at W48 had RNA >50 c/mL.

**Conclusions:** Nearly half of participants reported a reduction in POI 48W after initiating ART. Those who were younger and more educated were more likely to report a reduction in POI, whereas those of black race or with advanced disease were less likely to report a reduction. Overall, relatively few participants perceived themselves non-infectious – a salutary finding for prevention. Interestingly, actual viral suppression was not associated with POI. Understanding how POI changes due to therapy, and how it relates to condomless sex, will be critical for designing interventions to maximize the benefits of treatment as prevention.



**56 Structure of the HIV-1 RNA Packaging Signal**

**Michael F. Summers**, Howard Hughes Med Inst, Minneapolis, MN, USA

The 5'-leader of the HIV-1 genome contains conserved elements that direct selective packaging of the unspliced, dimeric viral RNA into assembling particles. Using a novel 2H-edited NMR approach, including a fragmentation-based approach that employs differential 2H labeling/editing, we determined the structure of a 155-nucleotide region of the leader that is independently capable of directing RNA packaging (Core Encapsulation Signal;  $\Psi^{CES}$ ). The RNA adopts an unexpected tandem three-way junction structure, in which residues of the major splice donor and translation initiation sites are sequestered by long-range base pairing, and guanines essential for both packaging and high-affinity binding to the cognate Gag protein are exposed in helical junctions. Mutation of these "junction guanines" inhibits binding by the cognate NC protein *in vitro* and leads to severe attenuation of competitive RNA packaging in cell assays. The structure reveals how translation is attenuated, Gag binding promoted, and unspliced dimeric genomes selected, by the RNA conformer that directs packaging.

**57 The Antiretroviral Activity of the SERINC Gene Family**

**Massimo Pizzato**, Univ of Trento, Trento, Italy

SERINC genes represent a highly conserved family encoding homologous multipass transmembrane proteins, which in humans includes five members. SERINC5, and to a lesser extent SERINC3, were recently found to inhibit HIV and MLV virion particle infectivity. Both SERINC proteins are efficiently incorporated into virions and inhibit an early step of the infection process with a mechanism which remains to be elucidated. Nef from primate lentiviruses and glycoGag from MoMLV are capable of counteracting SERINC5 and SERINC3 antiviral activity by causing their relocalization into the late endosomal compartment. The host factors are therefore removed from the cell surface and prevented from being incorporated into virus particles. In addition to glycoGag and Nef, we now identified the accessory protein S2, from equine infectious anemia virus (EIAV), as yet another retroviral factor capable of antagonizing SERINC5 and SERINC3. Reminiscent of Nef, the counteracting activity of S2 requires myristoylation and clathrin-mediated endocytosis, indicating a common mechanism of action. Remarkably, Nef, GlycoGag and S2 share no sequence homology and are derived from distinct regions in the retrovirus genome, suggesting that the anti-SERINC activity was acquired independently by the three retroviruses. In light of these results, we are now expanding our investigation in order to assess how general the ability of SERINC genes to inhibit retroviruses is, and how frequently SERINC-counteracting factors have arisen during retrovirus speciation.

For being restriction factors, SERINC5 and SERINC3 have unusual features, since they are highly conserved (from yeast to mammals) and their expression is not altered by treatment with interferon-beta. In addition, our analyses indicate that SERINC5 evolution is not driven by positive selection, an observation which would suggest the absence of selective pressure derived from a direct interaction with antagonizing factors. However, purified HIV-1 Nef and SERINC5 efficiently interact *in vitro*, demonstrating the ability of the two proteins to associate directly, in the absence of additional co-factors. SERINC proteins are therefore a unique and novel class of host factors with broad anti-retroviral activity. Mapping studies are currently under way to identify the molecular determinants crucial for the interaction with Nef and for the antiviral activity.

**58 How HIV-1 Nef Downregulation Hijacks Clathrin-Coated Vesicles****James H. Hurley**, *Univ of California Berkeley, Berkeley, CA, USA*

The lentiviruses HIV and simian immunodeficiency virus (SIV) subvert intracellular membrane traffic as part of their replication cycle. The lentiviral Nef protein helps viruses evade innate and adaptive immune defenses by hijacking the clathrin adaptor complexes AP-1 and AP-2. These in turn drive the downregulation of MHC-I, CD4, and SERINC3 and 5, among other cellular targets. We have been building up a structural picture that explains how HIV-1 Nef uses at least two different sites- the region around Asp123 and the dileucine motif- to hijack AP complexes in two different ways. In particular, HIV-1 Nef and the small G-protein Arf1 use a surprisingly complicated mechanism to hijack AP-1, which is responsible for MHC-I downregulation. Arf1 glues AP-1 into dimers and trimers that in turn form a hexagonal undercoat to clathrin. Nef promotes trimer formation and the generation of the hexagonal lattice. The mode of Nef action depends upon the presence of cargo. The action of Nef with respect to AP-1 appears to be highly regulated, and a novel Nef regulatory site involved in controlling the structure of AP-1 trimers will be described. Implications for AP-2 dependent downregulation of the SERINC3s, and similarities and differences between Nef hijacking of AP-1 and AP-2 will be considered.

**59 APOBEC3F-Vif Binding Interface Elucidated by Numerous Experimental Approaches****Reuben S. Harris**, *Howard Hughes Med Inst, Minneapolis, MN, USA*

APOBEC3 family DNA cytosine deaminases provide overlapping defenses against pathogen infections. However, most viruses have elaborate evasion mechanisms such as the HIV-1 Vif protein, which subverts cellular CBF-beta and scaffolds the assembly of a polyubiquitin ligase complex to degrade these enzymes. Despite advances in APOBEC3 and Vif biology, a full understanding of this direct host-pathogen conflict has been elusive. We combine virus adaptation, structural, and computational studies to interrogate the APOBEC3F-Vif interface and build a robust model for this vital host-pathogen interaction. First, new structures of the Vif binding domain of APOBEC3F were solved by x-ray crystallography. Second, a recurring compensatory amino acid substitution in HIV-1 Vif from virus adaptation experiments provided an initial docking constraint, and microsecond molecular dynamics simulations were used to optimize interfacial contacts. Third, single-cycle and spreading virus infectivity experiments were used to validate a long-lasting electrostatic interaction predicted by molecular dynamics simulations to occur between APOBEC3F E289 and HIV-1 Vif R15. Our studies have yielded a comprehensive structural model for the APOBEC3F-Vif interaction, which explains most prior genetic data. Taken together with the dynamic nature of this host-pathogen interaction, our data suggest a "wobble model" to explain how HIV-1 Vif has evolved to bind different APOBEC3 enzymes and, more generally, explain how pathogens may evolve to escape innate host defenses (*i.e.*, a molecular explanation for the Red Queen hypothesis). Our studies also suggest that the APOBEC-Vif interface may be a less favorable target for new antiviral drug development in comparison to other heterologous surfaces of the Vif/CBF-beta ubiquitin ligase complex.

**60 CNS HIV Infection: Cerebrospinal Fluid and Blood Biomarkers****Magnus Gisslén**, *Univ of Gothenburg, Gothenburg, Sweden*

Neurological complications are common in patients with HIV and the prevalence of neurocognitive impairment is high also among those on suppressive antiretroviral treatment (ART). Sometimes neurocognitive complications may be ascribed to CNS injury that occurred before treatment initiation (inactive disease), especially in patients with a low CD4 cell nadir; and other times to ongoing neuronal injury accompanied by chronic intrathecal immunoactivation (active disease). Cerebrospinal fluid (CSF) biomarkers of viral replication, immunoactivation, and neuronal injury provide an objective means of measuring ongoing HIV CNS infection and inflammation along with its effect on brain cells. This presentation will consider the usefulness of CSF biomarkers in measuring HIV CNS disease, particularly in HIV-infected patients on ART. Employing biomarkers for differential diagnostic purposes will also be covered. Biomarkers of CNS immunoactivation and brain injury may also be useful for research into latency and cure, and as tools for measuring the reservoir and the effect of latency-reversing agents on possible CNS immune activation and injury. However, the need to sample CSF limits the application of those measurements in a number of clinical and scientific contexts. A sensitive blood biomarker of neuronal injury continues to be sought. The recent development of a novel ultrasensitive Single Molecule Array (Simoa) immunoassay for the quantification of the neurofilament light chain protein (NFL) in blood will be presented.

**61 Neuroimaging of HIV in the Brain****Beau M. Ances**, *Washington Univ in St. Louis, St. Louis, MO, USA*

HIV enters the brain soon after seroconversion and can cause HIV associated neurocognitive disorders (HAND). While the more severe and progressive forms of HAND are now less prevalent due to combination antiretroviral therapy (cART), ~40% of HIV-infected (HIV+) patients continue to have cognitive impairment. Some HIV+ individuals who have effective plasma HIV-1 RNA suppression with cART still develop HAND due HIV reservoirs in the central nervous system. It is often difficult to diagnose HAND in the outpatient setting as detailed neuropsychological performance testing is required. Additional biomarkers that are relatively easy to obtain and are clinically relevant are needed for assessing HIV associated immune activation. Recently developed non-invasive magnetic resonance imaging (MRI) techniques have great potential to serve as biomarkers. This talk will review the application of advanced neuroimaging techniques [e.g. volumetric MRI, diffusion tensor imaging (DTI), functional MRI (fMRI)] in HIV+ individuals. Each neuroimaging methods can offer unique insight into mechanisms underlying neuroHIV, could monitor disease progression, and may assist in evaluating the efficacy of cART regimens in the CNS. It is hoped that continued development of neuroimaging methods will allow them to be easily incorporated across multiple sites and included in future HAND guidelines.

**62 CNS as an HIV Reservoir****Ronald I. Swanstrom**, *Univ of North Carolina at Chapel Hill, Chapel Hill, NC, USA*

Replication of HIV-1 within the CNS compartment can only be bad. At a minimum the detection by the immune system of replicating virus within the CNS leads to a neuro-toxic inflammatory response. If the clearance of virus is incomplete then a persistent inflammatory response could provide the basis for sustained cell damage within the CNS. Markers of viral replication in the CNS in living subjects must come from the analysis of virus in the CSF, or be inferred from indirect markers from non-invasive imaging techniques or from cell or inflammatory markers in the CSF or blood. Virus can be found in the CSF as the result of three distinct mechanisms. Infected T cells traffic into the CNS and release virus that by sequence looks just like the virus in the blood, and this virus can be present at a low level or, with pleocytosis, at a high level. Alternatively, some of this virus can be transiently clonally amplified by a poorly understood mechanism to give elevated viral loads but of surprisingly homogeneous viral populations. Finally, virus can take root within the CNS for sustained replication but in an environment that is poor in CD4+ T cells. This causes the virus to adapt to macrophage-like cells with their low levels of the surface CD4 protein needed for entry. The unique genetic lineage of virus replicating independently in the CNS and the phenotypic adaptation to this CD4 low environment provide clear evidence of a distinct viral population and site of ongoing viral replication. Therapy suppresses viral load in the blood and the CSF. However, antiviral drug levels are reduced in the CNS, a situation that would result in viral replication and the selection of resistance if it took place in the blood. In about 5-10% of subjects on therapy there is detectable virus in the CSF either in the absence of or at levels higher than virus in the blood. This CSF escape virus takes on several forms, including a complex viral population at least partially adapted to a CD4 low environment. We do not know how well the CSF records virologic events that may be ongoing deep within the brain parenchyma. Such replication when detected in the CSF represents evidence of an active viral reservoir on therapy, while the potential for a unique latent reservoir within the CNS is of considerable interest but challenging to study.

**63 Therapeutics of the CNS With HIV Infection****Andrea Calcagno**, *Univ of Torino, Torino, Italy*

Highly Active Antiretroviral Treatment, despite its striking systemic efficacy, might be incompletely effective in some organs and tissues thus favouring residual HIV replication and damage. The central nervous system (CNS) is a key organ in this setting and several data have reported a worrisome prevalence of neurocognitive deficits in effectively treated HIV-positive patients. In this talk data on the potential mechanisms underlying CNS disease in HIV-positive patients including early treatment, residual HIV replication,

potential drug-associated neuronal toxicity and vascular abnormalities will be presented. Following a brief description of the pharmacokinetic determinants of antiretroviral penetration and activity in the CNS (including cellular targets), the available evidence on the effect of different antiretrovirals on CNS HIV will be reviewed as well as the studies involving ARV combinations for treating patients with CNS disorders including the ongoing discussion on the use of the CNS concentration/effectiveness (CPE) score and the effect of unconventional antiretroviral regimens (based on more or less than three drugs). Finally, studies on adjunctive therapies will be presented and potential therapeutic targets reviewed.

#### 64 Hepatitis C: Global Epidemiology

**Imam Waked**, *Natl Liver Inst, Shebeen El Kom, Egypt*

Hepatitis C virus (HCV) is a major global health burden and the leading cause of liver disease and hepatocellular carcinoma in many parts of the world. New treatment options are available that have the potential to change the epidemiology and natural history of the disease, and there is a need to precisely characterize its epidemiology and disease burden. Total global sero-prevalence is estimated at 120-180 million cases, with around two thirds viremic (viremic infections estimated at 80-120 million infections). Egypt is the country with highest prevalence, with 12% of the population sero-positive, and close to 8% of the population chronically infected. The 5 countries with largest number of patients (China, Pakistan, Egypt, Nigeria and India) have close to 50% of the global viremic patients despite their limited resources.

Transmission routes are different in different areas globally. In developed countries where prevalence is low, transmission is mainly through needle sharing among PWID, as high-risk behavior and nosocomial transmission has decreased. In under-developed and limited resources countries, transmission is still ongoing mainly through blood transfusion and non-sterile equipment use in the healthcare settings, and reuse of needles for medical injections. Mass spread of HCV coincided in Egypt with the intravenous mass-treatment of schistosomiasis, and coincided in many areas of Africa with the introduction of population vaccination in the 1960s and 1970s.

In addition, HCV is genetically diverse, with seven genotypes and many subtypes. There are regional variations in genotype prevalence, and genotypes respond differently to different therapies. Genotype 1 (G1) is the most common worldwide (44%-48%) with around 50-60 million viremic cases, with most in East Asia, followed by G3 (20%-22%, 30-35 million cases), G2 (12%-15%, 15-20 million cases), and G4 (12%-15%, 15-20 million cases). Genotypes 5 and 6 comprise a limited minority. G1 and G3 are predominant in most countries, and genotypes 4 and 5 are more prevalent mainly in lower-income countries.

Not all countries report anti-HCV prevalence; and HCV viremic rates and genotype distribution are more scarce. More recent and accurate geographical epidemiological data is needed to direct treatment development and availability, and to guide vaccine development efforts.

#### 65 Hepatitis C in Egypt: A National Approach to Treatment

**Manal H. El-Sayed**, *Ain Shams Univ, Cairo, Egypt*

Egypt has one of the highest global burdens of hepatitis C virus (HCV) infection, with an estimated 7%, over 6 million people between 15-59 years, being chronically infected. Tragically, an estimated 150,000 new people are being infected annually, and thousands die every year. In recognition of the enormity of the problem, in 2006, the Ministry of Health established a National Committee for Control of Viral Hepatitis (NCCVH), a team of experts who published the first national strategy for control of viral hepatitis in Egypt and established a nationwide treatment program in 26 specialized viral hepatitis units treating 350,000 patients through the Ministry of Health program. In 2012, and in collaboration with stakeholders including WHO, US-CDC and Pasteur Institute, the NCCVH and MOH developed the "Plan of Action for the Prevention, Care & Treatment of Viral Hepatitis, Egypt" (PoA) which focuses on the seven main components of viral hepatitis prevention and control: surveillance, infection control, blood safety, hepatitis B virus (HBV) vaccination, care & treatment, communication, and research. In addition, in 2014, Sofosbuvir was introduced for nationwide treatment of HCV infection at 1% of its international price. The NCCVH is also introduced other approved direct antiviral agents consecutively during 2015, in addition to encouraging the local manufacturers to produce prequalified generics to effectively implement the elimination program in the shortest possible duration. A web-based registration system has been established mid-September 2014 to schedule patients' appointments to receive treatment at its specialized units. So far, more than one million patients with known HCV infection have registered and are being evaluated at the NCCVH centers. The numbers treated increase by at least 15,000 on monthly basis with almost 200,000 treated in by the end of 2015. The success of the Egyptian endeavor in collaboration with national and international partners would provide an exemplary model that can be replicated in other resource-limited countries.

#### 66 Hepatitis C Therapeutics: Pangenotypic Therapeutics on the Horizon

**Karine LaCombe**, *Sorbonne Univ, Paris, France*

With more than 185 million individuals affected worldwide, chronic hepatitis C virus (HCV) infection has emerged as a major public health problem. More than one million deaths are attributed to chronic HCV, together with chronic hepatitis B, ranking it among the top ten causes of death in the Global Burden of Diseases. The advent of direct antiviral agents, targeting multiple steps in the viral replication cycle, has led to substantial improvement in the management of chronically infected patients. By increasing rates of sustained virological response to 90% in the 12 weeks after end of treatment, HCV therapy has dramatically improved clinical outcomes for all patients, including those with more severe disease (i.e. cirrhotics or with liver transplants). However, there are still a number of issues needing to be addressed alongside the continuum of care, among which simplifying virological evaluation is paramount. One of the keys to alleviate the burden of costs will be the use of pangenotypic drugs. These agents help reduce the need for genotyping and possibly extensive viral load monitoring, thereby easing HCV-associated care for all. As we aim towards global elimination of hepatitis C, the use of pangenotypic drugs will constitute a major step forward.

#### 67 Hepatitis B Virus: Is a Cure Possible?

**Chloe Thio**, *Johns Hopkins Univ, Baltimore, MD, USA*

Worldwide, about 2 billion people have had a hepatitis B virus (HBV) infection and 248 million are chronically infected. Once an HBV infection occurs, a stable, intranuclear form, known as the cccDNA persists not only in those with chronic infection but also in those who resolve infection with production of protective hepatitis B surface antibody. Cure of hepatitis B can be defined as a functional cure or an eradication cure. A functional cure is similar to what occurs naturally with resolution of an acute infection where the immune system can control HBV replication without medication and protective hepatitis B surface antibody is produced. In the functional cure, the cccDNA is still present. An eradication cure is a therapy that eliminates the cccDNA. In this talk, I will discuss both types of cure and progress towards each of them.

#### 68 Delivery of PrEP: From Evidence to Practice

**C. Bradley Hare**, *Kaiser Permanente Northern California, San Francisco, CA, USA*

The efficacy and effectiveness of HIV pre-exposure prophylaxis (PrEP) have been shown in randomized clinical trials, demonstration projects and real-world cohort studies. Despite this, uptake of the use of PrEP in the US was initially slow after its FDA approval in July 2012. More recent data indicate increased awareness, acceptability and uptake in some – but not all – at-risk populations and geographic areas. As PrEP use increases, many questions remain unanswered about the optimal models for PrEP delivery. In what settings and by whom should PrEP be provided? What population(s) should be targeted, and how do we reach those who could most benefit from PrEP? What is the appropriate monitoring and follow-up? What additional services should be provided and how are they best integrated with PrEP? How do you predict interest and plan for increasing capacity and sustainability? What staffing and resources are needed to provide PrEP safely and effectively? This presentation will explore these questions using published reports and real-world experience to provide a framework for conceptualizing and implementing sustainable delivery models for PrEP.

**69 Sexually Transmitted Infection Control in the Era of PrEP****Sheena McCormack**, *Univ Coll London, London, UK*

Individuals at risk of catching HIV are also at risk of catching other sexually transmitted infections (STIs). Correct and consistent use of condoms virtually eliminates the risk of HIV, but may not prevent the acquisition of bacterial STIs. Syphilis and gonorrhoea have been notifiable diseases for decades in several countries. Trends in infections are explained by many factors including war, migration, access to the pill, as well as new tests and treatments. The emergence of HIV as a fatal infection had a dramatic impact on other STIs which reached an all-time low in gay and other men who have sex with men (MSM) in the late 1980s. This trend in MSM has been completely reversed, driven initially by changes in risk behaviours of HIV positive MSM on effective treatment, but also facilitated by the exponential growth in Social Apps. Conventional control measures (partner notification, epidemiological treatment, outreach in venues) contain transmission to an extent but the shift to group sex in private houses, frequently under the influence of recreational drugs, presents new challenges.

What can we anticipate in the era of PrEP? PROUD and IPERGAY enrolled MSM at 'high risk' of HIV in England and France respectively. Enrolment criteria were broad – condomless anal sex once in the last 3 months and likely to do so again in the next 3 months (PROUD); 2 or more condomless anal sex partners in the last 6 months (IPERGAY). However, the proportion of PROUD participants that reported rectal bacterial STIs (33%) or syphilis (10%) in the previous year was at least 5-fold higher than all MSM attending clinics in England in 2013 (5% and 2% respectively). So we can anticipate that MSM who are already catching STIs will be more likely than those who are not to present for PrEP, and that they will continue to be at risk of catching STIs whilst on PrEP. It is time to rethink our approach to control of STIs, to make screening, treatment and partner notification as easy as possible, and to find new methods of population control. One such method is the use of geospatial modelling and analysis to identify the sexual networks that are hotspots for STI transmission. We will need peer-navigators to engage with these networks and bridge the provision of services. As we move into the era of PrEP, it is time to be more creative with the methods we use to control STIs. On our march towards elimination of HIV, we must sort out the STIs on the way.

**70 PrEP-4-Love: Transmitting Desire Across Chicago****James Pickett**, *AIDS Frdn of Chicago, Chicago, IL, USA*

In Chicago, like much of the country, early uptake of PrEP has primarily been seen in middle-aged gay white men. To reach populations most vulnerable to HIV, in particular young gay black men and transgender women of color, more community outreach and creative effort is required. A sampling of other city media/social marketing campaigns will provide the jumping off point for a fast-paced discussion of the twisting, turning road followed by the Chicago PrEP Working Group – highlights of which include the securing of pro bono creative support, raising funds in new and innovative ways, and our community-led concept development, design execution and implementation strategy. The campaign, called "PrEP4Love", was just launched February 1 throughout Chicago and is positioning PrEP affirmatively in the oft-neglected realm of pleasure and intimacy. Focus populations are young gay Black men, transgender women of color, and cisgender Black heterosexual women.

**71 The Promise and Challenges of Sustained Delivery of PrEP****Ian McGowan**, *Univ of Pittsburgh Sch of Med, Pittsburgh, PA, USA*

Antiretroviral pre-exposure prophylaxis has huge potential for reducing the rates of new HIV infections in at risk populations. Oral and vaginal antiretroviral formulations have been evaluated in multiple Phase 2B/3 effectiveness trials and there is clear evidence that these products work when used. The converse is also true; antiretrovirals do not work when they are not used. As a consequence, long-acting (LA) injectable and implantable antiretroviral formulations are being developed for the prevention of HIV infection.

The two lead products are the non-nucleoside reverse transcriptase inhibitor rilpivirine and the integrase inhibitor cabotegravir. It is hoped that the use of LA antiretroviral PrEP will reduce the burden of product adherence associated with the use of oral and topical products and improve the level of HIV prevention associated with this form of PrEP. Although LA products have clear promise for HIV prevention, there are also challenges to consider. There are concerns about the possibility of idiosyncratic adverse events that would be difficult to manage given the irreversible nature of an LA injection. There are additional concerns about the long half life and extended PK 'tail' of these products that might lead to periods of subtherapeutic levels of antiretrovirals; infections acquired during this period might be associated with the development of resistance. The purpose of this presentation is to summarize recent preclinical and clinical research in this area of HIV prevention.

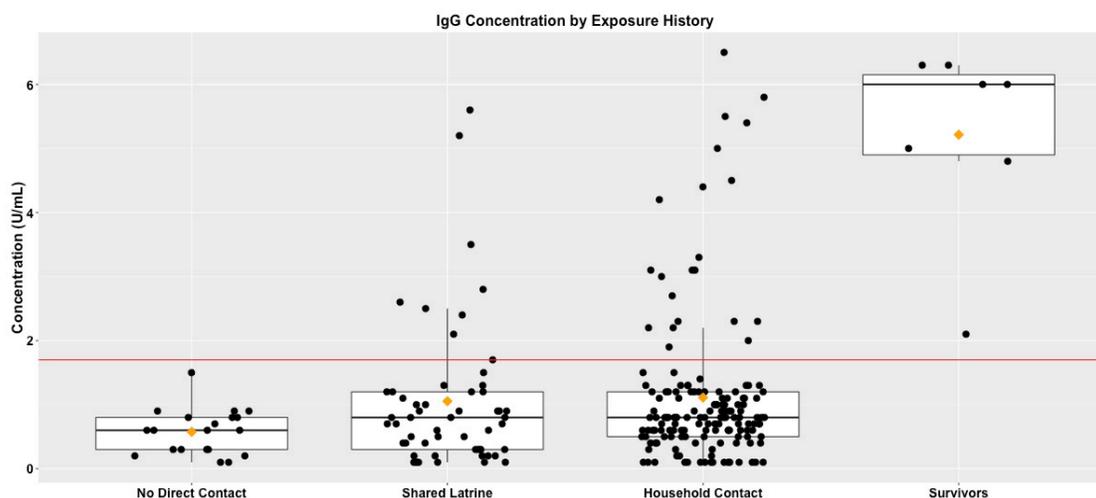
**72LB Unreported Cases and Asymptomatic Infection in an Ebola "Hotspot"****Eugene T. Richardson**<sup>1</sup>; J. Daniel Kelly<sup>2</sup>; Mohamed B. Barrie<sup>3</sup>; Annelies W. Mesman<sup>3</sup>; Komba Quiwa<sup>3</sup>; Sahr Karku<sup>3</sup>; George Rutherford<sup>2</sup>; James H. Jones<sup>1</sup>; Megan Murray<sup>4</sup>; Paul Farmer<sup>4</sup>  
<sup>1</sup>Stanford Univ, Stanford, CA, USA; <sup>2</sup>Univ of California San Francisco, San Francisco, CA, USA; <sup>3</sup>Partners In Hlth, Freetown, Sierra Leone; <sup>4</sup>Harvard Med School, Boston, MA, USA

**Background:** Evidence for asymptomatic Ebola infection is limited to the extent that, during the 2013-15 outbreak, it was not considered epidemiologically relevant to published epidemic models or projections of intervention effects. We conducted a cross-sectional IgG serosurvey in an Ebola 'hotspot' village in Sierra Leone, eight months after reported transmission ceased in that location. The surveyed village had a population of approximately 800 individuals distributed among 110 households. Throughout the entire outbreak, there were 25 cases (18 deaths and 7 survivors) reported by the District Ebola Response Center.

**Methods:** We sampled a total of 227 individuals in 30 of 31 previously quarantined households in the village. We assessed anti-glycoprotein IgG responses to Zaire Ebola virus by means of a commercial ELISA kit (Alpha Diagnostic International [ADI]), according to the manufacturer's instructions, with plasma diluted at 1:200. Optical density was read at 450 nm (subtracting OD at 630nm to normalize well background) on a ChroMate 4300 microplate reader. We used Welch's t-test to determine if mean antibody concentrations differed by exposure history. To discriminate between positive and negative IgG responses (cutoff), we used the mean concentration for individuals without direct contact with a confirmed case plus three standard deviations. We then performed a log-binomial regression using this seropositivity cutoff as the dependent variable and gender, age, occupational activity, and schooling as predictor variables.

**Results:** We identified an antibody concentration cutoff of  $\geq 1.7$  U/mL (roughly equal to 5.1 micrograms per mL). All 7 documented survivors demonstrated positive responses (range 2.1 - 6.3 U/mL). Plasma IgG was positive in an additional 30 of 227 quarantined individuals not known to have Ebola virus disease (Figure 1), 27 of whom denied being symptomatic during the period of active transmission in the village. Only having a higher level of education was significantly associated with seropositivity.

**Conclusions:** This is the first systematic exploration of asymptomatic infection in an Ebola 'hotspot.' These data support the hypothesis that the actual number of infections in the 2013-15 outbreak in West Africa is significantly higher than the reported cumulative incidence. The phenomenon of asymptomatic infection has implications for the management of future Ebola outbreaks, as well as for the definition—and treatment—of survivors.



**73LB Sequelae of Ebola Virus Disease in Surviving Patients in Guinea: Postebogui Cohort**

**Jean-François Etard**<sup>1</sup>; Mamadou Saliou Sow<sup>2</sup>; Sandrine Leroy<sup>1</sup>; Philippe Msellati<sup>3</sup>; Abdoulaye I. Toure<sup>4</sup>; Bernard Taverne<sup>5</sup>; Ibrahima Savane<sup>6</sup>; Moumié Barry<sup>7</sup>; Eric Delaporte<sup>8</sup>; for the Postebogui Study Group

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**Background:** On 29 December 2015, Guinea was declared free of Ebola virus disease (EVD) and 1268 patients were discharged from the Ebola treatment centers (ETC). In March 2015, survivors were started to be enrolled in a follow-up study in Conakry and Macenta to describe clinical, biological, virological, immunological, and psychosocial evolution. Recruitment is still on-going and we report here the main manifestations at inclusion in the cohort.

**Methods:** Clinical examination, psychological (CES-D scale) and social assessment, standard biological exams and Ebola virus RNA search in body fluids (semen...) were performed in adults and children at inclusion then every 3 months.

**Results:** As of 15 December 2015, 375 survivors were included. Mean age was 28.8 years (range: 1-67.4), 169 (45%) were male, 76 (20%) were children. The median delay between ETC discharge and Postebogui inclusion was 223 days (1-557 days). During the acute phase, mean viral load CT was of 33 (range: 16-36, n=53); 16 (4%) patients received favipiravir, and 37 (10%) plamaspheresis.

At inclusion, 1081 clinical events were registered in 296 (79%) surviving patients, more often in adults than children (82% vs. 64% resp.). Main signs were: general signs (39% - fatigue, fever, anorexia), neurological signs (32% - headache), rheumatologic signs (46% - join pain), ocular signs (16% - conjunctivitis, irdocyclitis, vision deficiency), infectious diseases (22%) pelvic-abdominal pain (21%), anemia (13% - Table).

Detailed ophthalmologic examination in 21 patients suffering from ocular signs showed 11 uveitis, including with bilateral corneal opacities, active chorioretinitis or pigmented scars, and panuveitis.

From the 98 semen screened for Ebola virus RNA (68 men), 10 samples (8 men) were positive up to 9 months after onset of EVD with a decreasing trend in both proportion of positive samples and viral RNA load.

Among 131 patients discharged from Conakry ETC since 5 months on average, 20% presented a breakdown score justifying psychologic/psychiatric care.

Two third of the 194 first included patients in Postebogui had more than one diseased person in their household (HH) with a mean number of 5.6 EVD person/HH, and a mean number of 2.5 death/HH.

**Conclusions:** Sequelae were from general, ocular, rheumatologic origins and major psychic pain, highlighting the heavy human and economic burden related to EVD. Moreover, the long-term viral persistence in semen raises the need of specific measures (screening/safe sex) in order to avoid secondary cases.

**Table.** Clinical events of Postebogui patients at inclusion

	All patients	Adults	Children
<i>Signs</i>	296 (79)	247 (82)	49 (64)
General signs	145 (39)	109 (36)	36 (47)
• Fever	81 (22)	56 (19)	25 (33)
• Fatigue	84 (22)	67 (22)	17 (22)
• Anorexia	51 (14)	32 (11)	19 (25)
Inflammation	193 (51)	172 (58)	21 (28)
Ophthalmologic signs	61 (16)	56 (19)	5 (7)
• Conjunctivitis	15 (4)	14 (5)	1 (1)
• Iridocyclitis	10 (3)	10 (3)	0 (0)
• Vision problems	43 (11)	40 (13)	3 (4)
• Ocular pain	16 (4)	14 (5)	2 (3)
Rheumatologic signs	172 (46)	154 (52)	18 (24)
• Join pain	149 (40)	133 (44)	16 (21)
Infectious diseases	81 (22)	64 (21)	17 (22)
• Malaria	49 (13)	39 (13)	10 (13)
• Helminthiasis	22 (6)	18 (6)	4 (5)
Stomatology, ENT signs	28 (7)	23 (8)	5 (7)
• Deafness	10 (3)	9 (3)	1 (1)
Abdominal signs	92 (25)	79 (26)	13 (17)
• Gastritis	11 (3)	11 (4)	0 (0)
• Abdominal pain	82 (22)	69 (23)	13 (17)
Neuro-psychiatric signs	123 (32)	98 (32)	25 (33)
• Headache	110 (29)	86 (29)	24 (32)
Hematological signs	18 (5)	14 (5)	4 (5)
• Anaemia	17 (13)	13 (4)	4 (5)
Gyneco-Urological signs	93 (25)	79 (26)	14 (18)
• Pelvic pain	77 (21)	64 (21)	13 (17)
Pneumo-Cardiological signs	18 (5)	15 (5)	3 (4)
Renal injury	1 (0)	1 (0)	0 (0)
Dermatologic signs	10 (3)	8 (3)	2(3)

**74LB A Cohort Study of Survivors of Ebola Virus Infection in Liberia (PREVAIL III)**

**Mosoka Fallah**; for the Prevail III Research Team , Ministry of Hlth, Monrovia, Liberia

**Background:** The Ebola virus disease (EVD) epidemic in West Africa was unprecedented. There were over 11,000 deaths and more than 26,000 infections. The outbreak left over 14,000 survivors with a myriad of health complications. To characterize the clinical sequelae in survivors and to assess whether they can transmit infection to household members and sexual contacts, the Partnership for Research on Ebola Virus in Liberia (PREVAIL) launched a cohort study of survivors and close contacts.

**Methods:** Following joint protocol development and IRB approval, enrollment of survivors  $\geq 12$  year began in June 2015 followed by contacts and pediatric survivors  $<12$  yrs in August 2015. Participants are seen at baseline and every 6 months for 5 years at 3 sites in Liberia. Data collection includes demographics, detailed medical exam, labs including serology to determine the presence of antibodies specific to EVD, and for survivors, information about ETU course, treatment, and discharge. Examinations may also include detailed an ophthalmologic exam, a comprehensive neurologic assessment, collection of semen, lumbar punctures, and the enrollment of newborns and breast milk anal

**Results:** Through December, 1022 survivors and 754 close contacts have been enrolled. The average age of survivors and close contacts is 30 and 27 years, respectively. For survivors, the median (IQR) time from EVD symptoms to enrollment is 352 days (307,395). A total of 76 individuals have had 1-4 semen samples obtained for PCR analysis. Overall 25% have been positive with 37% of subjects having at least 1 positive sample. The maximum amount of time between the onset of symptoms and a positive result was 488 days. 65% of survivors reported sexual activity without a condom since ETU discharge including 48% of those with a positive semen PCR for Ebola. EVD serology is in progress and has been completed for 527 survivors and 97 close contacts. 88% of survivors and 49% of close contacts had a positive EVD serology.

**Conclusions:** Preliminary findings indicate the prevalence of eye complications, musculoskeletal problems, and neurological findings are greater among EVD survivors than close contact controls. Most survivors report sexual contact and many do not use condoms. A surprising number of contacts have positive EVD serology, a finding now being confirmed with analysis of blood samples. Study findings have implications for EVD sexual transmission risk and indicate the importance of enrolling close contacts with EVD serology to use as controls.

Baseline characteristic	All close contacts (n=755)	Contacts with negative serology (n=49)	Contacts with positive serology (n=48)	All survivors (n=1023)	Survivors with negative serology (n=64)	Survivors with positive serology (n=463)
Age, mean (years)	28.53	31.35	33.73	30.14	30.29	30.85
Female (%)	55	59	48	54	56	57
Time in ETU mean (days)	NA	NA	NA	16.81	16.64	16.57
Vision problems on physical examination (%)	15	2	10	24	21	34
Musculoskeletal problems on physical examination (%)	4	2	4	8	17	11
Neurological problems on physical examination (%)	3	0	2	6	8	6
Systolic BP $\geq 140$ or diastolic BP $\geq 90$ mmHg (%)	20	22	44	18	17	16
Close contact: household member at time of diagnosis	83	88	92	NA	NA	NA
Household member with 1 or more symptoms (%)	41	29	42	NA	NA	NA

Table 1: Summary of PREVAIL III participants broken out by serology. A total of 1778 participants have enrolled of which 35% have had serology performed. Baseline refers to time of enrollment in study.

**75LB Dynamics of Ebola Virus Clearance in Semen in Guinea**

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**Background:** While it is well known that testes may be a reservoir for long term persistence of Ebola virus (EBOV), the dynamics of the persistence of EBOV in semen remained unexplored. We aimed to estimate the probability of EBOV RNA clearance in semen over time.

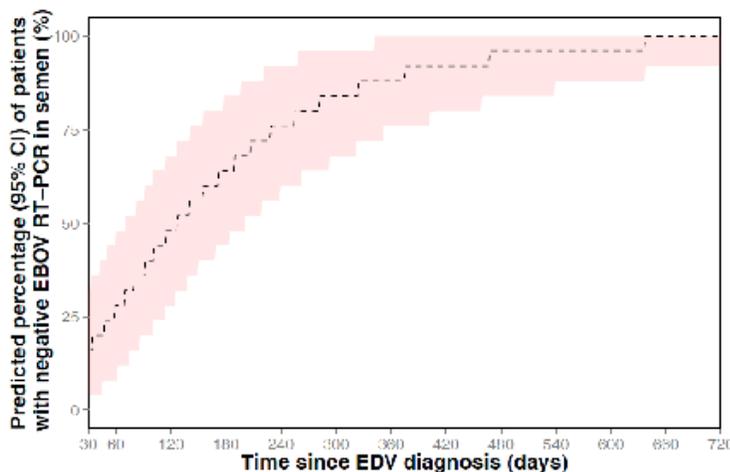
**Methods:** We enrolled a cohort of 25 men discharged from three Ebola Treatment Units (ETU) in Coastal region of Guinea between February 6, 2015 and July 6, 2015. Semen specimens were obtained every 3-6 weeks until reaching non-detectable levels of EBOV RNA (eg, Ct values  $\geq 45$ ) on two consecutive samples. Specimens were tested on-site using Altona RT-PCR. Furthermore, a nonlinear mixed effect model was used to estimate the slope of evolution of Ct values over time

**Results:** A total of 116 semen samples were collected. The median time between EVD onset and first semen collection was 49 days (IQR: 40-85). The median time between first and last semen collection was 193 days (IQR: 150-204). Of the 25 participants, 18 (72%) had a positive EBOV RT-PCR in the first semen sample. The median Ct value in the 18 positive semen was 28.1 (IQR= 22.4-36.3). The semen of the patient having the longest follow-up was still positive to EBOV at day 300 post-disease onset.

Using a linear mixed model, the mean increase of Ct in semen was estimated to +0.086 per day (SD 0.65), corresponding to a mean decrease of RNA of -0.87 log<sub>10</sub> copies/ml per month. Based on these numbers, the time to achieve negative RT-PCR in semen in 50% and 90% of the patients was predicted at 127 (95% Prediction interval -95% PI= 71-221) and 375 (95% PI=222- 638) days, respectively (Figure 1).

**Conclusions:** About 10% of patients may have detectable viremia in semen up a year after onset of disease. Therefore long term programs of care for EVD survivors should include implementation of semen testing, dedicated counselling and condom use in order to avoid potential sexual transmission. The evaluation of drug intervention among individuals harbouring EBOV in semen is highly desirable.

Figure 1 : Estimated cumulative probability distribution function for the time needed to achieve negative EBOV RT-PCR in semen



**76LB A Randomized Controlled Trial of the Safety and Immunogenicity of Two Ebola Vaccines**

**Fatorma Bolay**; for the Partnership for Research on Ebola Vaccines in Liberia (PREVAIL 1) Team, *Liberian Inst of BioMed Rsr–NIH Partnership, Bethesda, MD, USA*

**Background:** In November 2014, two candidate Ebola virus vaccines were in the late stages of phase 1 testing, the chimpanzee adenovirus 3 (ChZ) - based vaccine and the recombinant Vesicular Stomatitis virus (rVSVdeltaG-ZEBOV GP) - based vaccine. In order to rapidly evaluate these vaccines, a 28,170 subject randomized, placebo-controlled trial aimed at preventing Ebola virus disease (EVD) that included both vaccines was designed. As the number of new cases declined to a level that a phase 3 study was no longer feasible the study was converted to a 1,500 subject phase 2 safety and immunogenicity trial.

**Methods:** Consenting volunteers  $\geq 18$  years were randomized to saline or one of the two experimental vaccines. Temperature  $> 38^\circ\text{C}$ , history of EVD, pregnancy and breastfeeding were exclusions. Follow-up visits occurred at week 1, month 1, month 2 and then every 2 months thereafter through 12 months. Blood was collected for antibody measurements at baseline, week 1, month 1 and months 6 and 12. Chi-square tests were used to compare each vaccine versus the placebo group for injection site reactions, targeted symptoms, and antibody responses.

**Results:** From February 2015 through April 2015, 1,500 volunteers were enrolled at Redemption Hospital in Monrovia, Liberia (500 per group). Median age was 30 years and 37% were women. 0.7% reported recent contact with a patient with Ebola; 4.6% reported working in a job that required contact with Ebola patients. The percentages of participants with positive serostatus for HIV and syphilis at baseline were 5.2% and 5.1%, respectively. Overall follow-up visit attendance has exceeded 98%. Differences with placebo in injection site reactions, targeted symptoms (headache, muscle pain, feverishness, fatigue) and lymphocyte counts were noted at week 1, but not month 1 for both vaccines. Antibodies, measured using the FANG ELISA assay for 50% of participants, show that 8% had an antibody response to Ebola at baseline. Excluding these individuals, an antibody response at month 1 was noted in  $>85\%$  of participants in each of the vaccine arms and  $<10\%$  of participants in the placebo arm ( $p < 0.001$ )

**Conclusions:** It is possible to conduct a well-designed, randomized, placebo-controlled trial in the middle of an epidemic outbreak. Both vaccines were demonstrated to be safe and immunogenic. A number of individuals without a known history of Ebola virus disease were found to have evidence of past infection with Ebola. The prevalence of HIV-1 in this cohort is higher than expected.

**77LB PREVAIL II: A Randomized Controlled Trial of ZMapp™ in Acute Ebola Virus Infection**

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**Background:** Although there are currently no licensed treatments for Ebola virus disease (EVD), the triple monoclonal antibody cocktail ZMapp is one of the most promising immune-based approaches for treating EVD based upon non-human primate data. Given the changing patterns of morbidity and mortality for human EVD identified during the recent outbreak in West Africa, it was believed that the single most definitive means of determining the efficacy of such an intervention would be through performance of a randomized controlled trial (RCT) with a clinical endpoint.

**Methods:** In a collaboration sponsored by the U.S. Department of Health and Human Services and involving the Ministries of Health of Sierra Leone, Guinea and Liberia, Mapp Biopharmaceuticals, Inserm, and academic medical centers in the U.S., consenting patients of any age diagnosed with EVD by polymerase chain reaction (PCR) between March–November 2015 were enrolled in the affected countries. Patients were randomized 1:1 to receive either optimized standard of care (oSOC, defined minimally as intravenous (iv) fluid resuscitation plus electrolyte monitoring) or oSOC plus three iv infusions of 50 mg/kg ZMapp spread three days apart. Patients were stratified by baseline Cycle Threshold (CT) value ( $\leq 22$  vs.  $> 22$ ) on PCR and by treatment site (U.S. vs. Liberia/Sierra Leone vs. Guinea [where favipiravir was part of oSOC]). The primary endpoint was mortality 28 days following randomization. An independent Data and Safety Monitoring Board (DSMB) reviewed interim results.

**Results:** As of January 5, 2016, 72 patients were enrolled in the U.S. ( $n=1$ ), Liberia ( $n=5$ ), Sierra Leone ( $n=54$ ), or Guinea ( $n=12$ ). 30/72 (42%) and 42/72 (58%) had CT values  $\leq 22$  and  $> 22$ , respectively, with an overall mean CT value of 23.9 at enrollment. The mean age of the enrolled cohort was 26.1 years: 55.6% were female. Only 6.9% had prior occupational exposure to persons with known EVD. The mean #days since symptom onset was 4.2. Overall mortality at 28 days was 21/72 (29.2%).

**Conclusions:** In the only RCT of a putative therapeutic agent performed to date in the current crisis, 72 patients were randomized to receive either oSOC plus ZMapp versus oSOC alone over a 9 month period in the latter half of the 2014–15 EVD epidemic. Barring a resurgence of additional EVD cases and with DSMB concurrence, the study will be unblinded for safety and efficacy analyses on January 14, 2016, 42 days after the last confirmed EVD case in West Africa.

**78 Progress in Gene Therapy for HIV Cure**

**Paula M. Cannon**, *Univ of Southern California, Los Angeles, CA, USA*

A defining characteristic of the human immunodeficiency virus is its ability to permanently integrate into the genome of an infected cell. Since a subset of viruses become latent shortly after infection, a reservoir of HIV persists that is not impacted by antiretroviral therapy (ART), yet retains the ability to restart viremia should ART be discontinued. This viral trick creates a life-long requirement for ART in most individuals in order to maintain virologic control, and no cure.

Latent HIV shares many of the characteristics of a genetic disease, so it was no coincidence that HIV became an early target for gene therapy. Despite the complexity of these procedures, the potential for long-lasting effects, especially if stem cells could be modified, was appealing when measured against life-long ART. Initial studies focused on the idea of protecting uninfected cells by adding anti-HIV factors, and early trials established a portfolio of anti-HIV candidates and safety, although they stopped far short of demonstrating efficacy.

More recently, applications of gene therapy have been expanded to include consideration of a cure. However similar to ART, gene therapies aimed at simply protecting uninfected cells are not expected to impact the latent reservoir. Therefore approaches being considered combine transient drug treatments to 'shock' HIV awake with engineering HIV-specific immune cells that would deplete the infected cells so revealed. Other strategies under development are taking advantage of our deeper understanding of the biology of the host-virus interaction. Recent advances in genome editing based on targeted nucleases are allowing for the possibility of removing host dependency factors, such as the CCR5 coreceptor, or editing anti-viral restriction factors to shift the balance in favor of the host. Finally, the HIV genome itself is a tantalizing target for disruption, since nucleases can also be engineered to specifically recognize and disable this genetic parasite, if only the significant challenges of *in vivo* delivery could be met.

Antiretroviral drugs have made extraordinary progress over the last 30 years, turning HIV into a chronic and medically managed disease, but they have reached a limit in the latent HIV reservoir. Gene, immune and cell-based therapies may yet prove to be part of the final push that is needed to achieve a cure.

**79 HIV in Transgender Populations: Charted and Uncharted Waters**

**Tonia C. Poteat**, *Johns Hopkins Univ, Baltimore, MD, USA*

A growing body of research has demonstrated the heavy and disproportionate burden of HIV among transgender populations around the world. Transgender women, in particular, face an estimated global HIV prevalence of 19% with 49 times the odds of infection compared to the general adult population. Research among transgender men is quite limited with prevalence estimates that range from 0 - 10%. Estimates of the absolute number of transgender people living with HIV are lacking; however, recent data suggest they make up a key minority of HIV-infected adults in care.

This presentation will focus on what is known and not known about HIV and its drivers among transgender populations. Access to and utilization of exogenous hormone therapy and its impact on HIV risk as well as clinical implications for antiretroviral therapy will be discussed. What is known about the impact of genital reconstruction on HIV acquisition and transmission will be reviewed. We will examine the widespread use of illicit soft tissue fillers for body modification and their potential consequences for HIV disease progression. Relationships between stigma, sex work, and co-occurring syndemics of depression, victimization, substance use, and HIV will be described.

Modeling data suggest the need for combination high-impact prevention in order to significantly reduce HIV incidence in this population. Uptake of and adherence to PrEP has been limited by community concerns about potential drug-drug interactions between ART and hormone therapy; and preliminary unpublished data suggest associations between ART adherence and co-location of gender-related care with HIV services.

Current NIH-funded studies include behavioral, self-testing, PrEP adherence, and telemedicine interventions. However, more research is needed. Data on HIV prevalence in transgender populations is missing from the African continent as well as Eastern Europe/Central Asia. Little is known about the sexual partnerships and networks of transgender people; nor is there data on co-morbidities and co-infections among HIV-infected transgender people taking exogenous hormones. Implementation science studies are needed to inform how best to implement and scale up multi-component, high impact, prevention care and treatment interventions that address structural drivers, reduce HIV incidence, and improve the health and longevity of transgender people living with HIV.

**80 Investigating the Mechanisms that Control HIV Transcription and Latency In Vivo**

**Steven A. Yukt<sup>1</sup>**; Philipp Kaiser<sup>1</sup>; Peggy Kim<sup>1</sup>; Sunil K. Joshi<sup>1</sup>; Nicholas Kim<sup>1</sup>; Peilin Li<sup>1</sup>; Harry Lampiris<sup>1</sup>; Hongbing Liu<sup>2</sup>; Andrew Rice<sup>2</sup>; Joseph K. Wong<sup>1</sup>; for the DARE Study Group  
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**Background:** It is unclear what mechanisms maintain HIV latency in vivo. We hypothesized that the levels of different HIV RNA transcripts could be used to infer the sites of transcriptional blockade.

**Methods:** CD4+T cells from blood of 9 ART-suppressed individuals were isolated using negative selection and frozen or activated for 2 days with anti-CD3/CD28 beads. RNA was extracted using Trireagent. Aliquots from common RT reactions were used in ddPCR assays specific for different HIV transcripts indicative of transcriptional interference (U3-US; “read-through”), initiation (TAR), elongation (R-US-tRNA; “long”), completion (3’LTR-polyA; “polyA”), and multiple splicing (tat-rev). HIV RNA levels were expressed as absolute levels (normalized to cell counts) and ratios to total (TAR) and processive (long) transcripts.

**Results:** In unstimulated CD4+T cells, the relative abundance of HIV transcripts was: TAR (median 18,060 copies/10<sup>6</sup> cells) > long (1816) > polyA (257) and read-through (227) > tat-rev (4.7) [p<0.05], indicating blocks to proximal elongation, completion, and splicing. Most HIV transcripts were prematurely-terminated transcripts that had not elongated past the TAR loop (median long/TAR=0.073). Of processive transcripts, most had not completed transcription (polyA/long=0.15) and very few were spliced (tat-rev/long=0.0045). Read-through transcripts were detected in all individuals, but levels were modest in relation to processive transcripts (read through/long=0.15) and total transcripts (read-through/TAR=0.013). Activation increased all transcripts, but the increases in read-through (median d2/d0=3.1) and TAR (5.5x) did not exceed the global increase in cellular transcription, while “long” tended to increase more (7.4x) and the greatest increases (p<0.05) were seen in polyA (45.8x) and tat-rev (116x). Activation did not change read-through/TAR, tended to increase long/TAR (median d2/d0=3.4), and resulted in larger increases (p<0.05) in polyA/TAR (11.8x), tat-rev/TAR (61.8x), polyA/long (3.4x), and tat-rev/long (11.7x).

**Conclusions:** In unstimulated CD4+T cells, transcriptional interference plays a modest role in limiting HIV transcription, while blocks to elongation, completion, and splicing dominate. Activation selectively increased distal and spliced transcripts but had less effect on read-through or total transcripts, suggesting that the main reversible blocks to HIV expression are not interference or lack of initiation, but rather inhibition of elongation/polyadenylation and splicing.

**81 Human Galectin-9 Is a Potent Mediator of HIV Transcription and Reactivation**

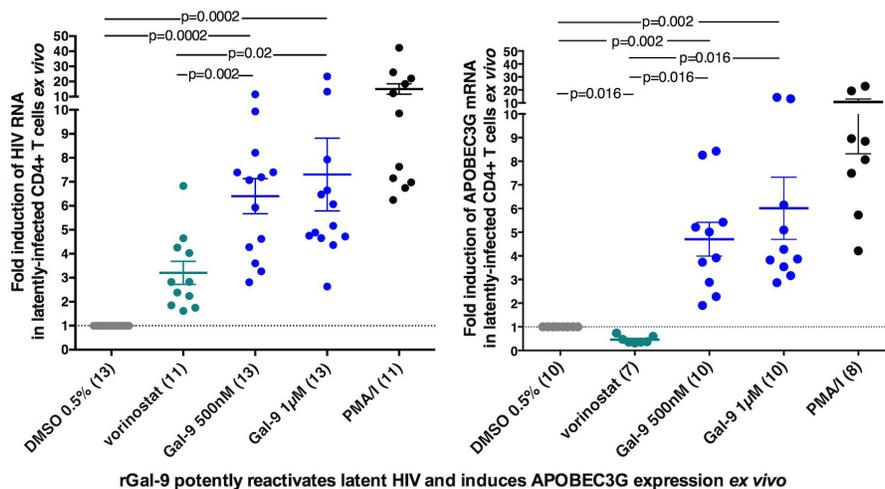
**Mohamed Abdel-Mohsen<sup>1</sup>**; Leonard Chavez<sup>1</sup>; Glen M. Chew<sup>2</sup>; Xutao Deng<sup>1</sup>; Ali Danesh<sup>1</sup>; Sheila M. Keating<sup>1</sup>; Rebecca Hoh<sup>2</sup>; Steven G. Deeks<sup>3</sup>; Lishomwa C. Ndhlovu<sup>2</sup>; Satish Pillai<sup>1</sup>  
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**Background:** Identifying host determinants governing HIV transcription and latency is critical to developing an HIV cure. Based on our recent finding that the host factor p21 regulates HIV transcription during antiretroviral therapy (ART), and published data demonstrating that the glycan-binding protein galectin-9 (Gal-9) regulates p21, we hypothesized that Gal-9 modulates HIV transcription.

**Methods:** Plasma Gal-9 levels were examined in relation to measures of the latent HIV reservoir in 72 HIV-infected ART-suppressed individuals. The ability of a recombinant, stable form of Gal-9 (rGal-9) to reactivate latent HIV was evaluated in the J-Lat 5A8 HIV latency model, and in primary CD4+ T cells isolated from 13 HIV-infected ART-suppressed individuals. Enzymatic and chemical deglycosylation was used to explore the requirement of glycans in viral reactivation by rGal-9. Effects of rGal-9 on the host transcriptome were evaluated using RNA-seq.

**Results:** Endogenous levels of plasma Gal-9 were associated with levels of CD4+ T cell-associated HIV RNA (p<0.02) and with the quantity and binding avidity of anti-HIV antibodies (p<0.009) *in vivo* during ART. Administration of rGal-9 reactivated virus in J-Lat cells (15.1%) more potently than anti-CD3/CD28 stimulation (4.8%, p<0.0001). In CD4+ T cells from HIV-infected individuals, rGal-9 induced a mean 7.3-fold increase in intracellular HIV RNA levels, as compared to DMSO negative control (p=0.002). Induction was significantly higher than vorinostat (p=0.02, 3.2 fold). Dosages of rGal-9 required to reverse HIV latency *ex vivo* were well-tolerated *in vivo* in Lewis rats. Cell surface N-linked oligosaccharides and O-linked hexasaccharides were essential for rGal-9-induced HIV reactivation, mediated by key transcription initiation, promoter proximal-pausing, and chromatin remodeling factors (FDR<0.05). rGal-9 induced expression of the host antiviral deaminase APOBEC3G up to 29-fold *in vitro* and *ex vivo* (FDR<0.006), resulting in 6.7-fold reduction in infectivity of progeny virus.

**Conclusions:** rGal-9 potently reactivates latent HIV and induces APOBEC3G expression *in vitro* and *ex vivo*. rGal-9-induced virus will likely be rendered replication incompetent as a result of APOBEC3G induction in the producer cell, ensuring that the reservoir will not be replenished when latency is reversed therapeutically, even in the setting of suboptimal ART suppression. Our data suggest that gal-9 and the glycosylation machinery should be explored as a foundation for novel HIV cure strategies.



**82 PD-1+ and Tfh Cells Represent the Major Source of HIV-1 Replication-Competent Virus**Riddhima Banga<sup>1</sup>; Francesco Procopio<sup>1</sup>; Matthias Cavassini<sup>2</sup>; Jean-Marc Corpataux<sup>2</sup>; Giuseppe Pantaleo<sup>3</sup>; Matthieu Perreau<sup>1</sup><sup>1</sup>CHUV, Epalinges, Switzerland; <sup>2</sup>Univ Hosp Lausanne, Univ of Lausanne, Lausanne, Switzerland; <sup>3</sup>CHU Vaudois, Lausanne, Switzerland**Background:** Recent studies have shown that Tfh cells from viremic HIV-1 infected individuals served as the major CD4 T-cell compartment for HIV infection, replication and production. However, their role in long-term treated aviremic HIV-1 infected subjects remains to be established.**Methods:** In the present study, we have investigated the distribution of HIV-1 infected CD4 T cells containing replication competent HIV within CXCR5<sup>+</sup>PD-1<sup>-</sup>, CXCR5<sup>+</sup>PD-1<sup>+</sup> and PD-1<sup>+</sup> memory CD4 T-cell populations isolated from blood and lymph nodes (LN) of long-term treated (1.5-14 years) aviremic (<20 HIV RNA copies per ml) subjects (n=11) using virus outgrowth assay (VOA). The LN PD-1<sup>+</sup> memory CD4 T-cell population contained about 65% of Tfh cells, i.e. CXCR5<sup>+</sup>PD-1<sup>+</sup> cells.**Results:** We demonstrated that the levels of HIV-1 RNA produced in the VOA supernatants were significantly higher in LN PD-1<sup>+</sup> CD4 T cells as compared to any other blood or LN CD4 T-cell subset and inversely correlated with the duration of treatment ( $P<0.05$ ). In depth analysis showed that the levels of HIV-1 RNA detected in the culture supernatants of LN PD-1<sup>+</sup> CD4 T cells were 810-1225 fold higher as compared to the other LN PD-1<sup>-</sup> cell populations ( $P<0.05$ ), and 5755-73123 higher as compared to blood CD4 T-cell populations ( $P<0.05$ ). In addition, P24 production was clearly detected in culture supernatants of LN memory PD-1<sup>+</sup> CD4 T cells in 6 out of the 11 long-term ART treated aviremic HIV-1 infected individuals and border line positive in 1 out of the 11 individuals tested in blood memory PD-1<sup>+</sup> CD4 T cells. Interestingly, HIV-1 produced by LN PD-1<sup>+</sup> CD4 T cells was also infectious as indicated by the transmission of infection *in vitro* to CD4 T cells of HIV-negative subjects. Finally, we have evaluated the mean frequencies of inducible replication competent virus from HIV-1 infected cells using both RUPM and IUPM for each sorted blood and LN memory CD4 T-cell populations isolated from 11 aviremic long-term treated patients and showed that LN PD-1<sup>+</sup> CD4 T cells were enriched with replication competent and infectious virus and represent the major source of replication competent and infectious virus in long-term treated aviremic HIV-1 infected individuals.**Conclusions:** Taken together, these results demonstrate that LN PD-1<sup>+</sup> CD4 T cells which include Tfh cells serve as the major CD4 T-cell compartment for HIV-1 replication competent and infectious virus in long-term treated aviremic HIV-1 infected subjects.**83 Rapid Accumulation of Defective Proviruses Complicates HIV-1 Reservoir Measurements**Katherine M. Bruner<sup>1</sup>; Ross Pollack<sup>1</sup>; Alexandra Murray<sup>1</sup>; Mary Soliman<sup>1</sup>; Sarah B. Laskey<sup>2</sup>; Matt F. Strain<sup>3</sup>; Douglas D. Richman<sup>3</sup>; Steven G. Deeks<sup>4</sup>; Janet Siliciano<sup>1</sup>; Robert Siliciano<sup>5</sup><sup>1</sup>Johns Hopkins Univ Sch of Med, Baltimore, MD, USA; <sup>2</sup>Johns Hopkins Univ, Baltimore, MD, USA; <sup>3</sup>Univ of California San Diego, San Diego, CA, USA; <sup>4</sup>Univ of California San Francisco, San Francisco, CA, USA; <sup>5</sup>Howard Hughes Med Inst, Baltimore, MD, USA**Background:** HIV-1 establishes latency in resting memory CD4<sup>+</sup> T cells, creating a major barrier to eradication. Although defective proviruses predominate in resting CD4<sup>+</sup> T cells from patients treated during chronic infection, we hypothesized that the fraction of defective proviruses is not constant and increases over the course of the infection as cells with genetically intact proviruses are eliminated. We sought to determine the time frame of this accumulation as it is relevant to HIV-1 reservoir measurements. We also sought to define the reservoir size in chronic and acutely-treated patients as well as investigate what types of proviruses would be detected by current HIV-1 assays and how a shock and kill strategy would affect those measurements.**Methods:** We characterized proviruses from patients treated in either acute or chronic infection and untreated viremic patients. We also performed an *in vitro* infection of CD4<sup>+</sup> T cells to determine the fraction of defective proviruses after a single round of infection. Proviruses were analyzed by an unbiased, limiting dilution, full genome PCR and direct sequencing of PCR products. The number of intact proviruses was quantified as a percentage for each patient and compared to total HIV-1 DNA ddPCR values and QVOA IUPMs. Proviruses were also analyzed at the DNA level to predict the likelihood of making HIV-1 RNA or protein, as required for detection by current HIV-1 assays and for elimination by a shock and kill strategy.**Results:** Following one round of *in vitro* replication, defective proviruses were readily detected and made up 40% of proviruses. Less than 5% of proviruses were intact in both patient groups; the remaining proviruses contained major defects. We also found that the QVOA underestimates the latent reservoir by a median of 64 fold in chronically treated patients and 20 fold in acutely treated patients while total DNA PCR measurements vastly overestimated the reservoir in both patient groups. Importantly, our analysis of the proviral sequences predicts that the majority of proviruses are unable to make protein and are unlikely to be affected by a shock and kill strategy.**Conclusions:** During acute infection, the fraction of defective proviruses likely increases very rapidly, quickly making up over 95% of proviruses in HIV-1 patients. Our analysis also indicates that most proviruses are unlikely to be affected by a shock and kill strategy. Thus, the high fraction of defective proviruses must be considered when evaluating latency reversing agents.**84 Clones of SIV-Infected Cells Are Present in Spleen and Lymph Nodes in Rhesus Macaques**Andrea Ferris<sup>1</sup>; Gregory Q. Del Prete<sup>2</sup>; Brandon Keele<sup>2</sup>; Xiaolin Wu<sup>2</sup>; Jeffrey Lifson<sup>2</sup>; Stephen H. Hughes<sup>1</sup><sup>1</sup>NCI, Frederick, MD, USA; <sup>2</sup>Frederick Natl Lab, Frederick, MD, USA**Background:** We and others have shown that there is clonal expansion of HIV infected cells in patients, and that, in some cases, the integrated viral DNA can cause the growth and persistence of infected cells. More recently, we showed that a clonally expanded cell carries an intact provirus and produces infectious HIV in a patient. Because there are limits on the samples that can be obtained from patients, we developed a model using SIV-infected rhesus macaques.**Methods:** The methods of Maldarelli et al (Science 345: 179, 2014) were used to generate an integration site library from rhesus macaque PBMCs infected in culture with SIV. Six additional libraries were generated from two rhesus macaques (two lymph node and one spleen sample from each animal) that were infected with SIV for four weeks and then treated for approximately one year with a cART regimen that fully suppressed the replication of the virus. Samples were taken during necropsy at the end of the treatment period.**Results:** The distribution of the SIV integration sites in the large integration site library (~50,000 independent sites) prepared from rhesus macaque PBMC infected *in vitro* was quite similar to the distribution of HIV integration sites in human PBMCs. We obtained approximately 380 independent integration sites from the monkey tissue sample and identified 13 clones of expanded cells. Cells from two of the clones were present in both the spleen and lymph node tissue samples.**Conclusions:** The presence of expanded clones in two SIV macaques that were treated after 4 weeks of infection shows the cells that are infected early can give rise to expanded clones. Cells from two clones were present in both lymph node and spleen, showing that the distribution of at least some clones was not tissue restricted. Having a large PBMC integration site library makes it possible to look for genes in which the integration of an SIV provirus provides the infected cells with a selective growth advantage in the animal. In this first experiment, there was no evidence for the selection of cells that have integration sites in either BACH2 or MKL2. Our results establish an SIV/macaque model that can be used to study the clonal expansion of infected cells using samples that cannot be obtained from patients.**85 ART Reduces Cellular HIV RNA but Not the Fraction of Proviruses Transcribing RNA**Feiyu Hong<sup>1</sup>; Jonathan Spindler<sup>2</sup>; Andrew Musick<sup>3</sup>; Anthony R. Cillo<sup>1</sup>; Michael Bale<sup>2</sup>; Wei Shao<sup>4</sup>; John M. Coffin<sup>5</sup>; John W. Mellors<sup>1</sup>; Mary F. Kearney<sup>3</sup><sup>1</sup>Univ of Pittsburgh, Pittsburgh, PA, USA; <sup>2</sup>HIV Dynamics and Replication Prog, Natl Cancer Inst, Frederick, MD, USA; <sup>3</sup>NCI, Frederick, MD, USA; <sup>4</sup>Leidos Biomed Rsr, Inc, Frederick, MD, USA;<sup>5</sup>Tufts Univ, Boston, MA, USA**Background:** Little is known about proviral expression at the single cell level before or during ART. We investigated both the fraction of infected cells that express HIV RNA and the levels of HIV RNA in single cells from untreated, viremic individuals and from those on suppressive ART.**Methods:** PBMCs from 5 viremic (median VL=5727 c/ml) and 5 ART-suppressed (VL<20 c/ml) individuals were analyzed for expression of unspliced viral RNA in single cells by 2 methods: Cell-Associated RNA (CAR) and DNA single-genome sequencing (CARD-SGS) and fractional proviral expression (fPVE). CARD-SGS was performed by extracting RNA from multiple PBMC aliquots diluted to an endpoint for HIV expressing cells. cDNA was synthesized from each RNA extraction and all cDNA molecules were sequenced by *gag-pol* SGS.

DNA was extracted from 1 PBMC aliquot to compare the genetics of CAR to the proviral population. fPVE was determined by serial dilution of PBMCs and measuring levels of HIV DNA and unspliced CAR by qPCR in 6 replicates at each dilution. The fraction of the infected cells expressing CAR was calculated by maximum likelihood estimates.

**Results:** The proportion of the proviruses that expressed unspliced CAR was not different between viremic and ART-suppressed individuals (median 7% vs. 6% respectively). By contrast, the fraction of cells that were “high CAR producers” (>20 CAR copies/cell by CARD-SGS and >50 CAR copies/cell by fPVE, corrected for the shorter amplicon used for fPVE) was greater in viremic than suppressed individuals (2.5% vs. <0.3%, p=0.02, by CARD-SGS and 12% vs. <2%, p=0.01, by fPVE). Frequent detection of identical CAR sequences across multiple aliquots of PBMCs from ART-suppressed individuals revealed that expanded clones commonly express unspliced HIV RNA.

**Conclusions:** The differences in HIV RNA expression levels in single cells between ART-suppressed and viremic individuals but not in the fraction of proviruses that express HIV RNA suggest that cells producing high levels of HIV RNA are associated with active virus replication and are eliminated by viral CPE or CTL responses, whereas the more frequent cells expressing low levels of HIV RNA can persist and expand despite ART. Expanded proviruses that express unspliced HIV RNA may be the source of rebound viremia when ART is interrupted.

**86 Restricted HIV-1 Diversity and Clonal Expansion Following Cytoreductive Chemotherapy**

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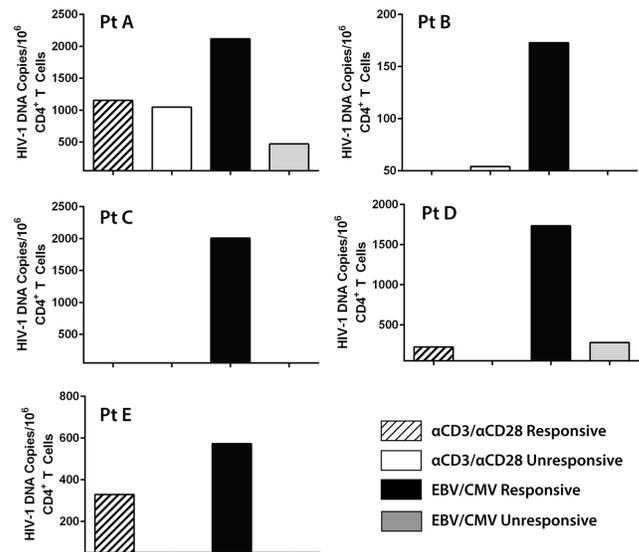
<sup>1</sup>Univ of California San Francisco, San Francisco, CA, USA; <sup>2</sup>Massachusetts General Hosp, Boston, MA, USA; <sup>3</sup>Ragon Inst of MGH, MIT, and Harvard, Cambridge, MA, USA; <sup>4</sup>Brigham and Women's Hosp, Harvard Med Sch, Boston, MA, USA; <sup>5</sup>Dana-Farber Cancer Inst, Boston, MA, USA; <sup>6</sup>Harvard Med Sch, Boston, MA, USA

**Background:** Cytoreductive chemotherapy for malignancies does not lead to consistent changes in HIV-1 DNA or RNA. However, observed reductions in CD4 T cells during chemotherapy suggest that total body reservoirs decrease. We hypothesize that constriction and subsequent oligoclonal expansion of HIV diversity may be a better measure of the reservoir response to cytoreductive therapy or novel HIV eradication strategies. We therefore examined HIV sequence evolution in the context of immune correlates, and quantified HIV levels in cells responding to non-HIV stimuli.

**Methods:** Longitudinal, single-genome analysis of HIV envelope sequences was performed in a cohort of 10 infected individuals on suppressive ART receiving chemotherapy. Findings were compared with changes in T cell subsets, activation/proliferation and HIV-specific immune responses measured by ELISpot. HIV DNA levels in CD4 T cells were quantified from cells sorted based on intracellular IL2 and/or INFγ staining after stimulation with αCD3/αCD28 antibodies or combined Epstein-Barr virus (EBV) and cytomegalovirus (CMV) lysates.

**Results:** Although CD4 T cell counts transiently decreased by up to 75% during chemotherapy, CD4 T cell HIV DNA did not change and RNA increased following completion of therapy (P=0.203 and P=0.037 by paired Wilcoxon test). Despite reductions in total cell counts, no changes in the percentage of naive, central or effector memory, or terminally differentiated CD4 and CD8 T cell subsets were observed before or after treatment (all P>0.05). Markers of activation (CD38/HLA-DR) and proliferation (Ki67) either decreased or remained stable in all populations. HIV-1 specific T cell responses either increased or developed novel peptide signatures following completion of chemotherapy. Furthermore, clonal expansion of HIV-1 envelope sequences following chemotherapy was observed in 3 of 6 patients for whom data was obtained; sequence clustering was only seen following completion of chemotherapy. Finally, CD4 T cells that responded to EBV/CMV lysates had higher HIV-1DNA levels compared to those that responded to αCD3/αCD28 stimulation or did not express IL2/INFγ (Fig 1).

**Conclusions:** Despite the lack of changes in peripheral blood HIV DNA, our results suggest that cytoreductive therapy reduces HIV reservoirs in some patients manifesting as a constriction of HIV sequence diversity. Our data also suggest that response to non-HIV antigens can lead to oligoclonal expansion of the DNA reservoir.



**87 Virological Remission After ART Interruption in African HIV-1 Seroconverters**

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**Background:** Increasing evidence supports virological remission in a subset of individuals after treatment with antiretroviral therapy (ART) in primary HIV infection (PHI). We present the first analysis of treatment interruption (TI) in individuals with PHI in Africa, to explore the prevalence and predictors of post-treatment control (PTC).

**Methods:** 137 individuals within 6 months of seroconversion were recruited to the SPARTAC RCT from South Africa and Uganda. We analysed samples from those randomised to ‘no treatment’ (NT) and 48 weeks of ART (ART48). We measured HIV-1 DNA in CD4 T cells (Total, Integrated), CD4 count, plasma viral load (VL) and markers of T cell activation (CD25, CD38, CD69, HLA-DR) and exhaustion (Lag-3, PD-1, Tim-3). We explored associations with both clinical progression and time to viral load rebound. Data were compared with those for UK SPARTAC participants (n=151) recruited under the same protocol.

**Results:** Of individuals recruited to SPARTAC in Africa, 91 were randomised to ART48 or NT, and of these, samples were available for 44 and 38, respectively. Of those in the ART48 arm, 22 received the full 48 weeks of ART and had VL < 400 copies/mL at TI. All were female; 19/22 were infected with subtype C. Of UK patients all were MSM infected with subtype B. Pre-therapy VL was significantly lower in Africans compared with the UK cohort (median 4.16 vs 4.62 log<sub>10</sub> copies/mL; p<0.001). CD4 T cell count and Total HIV-DNA were similar in UK and African patients, although Integrated HIV-1 DNA was lower in the latter (median 3.60 vs 3.06 log<sub>10</sub> copies/million CD4+ T-cells; p < 0.001). Measured pre-ART, Total HIV-1 DNA, VL and CD4 count in Africans were associated with clinical progression (p=0.001, HR (CI)<sub>95</sub> 5.37 (1.95-14.79); p<0.001, 1.99 (1.35-2.94) and p<0.001, 0.34 (0.24-0.48), respectively).

From TI after 48 weeks of ART, 5/22 (22.7%) Africans maintained VL<400 copies/ml over a median 188 weeks follow-up (range 147-203). In multivariable analyses, Africans experienced significantly longer duration of viral remission than UK participants (p<0.001; HR 3.90 (1.75-8.71). Total DNA at TI was a predictor of time to rebound, but the effect was much weaker in African than in UK patients.

**Conclusions:** We present the first analysis of PTC in Africans with PHI. The difference in virus-free remission post-TI in these individuals compared to the UK patients is striking. Further studies are needed to explore mechanisms and discriminate the distinct effects of sex, ethnicity and HIV subtype.

**88 An Infant bNAb With Low Somatic Hypermutation Contributes to Polyclonal Breadth**

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**Background:** Rapid development of plasma cross-clade neutralization breadth has been described in infants compared to adults. The characteristics of infant HIV-specific antibodies contributing to plasma neutralization breadth have not yet been studied, but may provide unique insights into the process of developing HIV-1-specific broadly neutralizing antibody (bNAb) responses.

**Methods:** We isolated and characterized neutralizing antibodies (NAb) from an infant sample from 450 days of age (~15 months), an estimated 336 days (~11 months) post-infection – a time when cross-clade NAb breadth was detected in plasma. We used large-scale culture of memory B cells followed by high-throughput neutralization assays to identify HIV-specific B cells. The neutralization breadth of isolated infant-derived NAb was defined using a diverse panel of envelope variants. The epitope target of the infant-derived antibody demonstrating the greatest neutralization breadth was defined by identifying mutations in known neutralizing epitope targets that confer neutralization resistance.

**Results:** To date, 9 infant-derived HIV-1-specific neutralizing antibodies have been identified and all are produced from distinct lineages of B cells. The level of somatic hypermutation (SHM) of infant neutralizing antibodies is significantly lower than adult bNAbs and also NAb from adults with Tier 1 neutralizing activity but limited cross-clade Tier 2 activity. Several infant NAb demonstrate heterologous Tier 2 neutralization; one demonstrates cross-clade Tier 2 neutralization breadth against clades A, B and C, and neutralizes 7 of 12 viruses from a standardized panel of global reference viruses. For this antibody, neutralizing activity is dependent on the N332 residue in V3, a known site of vulnerability and target of adult bNAbs such as PGT121 and PGT128. However, the VH-gene usage of this antibody is not the same as known adult bNAbs that target this site, and SHM is 6.8% at the nucleotide level compared to 17% for PGT121 and 19% for PGT128.

**Conclusions:** These data suggest that infant responses may be polyclonal and a component of the response includes antibodies targeting a glycan epitope. Furthermore, these data suggest that this infant's response may target some similar epitopes as adults, but do so using distinct gene families and less SHM.

**89LB Virus, Host, and Disease Factors Govern HIV-1 Broadly Neutralizing Antibody Induction**

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**Background:** Development of an effective AIDS vaccine that elicits broadly neutralizing antibodies (bnAbs) urgently awaits the identification of parameters that steer their induction. Here we report on a systematic analysis of bnAb activity in 4484 HIV-1 infected individuals designed to explore and identify viral, host and disease factors that affect bnAb generation.

**Methods:** Plasma neutralization activity was determined in a TZM-bl based pseudovirus neutralization assay against an 8 multi-clade virus panel. Top ranked neutralizers were further characterized in a 40 virus panel and the top 87 plasmas subjected to a bnAb fingerprinting analysis to delineate specificities.

**Results:** By linking information on the obtained bnAb activity with comprehensive longitudinal patient and virus data available in our cohort, we confirmed using logistic regression that three parameters reflecting the exposure to antigen – namely the viral load, the length of untreated infection and viral diversity – independently of each other drive bnAb induction. Black ethnicity proved to be associated with significantly higher rates of bnAb induction than white ethnicity independently of all other probed variables including the infecting HIV-1 subtype. In total we identified 239 individuals with elite or broad neutralization activity. Neutralization finger print analysis of the bnAb specificity of the top 87 ranked plasmas identified a strong predisposition of individual HIV-1 subtypes to induce certain bnAb types. Subtype B infection induced CD4bs bnAbs more frequently than non-B infection ( $p=0.02$ ; Fisher's exact test) whereas V2-glycan bnAbs were highly effectively stimulated by non-B but not subtype B viruses ( $p=5 \times 10^{-6}$ , Fisher's exact test).

**Conclusions:** Our study provides a number of novel and exciting clues for vaccine design. Three parameters, viral load, diversity and length of infection, previously implicated in bnAb evolution proved to influence bnAb generation independently of each other and thus individually could have a potential to advance vaccine strategies. The advantage of black ethnicity in inducing bnAbs highlights the need to uncover host genome determinants in bnAb inducing individuals of all ethnicities to understand their importance and implications for vaccines. Lastly, the subtype dependence in bnAb type development strongly implies that distinct subtype specific structural features of the viral envelope trimer must exist, which need to be unraveled and harnessed for vaccine immunogen design.

**90 Clinical Safety and Pharmacokinetics of IV and SC VRC01, a Broadly Neutralizing mAb**

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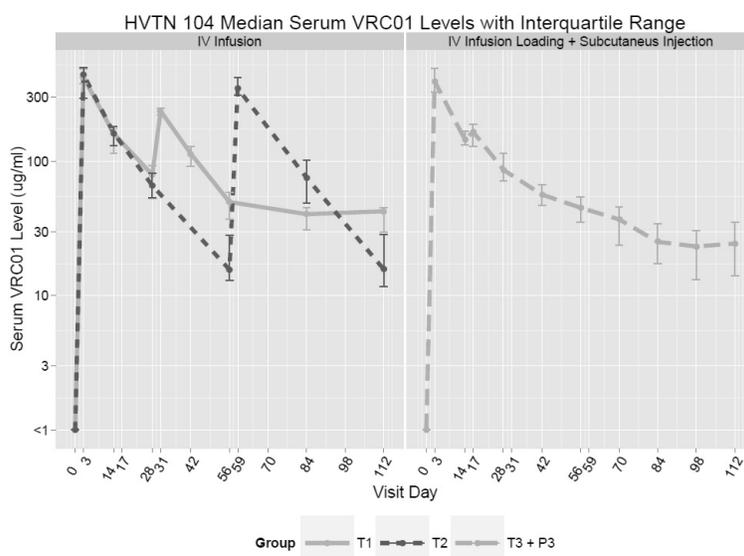
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**Background:** VRC01 is a monoclonal antibody (mAb) directed at the CD-4 binding site that neutralized most HIV isolates that it was tested against in vitro, protected simians from retroviral challenges, decreased HIV plasma RNA in treatment-naïve HIV-infected participants (ppt), and offers the potential to prevent HIV transmission in humans.

**Methods:** In HVTN 104, 88 low risk, healthy men ( $n=44$ ) and women ( $n=44$ ) were enrolled and assigned to receive either a 40 mg/kg IV loading dose, followed by 20 mg/kg IV every 4 weeks (Group 1), or 10, 30 or 40 mg/kg of VRC01 administered intravenously (IV) every 8 weeks (Groups 2, 4, or 5), or a 40 mg/kg IV loading dose, followed by 5 mg/kg subcutaneously, every 2 weeks for 5.5 months (Group 3, which included a placebo arm). Safety and tolerability were evaluated by site clinicians and reviewed weekly by the protocol safety team. VRC01 drug levels in serum were measured by a VRC01 anti-idiotypic ELISA binding antibody assay. Safety data from all 5 groups and pharmacokinetic data from groups 1 to 3 are presented here.

**Results:** The median age of enrollees was 27 yrs (range: 18-50); 46.5% were non-White; 11.4% were Hispanic. Infusions and injections were generally well-tolerated, with 28% of infusions and 14% of injections resulting in mild pain/tenderness reactions, and very few erythema/induration reactions. 57% of participants had a systemic reaction, and of the 3 that were severe, 2 had concurrent viral infections, and 1 had malaise lasting 1 day. Most participants (72.7%) reported at least 1 adverse event, 73.6% of these events were graded as mild; with only 6.2% deemed product-related, and all of those were mild. Study product was discontinued after AEs in 3 ppt, out of caution. As of Sept. 4, 2015, 226 infusions and 167 injections were completed. Median peak levels after a 40 mg/kg infusion generally exceeded 300 mcg/mL and median VRC01 levels at 112 days were ~30 mcg/mL whether VRC01 was administered at 20 mg/kg at 28 days, 40 mg/kg at 56 days, or 5 mg/kg SC every 2 weeks. Drug levels from the first 3 groups are depicted in the accompanying figure.

**Conclusions:** VRC01 administered IV or SC was well-tolerated. Product-related AEs were generally transient and mild. After a 40 mg/kg IV loading dose, VRC01 levels could be maintained >30 mcg/mL for several months through either IV or SC administration. These findings support the rationale that VRC01 administered every 2 weeks SC or 2 months IV should be evaluated for HIV immunoprophylaxis



**91 Neutrophil Functions Induced by Gp-120 Specific IgA and IgG; Clues for Immunotherapy**

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**Background:** While neutropenia has been associated with increased risk of HIV infection, the antiviral role of neutrophils in HIV infection has been poorly studied. However, neutrophil extracellular traps have been shown to capture HIV virions that were subsequently eliminated by myeloperoxidase and α-defensins. Moreover, neutrophils are the most abundant cells in the blood, the most rapid responders to infection and the most potent mediators of Fc-effector functions. Thus functional antibodies against HIV may have the capacity to rapidly recruit and direct the potent antiviral activity of this cell subset. Given the high levels of both IgG-responsive FcγRs and IgA-responsive FcαR, here we aimed to determine whether HIV-specific antibodies may be functionalized naturally during HIV infection or whether monoclonal therapeutics can be designed to selectively recruit the antiviral activity of neutrophils.

**Methods:** Serum IgA and IgG were purified from HIV-infected subjects and healthy controls. Their ability to activate different neutrophil functions, such as phagocytosis against gp120 antigen, release of elastase and mediators of oxidative stress, were assessed on peripheral blood neutrophils from healthy donors.

**Results:** Significant differences in HIV-specific IgA titers were observed in HIV infected patients, with controllers exhibiting significantly higher gp120-specific IgA1 and IgA2 titers than progressors. Both HIV-specific IgA and IgG induced rapid phagocytosis (as early as 40 minutes post-stimulation) with the same potency, however IgGs were significantly better at promoting continual phagocytosis over 24 hours. IgA, on the other hand, were more potent in inducing neutrophil degranulation. Gp120 specific IgA2 and IgG1 titers correlated positively with phagocytosis, however only IgA2 strongly correlated with neutrophil degranulation. Additionally, antibody glycosylation profiles were linked to neutrophil activity, highlighting antibody biophysical features that drive enhanced neutrophil mediated activity.

**Conclusions:** HIV infection, and specifically durable control of HIV in Controllers, is associated with the production of antibodies with the selective capacity to rapidly recruit and deploy the antiviral activity of neutrophils through the production of both IgG and IgA antibodies. These data highlight a novel means by which vaccine strategies or monoclonal immunotherapies may be designed to harness tremendous anti-viral potential of neutrophils in the context of prophylaxis or cure strategies.

**92 Potency and Kinetics of Autologous HIV-1 Neutralizing Antibody Responses During ATI**

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**Background:** Studies utilizing brief analytical treatment interruption (ATI) of cART in HIV-1 infected subject have consistently shown that viral rebound occurs rapidly upon treatment interruption. The viral dynamics and relevant host immune pressures at this juncture, however, remain unclear. To test the hypothesis that the autologous antibody response is a significant driver of virus selection during ATI, we identify the viral populations arising from latency and measure the potency and kinetics of the autologous antibody response.

**Methods:** Plasma samples from 11 chronically infected, cART-suppressed subjects undergoing ATI were studied by single genome sequencing (SGS) and maximum likelihood analysis of gp160 env. Consensus envs from each lineage were cloned and tested for neutralization sensitivity in TZM-bl assays.

**Results:** We generated 231 SGS-derived envs (range: 16-29/subject timepoint). Multiple low-diversity lineages (2 to >10/subject) comprised the rebound quasispecies at first detectable viremia. Envns representing initial rebound lineages displayed a range of neutralization sensitivities to autologous plasmas (IC<sub>50</sub> <1:50 to >1:5,000). In three subjects studied over 12 weeks of ATI, neutralization resistant viruses persisted while more sensitive lineages substantially decreased in frequency. For example, subject S05 rebounded with four genetically distinct lineages (total env diversity of 1.48%; within-lineage diversity of <0.17%); each lineage was moderately sensitive to week 4 plasma (IC<sub>50</sub> range: 1:240-1:420). Over time, titers to the largest lineage (8/20 sequences at week 4) rose to >1:3000 and this lineage was not sampled subsequently (0/18 sequences, week 10). Minor lineages, in contrast, became more resistant to longitudinal plasmas (IC<sub>50</sub> ~1:100 to week 10 plasma) and expanded to comprise the majority of the viral quasispecies (15/18 sequences, week 10).

**Conclusions:** This study demonstrates that HIV-infected individuals undergoing ATI have multiple genetically distinct rebounding viral lineages exhibiting a range of sensitivities to autologous antibodies. Results suggest that the autologous neutralizing antibody response during ATI exerts potent selective pressure and is a significant factor determining viral quasispecies composition. These findings contribute to our understanding of relevant immune pressures during ATI and suggest that autologous antibodies may contribute to virus suppression achieved with novel immunotherapies currently being tested in the context of ATI.

**93LB PD-1 Blockade as an Adjunct Therapy to ART and Potential to Destabilize SIV Reservoir****Geetha Mylvaganam**<sup>1</sup>; Sakeenah Hicks<sup>1</sup>; Benton Lawson<sup>1</sup>; Melon Nega<sup>1</sup>; Vijayakumar Velu<sup>1</sup>; Rafi Ahmed<sup>1</sup>; Gordon J. Freeman<sup>2</sup>; Rama Amara<sup>3</sup><sup>1</sup>Emory Univ, Atlanta, GA, USA; <sup>2</sup>Dana-Farber Cancer Inst, Boston, MA, USA; <sup>3</sup>Yerkes Natl Primate Rsr Cntr, Emory Univ, Atlanta, GA, USA

**Background:** The expression of the inhibitory receptor programmed death-1 (PD-1) on anti-viral CD8 T cells and virally infected CD4 T cells provides an immunological signature for both T cell dysfunction and viral latency during chronic SIV/HIV infection. We hypothesized that PD-1 blockade administered during the initiation of anti-retroviral therapy (ART) and under fully suppressive ART would have direct effects on both dysfunctional CD8 T cells and latently infected CD4 T cells. To test our hypothesis we developed a primatized anti-human PD-1 Ab to allow for repeated infusions in rhesus macaques (RMs) and administered PD-1 blockade to chronically SIV infected RMs in combination with ART. **Methods:** SIVmac251 infected RMs were administered 5 infusions (over 14 days) of a 3mg/kg dose of primatized anti-PD-1 Ab 10 days prior to the initiation of ART or saline. 26-30 weeks post ART, RMs received 3 monthly infusions of 10mg/kg anti-PD-1 or saline. ART was interrupted 2 weeks after the final PD-1 Ab infusion.

**Results:** PD-1 blockade administered during the initiation of ART enhanced proliferation of anti-viral CD8 T cells ( $p=0.02$ ), increased their cytotoxic potential ( $p=0.04$ ) and polyfunctionality ( $p=0.01$ ). Importantly, the PD-1 Ab treated animals showed more rapid viral suppression (42 days in the PD-1 group versus 140 days in saline group;  $p=0.01$ ) and greater reconstitution of Th17 cells in the rectal mucosa ( $p=0.01$ ) following initiation of ART. Moreover, PD-1 blockade administered under suppressive ART resulted in significant and detectable plasma viral blips, suggesting possible effects on destabilizing the latent viral reservoir. Following ART interruption, PD-1 Ab treated animals showed up to an 80-fold reduction in set point viremia compared set point levels prior to initiation of ART.

**Conclusions:** These results reveal for the first time the potential of PD-1 blockade both on restoring anti-viral CD8 T cell function and possibly destabilizing the viral reservoir under ART. They highlight the potential of PD-1 blockade to work synergistically with other therapeutic agents such as vaccines and latency reversing agents to effectively diminish HIV reservoir under ART as a means to establish a functional cure.

**94 Follicular CTL Accumulate in SIV-Infected Lymph Nodes Due to Immune Activation****Costantinos Petrovas**<sup>1</sup>; Sara Ferrando-Martinez<sup>1</sup>; Amarendra Pegu<sup>1</sup>; Sarah F. Andrews<sup>1</sup>; David Ambrozak<sup>1</sup>; Adrian B. McDermott<sup>1</sup>; Jason M. Brenchley<sup>2</sup>; John R. Mascola<sup>3</sup>; Richard A. Koup<sup>3</sup><sup>1</sup>NIH, Bethesda, MD, USA; <sup>2</sup>Frederick Natl Lab for Cancer Rsr, Frederick, MD, USA; <sup>3</sup>VRG, NIAID, NIH, Bethesda, MD, USA

**Background:** Strategies aimed to eradicate HIV/SIV infection require the characterization of cytolytic CD8 T cells that can migrate and recognize infected cells at the sites of active replication. Follicular CD4 T cells ( $T_{FH}$ ), located within the germinal centers (GC) of the lymph node (LN) B cell follicles support active HIV/SIV replication even under suppressive combined antiretroviral therapy (cART). Thus, a comprehensive analysis of CD8 T cell dynamics in the LN, particularly in the GC area, will provide valuable information towards HIV/SIV eradication.

**Methods:** Multi-parametric flow cytometry, multiplexed confocal imaging, histocytometry and fluidigm-based analysis were used to characterized PBMC, LN-derived cells and LN tissues from (1) SIV-negative rhesus macaques (RM), (2) acute and chronic SIV-infected RM and (3) chronic SIV-infected African green monkeys (AGM). CM9 (Gag) and TL8 (Tat) tetramers and intracellular cytokine staining after peptide pool stimulation identified virus-specific CD8 T cells while in vitro killing assays using sorted LN CD8 T cell populations and an anti-CD3/VRG07 antibody proved the cytolytic activity of LN-resident CD8 T cells.

**Results:** Chronic SIV infection in RM was characterized by the accumulation of memory (CD28<sup>lo</sup>CD95<sup>hi</sup>) and effector (CD28<sup>lo</sup>CD95<sup>hi</sup>) CD8 T cells with a follicular (CCR7<sup>lo</sup>CXCR5<sup>hi</sup>) phenotype (fCD8). Imaging and histocytometry confirmed the accumulation of fCD8 within the GC. fCD8, when compared to CCR7<sup>hi</sup>CXCR5<sup>lo</sup> non-fCD8, express higher amounts of Granzyme B and exert a higher capacity to mediate in vitro bispecific-mediated redirected killing of infected cells. Chronically infected AGM, on the other hand, did not accumulate fCD8. Fluidigm analysis showed that both memory and effector fCD8 clustered together and were mostly affected by the SIV-infection status. Besides, the frequency of LN CD8 T cells was significantly correlated with LN CD14<sup>hi</sup>CD16<sup>hi</sup> monocytes. Upon stimulation, monocytes from SIV-infected RM secreted increased amounts of CXCL10, a CXCR3 ligand. Confocal imaging confirmed the proximity of CXCR3-expressing cells and monocytes within the LN.

**Conclusions:** Cytolytic fCD8 T cells are able of killing infected cells, further justifying the use of the SIV model to develop new eradication strategies. Our results suggest that chronic immune activation is a major force driving the accumulation of fCD8, pointing to specific targets as mediators of the intra-follicular CD8 trafficking.

**95LB Repeated TLR7 Agonist Treatment of SIV+ Monkeys on ART Can Lead to Viral Remission****James B. Whitney**<sup>1</sup>; So-Yon Lim<sup>1</sup>; Christa E. Osuna<sup>1</sup>; Srisowmya Sanisetty<sup>1</sup>; Tiffany L. Barnes<sup>2</sup>; Tomas Cihlar<sup>2</sup>; Michael Miller<sup>3</sup>; Romas Geleziunas<sup>2</sup>; Joseph Hesselgesser<sup>2</sup><sup>1</sup>Beth Israel Deaconess Med Cntr/Harvard, Boston, MA, USA; <sup>2</sup>Gilead Sciences, Inc, Foster City, CA, USA; <sup>3</sup>Gilead Scis, Inc, Foster City, CA, USA

**Background:** The identification of pharmaceutical agents capable of safely reversing HIV-1 latency in ART-treated patients is urgently needed. We have previously reported that an oral TLR7 agonist GS-986, at doses that produce peripheral IFN- $\alpha$ , induces transient plasma viremia in SIV-infected rhesus macaques (RMs) on antiretroviral therapy (ART). In this follow up study, we assessed if lower clinically relevant doses of the TLR7 agonist GS-986 and the clinical compound GS-9620, which produce low or no detectable peripheral IFN- $\alpha$ , could induce transient plasma viremia or perturb viral reservoirs.

**Methods:** 11 RMs were infected with SIVmac251 and started on daily suppressive ART at 65 days post-infection. Virologic suppression ( $<50$  SIV RNA copies/mL) was achieved and maintained through 67 weeks. The cohort was divided into 4 groups receiving: (1) 19 doses of vehicle ( $n=2$ ), (2) 19 doses of 0.1mg/kg GS-986 ( $n=3$ ), (3) 19 doses of 0.05mg/kg GS-9620 ( $n=3$ ), or (4) 10 doses of 0.15mg/kg GS-9620 ( $n=3$ ) once every two weeks while maintaining ART. We longitudinally assessed plasma SIV RNA, total SIV DNA in PBMCs, lymph node (LN) and colon biopsies, and *ex vivo* SIV production from LN mononuclear cells and PBMC cultures stimulated with ConA.

**Results:** The first two TLR7 doses did not induce detectable plasma viremia. However, doses 3-10 led to transient but inconsistent production of plasma SIV RNA "blips" in all TLR7-treated RMs, while additional doses (11 to 19) did not induce SIV. After completing TLR7 dosing but prior stopping ART, SIV DNA levels were reduced in PBMC, colon and LN biopsies, and levels of *ex vivo* stimulated virus production were also diminished, with no such changes in the control group. To assess the impact of TLR7 treatment on viral reservoirs, ART was discontinued 2 weeks after the last TLR7 dose. Plasma virus rebound in 7 of the 9 TLR7-treated RMs was similar to that of the control group. However, two RMs having received either 19 doses of GS-986 or 10 doses of GS-9620 maintained undetectable plasma viral load for  $>60$  days after stopping ART. Prior to ART cessation, these same two RMs were negative for *ex vivo* virus induction.

**Conclusions:** Repeated low doses of TLR7 producing minimal to undetectable peripheral IFN- $\alpha$  in SIV-infected ART-suppressed RMs induced transient plasma viremia, decreased viral DNA levels, and delayed plasma virus rebound after stopping ART in some RMs. These data support the ongoing clinical testing of GS-9620 in HIV-infected persons on ART.

**96 Pharmacologic Measures of Adherence and Relationship to Drug Response****Peter L. Anderson**, Univ of Colorado, Denver, CO, USA

Non-adherence occurs in about 40% of patients on chronic therapies. In studies, it complicates interpretation of study outcomes and may lead to inaccurate conclusions regarding biological drug efficacy. One approach to deal with this is to incorporate pharmacologic monitoring for adherence assessment. This has become commonplace for PrEP studies and has been evaluated for other HIV-related disease states. This presentation will discuss lessons learned from pharmacologic measures of adherence in PrEP studies and their utility in interpreting study outcomes, including guidance about pharmacokinetic forgiveness, predicted efficacy for intermittent dosing strategies, and estimation of concentration-response relationships. Parallels will be drawn for other HIV-related disease states such as virologic breakthrough or development of drug resistance.

**97 HIV-1 Combinectin BMS-986197: A Long-Acting Inhibitor With Multiple Modes of Action**

**Mark Krystal**<sup>1</sup>; David Wensel<sup>2</sup>; Yongnian Sun<sup>1</sup>; Jonathan Davis<sup>2</sup>; Zhufang Li<sup>1</sup>; Thomas McDonagh<sup>2</sup>; Sharon Zhang<sup>1</sup>; Matt Soars<sup>1</sup>; Mark Cockett<sup>1</sup>; for the Combinectin Working Group  
<sup>1</sup>Bristol-Myers Squibb, Wallingford, CT, USA; <sup>2</sup>Bristol-Myers Squibb, Waltham, MA, USA

**Background:** Long-acting antiretrovirals could provide a useful alternative to daily oral therapy for HIV-1 infected individuals. However, the need for combination therapy and the potential for multiple IV/IM injections or tolerability issues may create roadblocks to this type of therapy. Adnectins are small proteins derived from the 10<sup>th</sup> type III domain of the human fibronectin protein that possess modifiable binding loops akin to the complementarity determining region of an antibody. Using Adnectins, we have developed the Combinectin inhibitor BMS-986197, a long-acting biologic with 3 independent and synergistic modes of HIV entry inhibition that potentially could be self-administered as a long-acting subcutaneous injection.

**Methods:** Adnectins targeting CD4 and a region of gp41 were isolated and optimized for antiviral potency and biophysical characteristics. The anti-gp41 Adnectin was joined at its amino terminus to the anti-CD4 Adnectin via a peptide linker. A third inhibitor, an alpha-helical peptide fusion inhibitor, was linked to the carboxy end of the anti-gp41 Adnectin via another linker. Finally, a human serum albumin (HSA) molecule was attached to amino terminus of the anti-CD4 Adnectin to optimize *in vivo* PK.

**Results:** The EC<sub>50</sub>s of the isolated anti-CD4 Adnectin, anti-gp41 Adnectin, and fusion inhibitor peptide were 8.5 nM, 5.4 nM, and 0.4 nM, respectively. Various synergies were obtained through linking all three inhibitors into a single molecule. Optimally combining the two Adnectins increased potency over 100-fold to ~30 pM. Addition of the fusion inhibitor peptide resulted in an increased resistance barrier compared to the separate components, as virus resistant to any one of the three components did not affect the potency of BMS-986197. Addition of HSA to the amino terminus decreased potency to 0.27 nM, but improved PK, as subcutaneous dosing in a cynomolgous monkey model produced a t<sub>1/2</sub> of 30 h. BMS-986197 has the biophysical characteristics and expression levels in stable cell lines compatible for further drug development, with a projected weekly subcutaneous human dose.

**Conclusions:** BMS-986197 is a novel recombinant biologic molecule containing three independent HIV inhibitors that has been developed as a potential single long-acting regimen for HIV-1. This molecule has the biophysical characteristics amenable for a self-administered subcutaneous weekly injection.

**98 Long-Acting Oral and Parenteral Dosing of MK-8591 for HIV Treatment or Prophylaxis**

**Jay Grobler**<sup>1</sup>; Evan Friedman<sup>2</sup>; Stephanie E. Barrett<sup>1</sup>; Sandra L. Wood<sup>1</sup>; Wendy Ankom<sup>1</sup>; Kerry L. Fillgrove<sup>1</sup>; Ming-Tain Lai<sup>1</sup>; Marian Gindy<sup>1</sup>; Marian Iwamoto<sup>3</sup>; Daria J. Hazuda<sup>1</sup>; for the MK-8591 Early Development Team

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**Background:** The potential to enhance adherence with less frequent dosing would represent a major advance for the treatment and prevention of HIV. MK-8591 is a nucleoside reverse transcriptase translocation inhibitor (NRTTI) with sub-nM potency that is in early stage clinical development. The phosphorylated anabolites of MK-8591, including the active triphosphate (MK-8591-TP), exhibit protracted intracellular persistence in human PBMCs and macrophages, protecting cells from infection in the absence of continued exposure *in vitro*. The potency, pharmacokinetic, and physical properties of MK-8591 are ideal for extended duration dosing.

**Methods:** MK-8591 efficacy was evaluated in a SIVmac251-infected rhesus macaques dosed once weekly (QW) with 1.3 to 18.2 mg/kg. Doses were chosen on the basis of rhesus PK and intracellular NTP levels designed to match levels associated with antiviral efficacy in PBMCs. Plasma viral loads and MK-8591 concentrations (measured pre-dose through day 42) were used to develop a PK/PD model and select ph1 doses for evaluation as QW oral dose in healthy subjects and enable dose selection for the development of long-acting parenteral formulations.

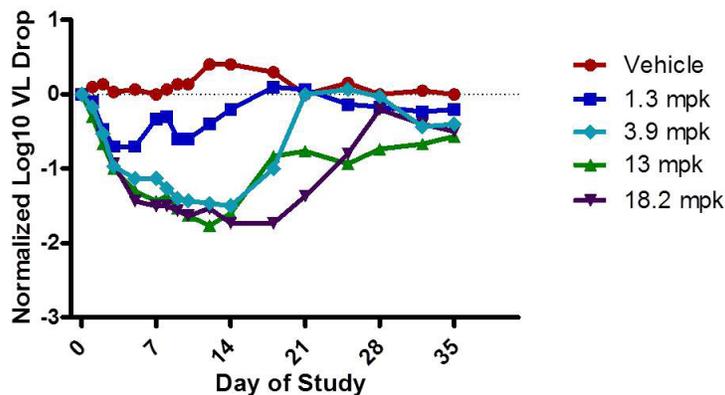
**Results:** Baseline SIV viral loads in monkeys ranged from 10<sup>6</sup> to 10<sup>8</sup> copies per ml. After administration of QW doses of 3.9 to 18.2 mg/kg MK-8591, monkeys with viral loads < 10<sup>8</sup> exhibited near maximal 2-log drops in viral loads and maintained suppression of viremia for at least 7 days. MK-8591-TP concentrations of ≥0.53 pmol/10<sup>6</sup> PBMCs were associated with QW efficacy.

In healthy volunteers, single doses of 10 mg and greater were able to achieve these levels of MK-8591-TP for at least 7 days, suggesting the potential for QW antiviral efficacy at a low dose.

Finally, long-acting parenteral formulations of MK-8591 exhibited continuous, extended-duration drug release in rodents with MK-8591 plasma levels comparable to those achieved in rhesus and humans and duration of release exceeding 6 months.

**Conclusions:** The antiviral efficacy in an SIV rhesus macaque model when dosed QW together with the human PK data, suggest the potential for MK-8591 QW oral dosing in the clinic and a low dose compatible with delivery via long-acting formulations. MK-8591 QW oral and long-acting parenteral formulations with potential for six months or longer duration would represent a potential paradigm shift as a single agent for prevention of HIV infection or as a component of an extended dosing regimen for HIV treatment.

**SIV-Infected Rhesus Treated with EFdA Dosed on D0 and D7 PO**



**99 Lower Ribavirin Exposures in HIV+ Patients That Relapsed to Acute HCV Treatment**

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**Background:** ACTG 5327 (SWIFT-C) Cohort I determined the efficacy of sofosbuvir (SOF) and weight-based ribavirin (RBV; 1000 or 1200mg daily) in 17 HIV-1 infected individuals with acute hepatitis C virus (HCV). The rate of relapse following 12 weeks of treatment was 41% in this Cohort. The objective of this analysis was to determine the contribution of RBV pharmacokinetics to viral relapse.

**Methods:** RBV plasma concentrations were determined using a validated LC/MS-MS method. RBV concentrations at weeks 4, 8, and 12 of treatment, RBV dose, IL28B genotype, ITPA phenotype, race, and antiretroviral (ARV) regimen were compared in those who achieved sustained virologic response (SVR) vs. relapse using the Mann-Whitney U test. Self-reported adherence was measured using a written four-day recall at each visit.

**Results:** Seventeen HIV-infected males (11 Hispanic, 6 Caucasian, mean  $\pm$  SD age 42.8 $\pm$ 10.6yrs, weight 75.9 $\pm$ 10.3kg, and CrCl 120.2 $\pm$ 27.3 mL/min, 11/2/1/3 HCV genotype 1a/1b/2b/indeterminate, 4/10/3 IL28B CC/CT/TT, 12/3/2 with 100%/60%/30% ITPA activity) received 12 weeks of SOF/RBV for acute HCV. No patient required a RBV dose reduction. Self-reported adherence to SOF/RBV was >95%. RBV concentrations are shown in the table. Median RBV exposures were 34% lower (p=0.01) at week 12 in those that relapsed vs. those that achieved SVR. The other variables tested were not significantly associated with relapse.

**Conclusions:** RBV exposures were lower at end of treatment in HIV-infected patients that relapsed to SOF/RBV therapy for acute HCV compared to those that achieved SVR. The cause of this discrepancy in exposures is unclear, but could relate to pharmacokinetic variability, a chance imbalance between groups, or differences in adherence despite the high levels of self-reported adherence.

	Relapse (median, range), (sample size)	SVR (median, range), (sample size)	Ratio of medians (relapse/SVR)	P-value
<b>Week 4</b>	1675 (632-3092) (n=7)	2005 (1623-4091) (n=10)	0.84	0.46
<b>Week 8</b>	2154 (655-3157) (n=7)	2393 (655-3378) (n=10)	0.90	0.26
<b>Week 12</b>	1745 (664-2504) (n=7)	2655 (1510-3603) (n=9)*	0.66	0.01

\*Week 12 concentration was missing for one patient. Results were similar when this subject's data was omitted from analyses at weeks 4 and 8.

**100 Drug Interaction Studies Between Sofosbuvir/Velpatasvir and Boosted HIV ARV Regimens**

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**Background:** A once-daily fixed-dose combination tablet composed of sofosbuvir (SOF; nucleotide analog NS5B inhibitor) and velpatasvir (VEL, GS-5816; pangenotypic NS5A inhibitor) is in clinical development for the treatment of chronic HCV infection. Phase 1 studies were conducted in healthy volunteers to evaluate potential drug-drug interactions (DDIs) between SOF/VEL and HIV antiretroviral (ARV) regimens containing a pharmacokinetic "booster" (RTV or COBI) to support their use together in HIV/HCV co-infected patients.

**Methods:** These were multiple-dose, randomized, cross-over DDI studies. Subjects received SOF/VEL and ARV regimens (EVG/COBI/FTC/TDF, FTC/TDF+DRV/r, FTC/TDF+ATV/r, FTC/TDF+LPV/r, and EVG/COBI/FTC/TAF) alone and in combination. Steady-state plasma concentrations of SOF, its predominant circulating nucleoside metabolite GS-331007, VEL, and ARVs were analyzed on the last day of dosing for each treatment and PK parameters were calculated. Geometric least-squares means ratios and 90% confidence intervals (combination vs. alone) for SOF, GS-331007, VEL, and ARV AUC<sub>tau</sub>, C<sub>max</sub>, and C<sub>tau</sub> were estimated and compared against lack of PK alteration boundaries of 70-143% for all analytes. Safety assessments were conducted throughout the study.

**Results:** 123 of 129 enrolled subjects completed the studies; 5 subjects withdrew consent and 1 discontinued due to pregnancy. The majority of adverse events (AEs) were Grade 1 and there were no discontinuations due to AEs and no serious AEs. SOF AUC increased 37% with EVG/COBI/FTC/TAF, decreased ~30% with FTC/TDF+DRV/r or LPV/r, and was unchanged with other regimens. GS-331007 AUC increased 48% with EVG/COBI/FTC/TAF and was unchanged with other regimens. VEL AUC increased 58% with EVG/COBI/FTC/TAF, increased 142% with FTC/TDF+ATV/r, and was unchanged with other regimens. No clinically significant changes in the PK of EVG, COBI, DRV, ATV, LPV, RTV, FTC, and TAF were observed when administered with SOF/VEL. Increased TFV exposure (~40%) was observed with SOF/VEL when administered as TDF. No increase in TFV exposure was observed when administered as TAF.

**Conclusions:** Study treatments were generally well tolerated. Results from this study demonstrate that SOF/VEL may be administered with EVG, COBI, DRV/r, ATV/r, and LPV/r with a backbone of FTC/TDF or FTC/TAF. The safety and efficacy of SOF/VEL and ARVs will be evaluated in clinical studies of HIV/HCV coinfecting subjects.

**101 EFV Reduced PK of Piperaquine for Malaria Prevention in HIV+ Ugandan Pregnant Women**

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**Background:** Improving health of HIV+ pregnant women living in sub-Saharan Africa requires strategies combating both HIV and comorbidities including placental malaria. Monthly chemoprevention for malaria with dihydroartemisinin(DHA)-piperaquine(PQ) is currently under study. For HIV+ pregnant women, DHA-PQ is administered with combination ART (cART), primarily EFV-cART as per 2013 WHO ART guidelines, without prior data on drug interactions, even though PQ is cytochrome p450 metabolized. We have included pharmacokinetic (PK) into our trials in Tororo, Uganda and report PK results for PQ with EFV cART to inform dosing guidelines.

**Methods:** PROMOTE includes double blind, placebo controlled studies investigating DHA-PQ for malaria prevention in HIV- and HIV+ pregnant women (NCT02163447 and NCT02282293, respectively). Women are enrolled from 12-20 (HIV-) or 12-28 (HIV+) wks gestation. Those randomized to DHA-PQ receive a standard regimen (qd x 3d) either monthly or bimonthly (HIV-) or monthly only (HIV+). PK of DHA-PQ was studied using an intensive design in a subset of women at 28 wks gestation with comparisons made between HIV- (no cART) and HIV+ (EFV-cART). The area under the concentration-time curve (AUC) was measured over 21d via venous (0-8 hr) and capillary plasma on Day 3,4,7,14 and 21 with correlation studies allowing conversion of all PK to venous plasma. Samples were measured by LC tandem MS. EKGs were performed at baseline and at time of peak (C<sub>max</sub>) levels.

**Results:** 30 HIV- women (13 DHA-PQ monthly; 17 DHA-PQ bimonthly), median age 23 yrs, and 17 HIV+ women (all DHA-PQ monthly), median age 28 yrs, provided PK results. We found highly significant decreases for HIV+ women compared to HIV- women in PQ AUC (GMR:0.62-0.68) and Day 7,14 and 21 levels (GMR:0.21-0.40). Limiting the comparison to

women who received monthly DHA-PQ only, an even greater decrease for HIV+ women was found. For all women, mean PQ on Days 14 and 21 were consistently <10 ng/mL, lower than previously estimated for effective chemoprevention (30 ng/mL), and for HIV+ women, Day 7 values were also <10 ng/mL. No significant link between QTc change or QTc at C<sub>max</sub> with either C<sub>max</sub> or AUC was found.

**Conclusions:** EFV results in significant reductions in PQ exposure in HIV+ pregnant women as measured by AUC with terminal concentrations persistently lower than previously suggested thresholds. Clinical correlates of these lower levels need to be determined, and dose escalation strategies may merit study.

Table: Pharmacokinetics of PQ in HIV+ and HIV- pregnant women receiving DHA-PQ as chemoprevention

Parameters	HIV+	HIV-	GMR	HIV-	GMR
	monthly DHA-PQ	monthly or bimonthly DHA-PQ		monthly DHA-PQ	
	GM; 95%CI (n=17)	GM; 95%CI (n=30)		GM; 95%CI (n=13)	
C <sub>max</sub> (ng/mL)	359 (275, 468)	391 (323, 474)	0.918	339 (249, 462)	1.06
t <sub>1/2</sub> (hr)	133 (106, 168)	185 (163, 211)	0.719*	191 (148, 247)	0.696
AUC <sub>0-21d</sub>	7.75 (6.14, 9.79)	11.4 (9.64, 13.6)	0.680**	12.8 (10.2, 16.2)	0.605**
AUC <sub>0-6h</sub>	8.09 (6.40, 10.2)	13.0 (11.0, 15.4)	0.622**	15.0 (12.2, 18.5)	0.539**
C <sub>7d</sub>	7.28 (5.43, 9.76)	18.3 (14.7, 22.8)	0.398***	21.8 (14.6, 32.4)	0.334**
C <sub>14d</sub>	2.68 (1.93, 3.72)	7.00 (5.41, 9.05)	0.383***	9.44 (6.68, 13.3)	0.284**
C <sub>21d</sub>	1.05 (0.579, 1.89)	5.09 (4.19, 6.17)	0.206***	6.43 (4.79, 8.65)	0.163***

GM: geometric mean; CI: confidence interval; GMR: geometric mean ratio (BC2/BC1). AUC unit is hr\*ng/mL; concentration unit is ng/mL. C<sub>max</sub> is maximum concentration; t<sub>1/2</sub> is half-life; C<sub>7d</sub>, C<sub>14d</sub>, and C<sub>21d</sub> are conc on day 7, 14, and 21 post-1st dose of a 3-day DHA-PQ dose. \*p<0.05; \*\*p<0.005; \*\*\*p<0.0001

**102LB Concentrations of TFV and TFVdp in Female Mucosal Tissues After a Single Dose of TAF**

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**Background:** The administration of oral tenofovir disoproxil fumarate (TDF) results in 100-fold higher exposure of tenofovir (TFV) and its active moiety, tenofovir diphosphate (TFVdp), in colorectal tissues compared to female genital tract tissues. This may contribute to PrEP adherence forgiveness in MSM compared to women. Tenofovir alafenamide (TAF), a novel TFV prodrug, achieves higher concentrations of TFVdp in peripheral blood mononuclear cells (PBMCs) compared to TDF. We sought to characterize genital and rectal tissue pharmacokinetics (PK) in women after a single dose of TAF.

**Methods:** A phase I PK study to describe TAF, TFV, and TFVdp exposure over 14 days in blood and tissues was conducted in 8 healthy women given one 25mg dose of TAF. Each participant provided 10 plasma, 9 PBMC, 9 cervicovaginal fluid (CVF) samples, and 2 biopsies from the cervix, vagina, and rectum. TAF, TFV, and TFVdp concentrations were determined by validated LC-MS/MS methods. TAF lower limit of quantification (LLOQ) in plasma was 0.05ng/ml and 0.005ng/ml in tissues. TFVdp LLOQ was 0.02ng/ml in tissues and PBMCs. TFV LLOQ was 0.25ng/ml in plasma and 2ng/ml in CVF. Noncompartmental PK analysis was conducted using WinNonlin v6.4. 48h PK parameters, from an earlier single 300mg dose of TDF in healthy women were used as reference values. Data are reported as median (min-max) except for tissues where pooled sample analysis was used.

**Results:** PK data are listed in the table. In plasma, compared to TDF, the area under the TFV concentration time curve (AUC)<sub>0-48hrs</sub> was 95% lower with TAF. By 6h, TAF was mostly unquantifiable in plasma, with an AUC<sub>0-6hrs</sub> of 38 (20.5-119.6) ng<sup>2</sup>h/ml. TAF was undetectable in tissue. Conversely, in PBMCs, TFVdp AUC<sub>0-48hrs</sub> was 808% higher with TAF. In all mucosal tissues TFV AUC<sub>0-48hrs</sub> was 20-90% lower with TAF. TFVdp was detectable in only 2 vaginal and cervical (12.5%) and 4 rectal (25%) tissue samples of women dosed with TAF, compared to 50%, 100%, and 100% for TDF, respectively.

**Conclusions:** After TAF dosing, plasma TFV PK and PBMC TFVdp PK were consistent with previous reports. Unlike TDF dosing, TFVdp was undetectable in most (83%) tissues after TAF dosing. Although the appropriate biomarkers of HIV protection for PrEP are currently unknown, these biopsy findings warrant further investigation.

	TAF 25mg		TDF 300mg	
	TFV	TFVdp	TFV	TFVdp
Plasma TFV	N=48, 0% BLQ		N=103, 0% BLQ	
AUC <sub>0-48hrs</sub> (ng*hr/ml)	92.1 (58.9-126)		1,777.8 (1,402.2-3,380.8)	
PBMC TFVdp	N=40, 0% BLQ		N=54, 2% BLQ	
AUC <sub>0-48hrs</sub> (hr*fmol/10 <sup>6</sup> cells)	3,977.9 (2,551.1-6,979.1)		437.8 (265.3-675.3)	
CVF TFV	N=40, 57.5% BLQ		N=95, 23% BLQ	
AUC <sub>0-48hrs</sub> (ng*hr/ml)	383.3 (110.9-2165)		5,082.8 (1,169.8-15,525.1)	
Cervical Tissue	TFV N=8, 12.5% BLQ	TFVdp N=8, 75% BLQ	TFV N=8, 0% BLQ	TFVdp N=8, 50% BLQ
AUC <sub>0-48hrs</sub> (ng*h/g or fmol*h/g)	382.5	Unable to calculate	994.6	113,373.2
Vaginal Tissue	TFV N=8, 0% BLQ	TFVdp N=8, 75% BLQ	TFV N=8, 0% BLQ	TFVdp N=8, 0% BLQ
AUC <sub>0-48hrs</sub> (ng*h/g or fmol*h/g)	620.9	Unable to calculate	777.8	224,437.5
Rectal Tissue	TFV N=8, 0% BLQ	TFVdp N=8, 62.5% BLQ	TFV N=8, 0% BLQ	TFVdp N=8, 0% BLQ
AUC <sub>0-48hrs</sub> (ng*h/g or fmol*h/g)	3,790	137,750.9	38,493.1	2,046,546

**103 HPTN 069/ACTG 5305: Phase II Study of Maraviroc-Based Regimens for HIV PrEP in MSM**

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**Background:** Maraviroc (MVC) is an HIV entry inhibitor that concentrates in the genital tract/rectum and can be given orally once-daily, making it a possible alternative PrEP agent.

**Methods:** Prospective, randomized, double-blinded, multisite, safety/tolerability study of 4 regimens for HIV PrEP: (1) MVC alone; (2) MVC + emtricitabine (FTC); (3) MVC + tenofovir (TDF); (4) TDF + FTC. Study regimens consisted of 3 pills once-daily -- MVC 300 mg, FTC 200 mg, TDF 300 mg, with matching placebos. Eligible participants (pts) were adult HIV-uninfected men who reported a history of condomless anal intercourse with  $\geq 1$  HIV-infected or unknown-status man within 90 days, and had adequate safety laboratory parameters including calculated creatinine clearance  $\geq 70$  ml/min. Pts received randomized study regimens for 48 weeks with follow-up visits at weeks 2, 4, 8, and then every 8 weeks. At each study visit, interval history, physical exam, safety laboratories, blood plasma for drug levels, and HIV and adherence counseling and testing were conducted. All analyses were intent-to-treat; primary analyses used Kaplan-Meier survival analysis and comparisons between study arms used chi-square, t-test, or log-rank testing.

**Results:** 12 HPTN and ACTG sites enrolled 406 men with a median age of 30 (range 18-70; 30% <26), including 28% black, 62% white, and 10% other races; with 22% Latino. 340 (84%) completed study follow-up; 38 (9%) were lost to follow-up. 37 (9%) permanently discontinued the study regimen prior to 48 weeks; time to discontinuation did not differ among the study arms ( $p=0.6$ ). The rates of grade 2-4 adverse events did not differ in pairwise comparisons between the study arms ( $p>0.5$ ). Calculated creatinine clearance decreased 3-8 ml/min from baseline to week 48, without differences among the study arms ( $p=0.6$ ). In a random subset of participants ( $n=122$ ) at random study time points, 93% had detectable study drug plasma levels. 5 HIV seroconversions occurred: 2 had no detectable study drug levels at any study visit (1 on MVC alone, 1 on MVC+TDF), 3 others (all on MVC alone) had MVC levels of 0.7, 6.7, and 145 ng/ml (limit of quantification 0.5) at the documented seroconversion study visit; all had R5 virus and none had genotypic resistance.

**Conclusions:** Given as HIV PrEP in MSM, MVC-based regimens were comparably safe and well-tolerated versus the control regimen of TDF+FTC. Of 5 seroconversions, 4 were associated with low or undetectable drug levels. MVC regimens may be alternatives for oral PrEP.

**104 PrEP Impact on T-Cell Activation and Explant Infection: HPTN 069/ACTG 5305 Substudy**

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**Background:** Studies of maraviroc (MVC) intensification in HIV-infected individuals have suggested that exposure to MVC is associated with increased gut-associated lymphoid tissue (GALT) T cell activation as well as increased HIV-coreceptor (CCR5) expression. Neither of these outcomes would be desirable for a PrEP regimen and so a tissue substudy was added to the HPTN 069/ACTG A5305 study to evaluate GALT responses to four antiretroviral (ART) regimens (MVC, MVC + emtricitabine (FTC), MVC + tenofovir (TDF), and TDF + FTC) and to determine whether ART exposure was associated with suppression of *ex vivo* / *in vitro* colorectal explant HIV infection.

**Methods:** CCR5 genotype was characterized from blood sample derived DNA using PCR. Participants received 48 weeks of ART. Colorectal tissue was collected by flexible sigmoidoscopy at Baseline, +24 weeks, +48 weeks, and +49 weeks. Biopsies were enzymatically digested using 2-3 rounds of collagenase II and mechanical disassociation. Mucosal mononuclear cells were stained with antibodies to cell surface markers (CD45, CD3, CD4, CD8, CCR5, CXCR4, CD38, HLA-DR, and CD69) and the Ki-67 intracellular activation marker and run on a BD LSRFortessa flow cytometer. Four biopsies from each participant/per time point were challenged with  $10^5$  TCID<sub>50</sub> of HIV-1<sub>BAL</sub> for two hours, washed, and incubated for 2 weeks with supernatant collection at Days 4, 7, 10, and 14 ( $\pm 1$  day). Day 14 supernatant HIV-1 p24 was quantified using the Alliance assay.

**Results:** Of the 55 men enrolled in the tissue substudy (MVC [ $n=13$ ]; MVC + FTC [ $n=19$ ]; MVC + TDF [ $n=13$ ]; and TDF + FTC [ $n=10$ ]), 51 participants were CCR5 wild type and four were heterozygotes (MVC + FTC [ $n=2$ ]; TDF + FTC [ $n=2$ ]). There were no significant differences in CD4 T cell activation phenotypes (CD38, HLA-DR, CD69, or Ki-67) between Baseline and Week 24/48 samples or in CCR5 phenotype in any study arm. While significant Day 14 explant viral suppression was seen between Baseline and Week 24 with all the PrEP study regimens, at Week 48 no significant suppression was seen in samples from those randomized to MVC alone.

**Conclusions:** Increased GALT T cell activation or CCR5 phenotype was not seen in any of the study arms. The explant data suggest that MVC alone may be less effective than combination ART regimens. These observations need to be correlated with pharmacokinetic adherence data. It is also possible that reduced explant viral suppression with MVC may be due to previously reported limitations of testing MVC in the explant model.

	Day 14 explant supernatant HIV-1 p24 (pg/mL)					
	Baseline		Week 24		Week 48	
	N	Mean (SD), Median	N	Mean (SD), Median	N	Mean (SD), Median
MVC only	3	14179 (10596), 12015	10	4977 (5023), 3535*	9	6085 (4000), 8082
MVC+FTC	21	8676 (8610), 5951	18	1679 (5218), 2*	18	357 (888), 1**
MVC+TFV	3	12530 (10664), 10420	12	1419 (4118), 8*	9	502 (1212), 0**
FTC+TFV	12	6431 (3779), 7471	12	2 (4.0), 0*	12	33 (98), 1**

\*Baseline versus Week 24 ( $p < 0.05$ ), \*\*Baseline versus Week 48 ( $p < 0.05$ )

**105 Cabotegravir Long-Acting Injection Protects Macaques Against Intravenous Challenge**

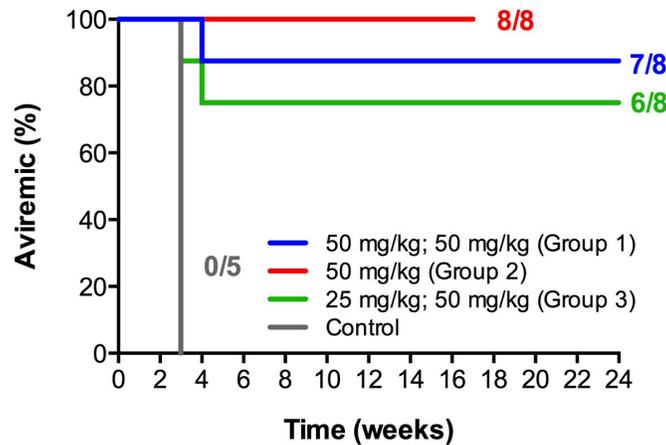
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**Background:** Cabotegravir (CAB; GSK1265744 or GSK744) long-acting (LA) is an INSTI formulated as a 200 mg/mL injectable nanoparticle suspension. CAB LA is an effective pre-exposure prophylaxis (PrEP) agent against rectal and vaginal SHIV exposures in macaques. This study was performed to evaluate the effectiveness of CAB LA as PrEP against intravenous (IV) SIV challenge in a model that mimics blood transfusions based on the per-act probability of infection.

**Methods:** Three groups of rhesus macaques ( $n=8$ /group) were injected IM with CAB LA and challenged IV with 17 AID<sub>50</sub> SIVmac251 on week 2. Group 1 was injected with 50 mg/kg on week 0 and 4 to evaluate the protective efficacy of the CAB LA dose used in macaque studies mimicking sexual transmission. Group 2 was injected with 50 mg/kg on week 0 to evaluate the necessity of the second injection of CAB LA for protection against IV challenge. Group 3 was injected with 25 mg/kg on week 0 and 50 mg/kg on week 4 to correlate CAB plasma concentrations at the time of challenge with protection. Five additional macaques remained untreated as controls. Infection status was monitored by real-time PCR amplification of viral *gag* sequences from plasma obtained weekly. Plasma CAB concentrations were measured by HPLC-MS/MS.

**Results:** Plasma vRNA was detected in all five control macaques one week after challenge (see figure). Two 50 mg/kg doses of CAB LA resulted in 7 of 8 macaques remaining aviremic through week 24 ( $p=0.0047$ ; Fisher's exact test). All 8 macaques given a single 50 mg/kg CAB LA dose remained aviremic through week 17 ( $p=0.0008$ ; Fisher's exact test). Six of the 8 macaques given a 25 mg/kg dose followed by a 50 mg/kg dose of CAB LA remained aviremic through week 24 ( $p=0.021$ ; Fisher's exact test). The mean plasma CAB concentration in the macaques that remained aviremic was 2.58 (range 1.00 to 5.56;  $n=21$ )  $\mu$ g/mL compared with 1.17 (range 0.67 to 1.93;  $n=3$ )  $\mu$ g/mL for the animals that became infected ( $p=0.0524$ ; t-test).

**Conclusions:** In an IV challenge model, 21 of the 24 CAB LA-treated macaques remained aviremic resulting in 88% protection. The plasma CAB concentration at the time of challenge is more important for protection than sustaining plasma concentrations with the second CAB LA injection. These results support the clinical investigation of CAB LA as PrEP in people who inject drugs.



**106 ÉCLAIR: Phase 2A Safety and PK Study of Cabotegravir LA in HIV-Uninfected Men**

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**Background:** Cabotegravir (CAB, GSK1265744) is an INSTI in development as a long-acting (LA) injectable for treatment and prevention of HIV-1. ÉCLAIR evaluated CAB LA injections for HIV PrEP. Dose selection was based on Phase 1 studies of CAB LA and target trough concentrations of 4X PA-IC90 (0.664mg/mL), supported by animal studies of SHIV transmission.

**Methods:** Phase 2a, randomized, multicenter, double-blinded study in healthy males, excluding subjects at high risk of acquiring HIV-1 at screening. Subjects were randomized (5:1) to QD oral CAB 30mg or placebo (PBO) tablets for 4 weeks. Following a safety assessment, eligible subjects received gluteal IM injections of 800mg CAB LA or saline PBO Q12 weeks X3. Safety, tolerability, and PK results through three injection cycles (Week 41 primary endpoint) are presented.

**Results:** Of 127 subjects randomized (83% MSM 31% African-American and 15% Hispanic), 126 were treated (CAB: 105; PBO 21). Eighteen individuals withdrew from CAB during the study: Five during oral dosing, six after oral dosing but prior to injections, and seven during the injection phase. In total, Grade 2-4 adverse events (AEs) occurred in 13/21 (62%) PBO and 84/105 (80%) of CAB subjects. Of Grade 2-4 AEs, injection site pain (55/94, 59%) occurred most frequently for subjects receiving CAB LA. During the Oral Phase, 7/105 (7%) CAB subjects had AEs that led to withdrawal (WD) including 3 events each of neutropenia and blood CPK increased, and one event of fatigue. During the Injection Phase, injection intolerance led to WD in 4/94 (4%) CAB LA subjects (none attributed to individual AEs), and 1/21 (5%) of PBO subjects had an AE (HIV-1 seroconversion) that led to WD. Through Week 41, 14/105 (13%) of CAB and 1/21 (5%) of PBO subjects had Grade 2-4 drug-related lab abnormalities. Geometric mean CAB Ct values ranged from 0.302-0.387mg/mL; AUC(0-t) ranged from 3415-4021mg-h/mL, with minimal accumulation upon repeat administration. Following repeat injections, 67/91 (74%) of subjects favored continuing CAB LA compared to Oral CAB.

**Conclusions:** CAB LA was well tolerated through Week 41 with a majority of subjects reporting satisfaction with CAB LA IM injections. A higher peak to trough fluctuation driven by faster absorption from the depot site resulted in mean Ct below the minimum target of 4x PA-IC90. An alternative dosing strategy of 600mg Q8 weeks is being investigated with the aim of achieving targeted exposure prior to conduct of Phase 3 efficacy trials.

	Placebo; n (%)	CAB; n (%)	
<b>Grade 2-4 AEs by Overall Frequency- Oral Phase (&gt;2 subjects in any arm)</b>	<b>N=21</b>	<b>N=105</b>	
Any Grade 2-4 AE	4 (19)	24 (23)	
Blood creatine phosphokinase increased	0	4 (4)	
Neutropenia	0	3 (3)	
<b>Grade 2-4 AEs by Overall Frequency-Injection Phase (&gt; 5% in any arm)</b>	<b>N=21</b>	<b>N=94</b>	
Any Grade 2-4 AE	10 (48)	75 (80)	
Injection site pain	1 (5)*	55 (59)†	
Pyrexia	0	7 (7)*	
Injection site pruritus	0	6 (6)*	
Injection site swelling	0	6 (6)*	
<b>Injection Site Pain (Grade 1-4)</b>	<b>12 (57)</b>	<b>86 (91)</b>	
<b>Select Grade 2-4 Chemistry Abnormalities- Maximum On Treatment</b>	<b>N=21</b>	<b>N=105</b>	
Any Grade 2-4 Chemistry Abnormality	7 (33)	32 (30)	
Aspartate Aminotransferase (IU/L)	0	5 (5)	
Bilirubin (UMOL/L)	0	4 (4)	
Alanine Aminotransferase (IU/L)	0	3 (3)	
*Subject was misdosed with CAB LA on 3rd injection (Grade 2 injection site pain was reported after 3rd injection)			
†51 (54%) of CAB subjects had Grade 2 events and 18 (19%) had Grade 3 events			
‡All Grade 2 AEs			
<b>Summary (Geomean, [95% CI], (CVb%)) of CAB PK by Injection Visit</b>			
Plasma PK Parameter	Injection 1 (n=93)	Injection 2 (n=89)	Injection 3 (n=85)
AUC(0-t) (µg•h/mL)	3415 [3140, 3714](42.5)	3873 [3543, 4235](44.3)	4021 [3728, 4337](36.2)
Cmax (µg/mL)	4.26 [3.64, 4.98](88.6)	5.22 [4.52, 6.04](78.0)	4.91 [4.31, 5.60](66.6)
Cτ (ug/mL)	0.302 [0.237, 0.385] (157)	0.331 [0.253, 0.435] (165)	0.387 [0.296, 0.505] (150)

**107 Chemoprophylaxis With Oral FTC/TAF Protects Macaques From Rectal SHIV Infection**Ivana Massud<sup>1</sup>; James Mitchell<sup>1</sup>; Darius Babusis<sup>2</sup>; Frank Deyoungs<sup>1</sup>; Adrian Ray<sup>2</sup>; James Rooney<sup>2</sup>; Walid Heneine<sup>1</sup>; Michael Miller<sup>2</sup>; **Gerardo Garcia-Lerma<sup>1</sup>**<sup>1</sup>CDC, Atlanta, GA, USA; <sup>2</sup>Gilead Scis, Inc, Foster City, CA, USA

**Background:** Tenofovir alafenamide (TAF) is a novel oral prodrug of tenofovir (TFV) that at low doses achieves ~90% lower plasma TFV exposure and increased intracellular TFV-diphosphate (TFV-DP) levels compared with 300 mg of tenofovir disoproxil fumarate (TDF). An investigational fixed dose combination of emtricitabine (FTC) and TAF (200/25 mg) (FTC/TAF) is currently under regulatory review for HIV treatment. If FTC/TAF will be as effective as FTC/TDF for HIV pre-exposure prophylaxis (PrEP) is not known. We investigated in macaques the efficacy of FTC/TAF in preventing rectal SHIV infection to determine the potential use for PrEP.

**Methods:** The prophylactic efficacy of FTC/TAF was investigated using a repeat low virus dose macaque model that predicted efficacy of oral FTC/TDF in humans. Rhesus macaques (n = 12) were exposed rectally once a week to low (10 TCID<sub>50</sub>) doses of SHIV<sub>162p3</sub> for up to 19 weeks. Animals were exposed to a first stock of SHIV<sub>162p3</sub> for up to 5 weeks and to a second stock of the same virus isolate for up to 14 additional weeks. Six animals received FTC/TAF (20/1.5 mg/kg) orally 24h before each virus exposure and 2h thereafter and 6 received saline as a placebo. Infection was monitored by serology and RT-PCR. Intracellular TFV-DP and FTC-triphosphate (FTC-TP) concentrations in PBMCs were measured in FTC/TAF-treated animals at first dose and 7 and 14 weeks later to evaluate drug accumulation.

**Results:** All 6 macaques that received FTC/TAF remained seronegative and viral RNA negative during the 19 virus challenges and a follow up period of 10 weeks. In contrast, all 6 placebo controls were SHIV RNA positive after a median of 8 (range = 1-15) exposures. The median (IQR) intracellular TFV-DP and FTC-TP concentrations in PBMCs at first dose were 554 (310, 870) fmols/10<sup>6</sup> cells and 1065 (912, 2459) fmols/10<sup>6</sup> cells, respectively. Levels accumulated approximately to 2-fold after 7 to 14 weeks of dosing (median 1365 (976, 1689) and 1626 (1311, 3252) fmols/10<sup>6</sup> cells of TFV-DP and FTC-TP, respectively).

**Conclusions:** We show that FTC/TAF prevents rectal SHIV infection in macaques to a degree similar to that previously found with FTC/TDF but with a substantially reduced dose. As expected, low TAF doses result in high intracellular TFV-DP concentrations in PBMCs with levels that exceed those previously seen with oral TDF. Our results in macaques suggest that FTC/TAF may be a feasible alternative to FTC/TDF for PrEP against rectal HIV infection.

**108LB MTN-017: Rectal Phase 2 Extended Safety and Acceptability Study of 1% Tenofovir Gel**Ross Cranston<sup>1</sup>; Javier Lama<sup>2</sup>; Barbra A. Richardson<sup>3</sup>; Alex Carballo-Diéguez<sup>4</sup>; Ratiya Kunjara Na Ayudhya<sup>5</sup>; Cindy Jacobson<sup>5</sup>; Mark A. Marzinko<sup>6</sup>; Sherri Johnson<sup>7</sup>; Jeanna Piper<sup>8</sup>; Ian McGowan<sup>1</sup><sup>1</sup>Univ of Pittsburgh Sch of Med, Pittsburgh, PA, USA; <sup>2</sup>Impacta Peru, Barranco, Lima, Peru; <sup>3</sup>Univ of Washington, Seattle, WA, USA; <sup>4</sup>New York State Psychiatric Inst., New York, NY, USA;<sup>5</sup>Magee-Womens Rsr Inst, Pittsburgh, PA, USA; <sup>6</sup>Johns Hopkins Univ, Baltimore, MD, USA; <sup>7</sup>FHI 360, Washington DC, DC, USA; <sup>8</sup>NIAID, NIH, Bethesda, MD, USA

**Background:** Men who have sex with men (MSM), and transgender women (TGW) are disproportionately affected by HIV. Safe and acceptable HIV prevention methods that target the site of initial rectal mucosal infection are needed.

**Methods:** MTN-017 was a Phase 2, three-period, randomized sequence, open-label, expanded safety and acceptability crossover study comparing rectally applied reduced-glycerin (RG) 1% tenofovir (TFV) and oral emtricitabine/TFV disoproxil fumarate (FTC/TDF). We enrolled healthy HIV-1 uninfected MSM and TGW ≥18 years at 8 sites. In each 8 week study period participants were randomized to RG-TFV rectal gel daily; or RG-TFV rectal gel before and after receptive anal intercourse (RAI) (or at least twice weekly in the event of no RAI); or took daily oral FTC/TDF. Participants were seen every 4 weeks. High product adherence was defined as >80% of expected doses taken, assessed by convergence scoring of daily texts and study product returns. Additionally, qualitative plasma TFV testing was done with results provided to participants at their next clinic visit. Generalized estimating equation models with exchangeable correlation structures and robust errors were used to compare safety, acceptability, and adherence between the three regimens.

**Results:** Participants (n=187) were recruited from the United States (4 sites, 42%), Thailand (2 sites, 29%), Peru (1 site, 19%), and South Africa (1 site, 10%) with mean age of 31.1 years (range 18-64). Twelve percent were TGW/women by self-report and 80% had a college education. There were no differences in Grade 2 or higher adverse event rates in participants using daily gel (Incidence Rate Ratio (IRR): 1.03, p=0.88) or RAI gel (IRR: 0.88, p=0.43) compared to FTC/TDF. High adherence was less likely during the daily gel regimen (Odds Ratio (OR): 0.35, p<0.001) and participants reported they would be less likely to use the daily gel regimen for HIV protection compared to FTC/TDF (OR: 0.38, p<0.001). Adherence to gel use at least twice weekly (RAI regimen) was similar to FTC/TDF (p=0.7) with no difference in intention to use product for HIV prevention (p=0.2).

**Conclusions:** Rectal application of RG TFV gel was safe in MSM and TGW. Similar adherence and intention to use product for HIV prevention was seen with gel applied at least twice weekly and FTC/TDF. Future topical rectal product development should focus on convenient dosing regimens.

**109LB A Phase III Trial of the Dapivirine Vaginal Ring for HIV-1 Prevention in Women**Jared M. Baeten<sup>1</sup>; Thesla Palanee-Phillips<sup>2</sup>; Elizabeth R. Brown<sup>3</sup>; Katie Schwartz<sup>4</sup>; Lydia E. Soto-Torres<sup>5</sup>; Annalene Nel<sup>6</sup>; Zeda Rosenberg<sup>7</sup>; Ian McGowan<sup>8</sup>; Sharon L. Hillier<sup>9</sup>; for the MTN-020/ASPIRE Study Team<sup>1</sup>Univ of Washington, Seattle, WA, USA; <sup>2</sup>Wits Reproductive Hlth and HIV Inst, Johannesburg, South Africa; <sup>3</sup>Statistical Cntr for HIV/AIDS Rsr & Prevention, Fred Hutchinson Cancer Rsr Cntr, Seattle, WA, USA; <sup>4</sup>FHI 360, Durham, NC, USA; <sup>5</sup>NIAID, NIH, Bethesda, MD, USA; <sup>6</sup>Intl Partnership for Microbicides, Paarl, South Africa; <sup>7</sup>International Partnership for Microbicides, Silver Spring, MD, USA; <sup>8</sup>Univ of Pittsburgh Sch of Med, Pittsburgh, PA, USA; <sup>9</sup>Magee-Womens Hosp of the Univ of Pittsburgh Med Cntr, Pittsburgh, PA, USA

**Background:** Antiretroviral medications used as prophylaxis can prevent acquisition of human immunodeficiency virus type 1 (HIV-1). However, in clinical trials among African women, HIV-1 incidence was not reduced because of low adherence to daily- or coitally-prescribed antiretroviral-containing pills and vaginal gels. Sustained drug-delivery products, including antiretroviral-containing vaginal rings, may improve adherence and provide protection against HIV-1 with lower systemic antiretroviral exposure.

**Methods:** We conducted a randomized, double-blind, placebo-controlled trial of a monthly vaginal ring containing dapivirine, a non-nucleoside HIV-1 reverse transcriptase inhibitor, among women aged 18-45 years in Malawi, South Africa, Uganda, and Zimbabwe. Dapivirine concentrations in plasma were measured in quarterly-collected samples; levels >95 pg/mL, a concentration nearly always achieved with >8 hours of use, were used to define adherence. Thus, plasma dapivirine could exclude those who were non-adherent but could potentially overestimate adherence if a ring was inserted only several hours before a visit.

**Results:** A total of 2629 women enrolled. Their median age was 26 years, median follow-up was 1.6 years, and women attended 91% of expected monthly visits. Among women assigned to the active dapivirine vaginal ring arm, approximately 80% had dapivirine detected in plasma. A total of 168 post-randomization HIV-1 infections occurred: 71 among those assigned the dapivirine vaginal ring (incidence 3.3 per 100 person-years) and 97 among those assigned the placebo ring (incidence 4.5 per 100 person-years). Compared to those assigned placebo, women assigned the dapivirine ring had a 27% (95% CI 1 to 46, p=0.046) relative reduction in HIV-1 incidence overall, a 37% (95% CI 12 to 56, p=0.007) reduction in an analysis excluding data from two sites with lower retention and adherence, and a 56% (95% CI 31 to 71, p=0.0003) reduction in an as-randomized analysis among women older than 21 years of age. Adherence was lower in women aged 18-21 compared to women older than 21. The rate of adverse medical events was similar between study arms.

**Conclusions:** A monthly dapivirine vaginal ring was safe and effective for HIV-1 prevention in African women. This multi-country study is the first to demonstrate HIV-1 protection for a sustained-release approach for delivery of an antiretroviral for HIV-1 prevention. HIV-1 protection was greater in as-randomized subgroups with evidence of better adherence to ring use.

**110LB Safety and Efficacy of Dapivirine Vaginal Ring for HIV-1 Prevention in African Women**

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**Background:** A monthly vaginal ring (Ring-004), containing 25 mg dapivirine, was evaluated for safety and efficacy against HIV-1 infection in two large Phase III clinical trials, IPM 027 and MTN-020. Higher than anticipated HIV-1 incidence was observed and final safety and efficacy analyses were performed for IPM 027 prior to the planned completion of this time-driven trial based on an independent DSMB recommendation. The final results of IPM 027 at the data cut-off point of 16 October 2015 are reported.

**Methods:** IPM 027 is a Phase III, multi-centre, double-blind, randomized, placebo-controlled trial, conducted at six research centers in South Africa, and one center in Uganda to assess the safety and efficacy of dapivirine vaginal ring, inserted once every 4 weeks over 24 months, in healthy, sexually active HIV-negative women, 18 to 45 years of age. The primary efficacy endpoint was the rate of HIV-1 seroconversion and the primary safety endpoint was incidence of adverse events (AEs).

**Results:** 1959 women (1762 in South Africa and 197 in Uganda) were randomized in a 2:1 ratio to receive either a dapivirine ring or a placebo ring. The median age at enrolment was 25 years, and 91% were unmarried. At the data cut-off point, the total number of person years of follow-up was 2805 and 761 women had completed the two year follow-up period. A total of 133 post-randomization HIV-1 infections occurred: 77 among women assigned to dapivirine ring (incidence 4.08 per 100 person-years) and 56 among women assigned to placebo (incidence 6.10 per 100 person-years). Dapivirine vaginal ring reduced the risk of HIV-1 infection by 30.7% (95% CI: 0.90-51.5%; p=0.0401) relative to placebo. A 37.5% (95% CI: 3.5-59.5%) reduction in HIV-1 infection was observed in a subgroup analysis of women older than 21 years. Product-related AEs included metrorrhagia, menometrorrhagia, pelvic discomfort/pain, suprapubic pain and application site pain. The rate of AEs, including product-related AEs, urogenital AEs, serious AEs and deaths, was similar between treatment arms. Additional analyses of adherence to product use as measured by dapivirine levels in plasma and residual dapivirine levels in used rings will be presented.

**Conclusions:** Dapivirine Vaginal Ring-004 was safe and effective in preventing HIV-1 infection via vaginal intercourse in women in sub-Saharan Africa and may be an important HIV prevention option for women at risk of HIV infection.

**111 Botswana Is Close to Meeting UNAIDS 2020 Goals of 90-90-90 Coverage**

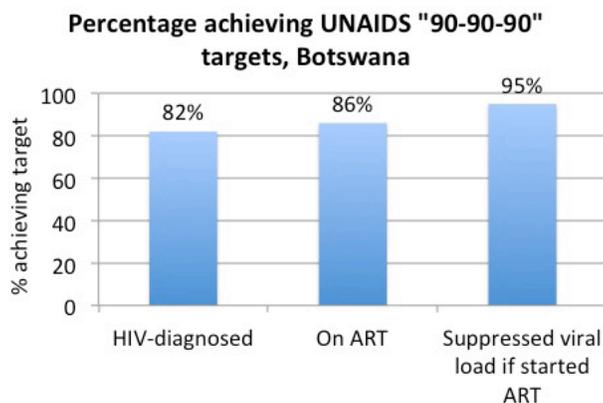
**Tendani Gaolathe**<sup>1</sup>; Kathleen Wirth<sup>2</sup>; Molly Pretorius Holme<sup>2</sup>; Joseph Makhema<sup>1</sup>; Sikhulile Moyo<sup>3</sup>; Eric Tchetgen Tchetgen<sup>2</sup>; Refeletswe Lebelonyane<sup>4</sup>; Lisa A. Mills<sup>5</sup>; M. Essex<sup>2</sup>; Shahin Lockman<sup>6</sup>  
<sup>1</sup>Botswana Harvard AIDS Inst Partnership, Gaborone, Botswana; <sup>2</sup>Harvard Sch of PH, Boston, MA, USA; <sup>3</sup>Botswana Harvard AIDS Inst Partnership, Gaborone, Botswana; <sup>4</sup>Ministry of Hlth, Gaborone, Botswana; <sup>5</sup>CDC, Gaborone, Botswana; <sup>6</sup>Brigham and Women's Hosp, Harvard Med Sch, Boston, MA, USA

**Background:** Many countries have experienced challenges with achieving high rates of HIV testing and treatment. Botswana may serve as a useful “demonstration case” in assessing the feasibility of approaching the new UNAIDS 2020 targets for 90% of HIV-infected persons knowing their status, 90% of these individuals receiving sustained ART, and 90% of those starting ART having undetectable HIV-1 RNA.

**Methods:** A population-based random sample of individuals was recruited and interviewed in 24 communities in Botswana from October 2013 to July 2015 as part of a large, ongoing PEPFAR funded pair-matched community-randomized trial designed to evaluate the impact of a combination prevention package on HIV incidence (the “BCPP” study). A random sample of 20% of households in each of these 24 communities was selected from a list of all households created using Google Maps. Consenting household residents age 16-64 years who were Botswana citizens were asked to participate in an individual questionnaire, and to have blood drawn for HIV testing in absence of written documentation of positive HIV status (and for HIV-RNA testing if HIV-infected, regardless of ART status).

**Results:** Seventy-nine percent of enumerated eligible household members took part in the survey (11% refused participation and 10% were absent). Among 9,780 participants, 2,727 (28%) were HIV-infected; 2,226 (82%) of the HIV-infected residents already knew their HIV status. Among those who knew their HIV status, 1,915 (86%) were receiving ART (this represented 95% of those eligible for ART by national guidelines). Overall, 70% of all HIV-infected persons were on ART. We obtained an HIV-1 RNA result in greater than 99% of HIV-infected. Of the 1,932 individuals who had already started ART (including 17 defaulters), 1,837 (95%) had HIV-1 RNA <400 copies/mL and 1,764 (91%) had HIV-1 RNA <40 copies/mL.

**Conclusions:** Botswana, a resource-constrained setting with high HIV prevalence, has achieved very high rates of HIV testing and treatment coverage. Rates of knowledge of HIV status, ART initiation, and virologic suppression are close to the UNADS 90-90-90 targets, at 82%, 86%, and 95%, respectively. Overall, 67% of HIV-infected persons had HIV-1 RNA<400 copies/mL, approaching the UNAIDS target of 73%.



**112 Streamlining Antiretroviral Therapy Uptake: A Stepped-Wedge Cluster Randomized Trial**

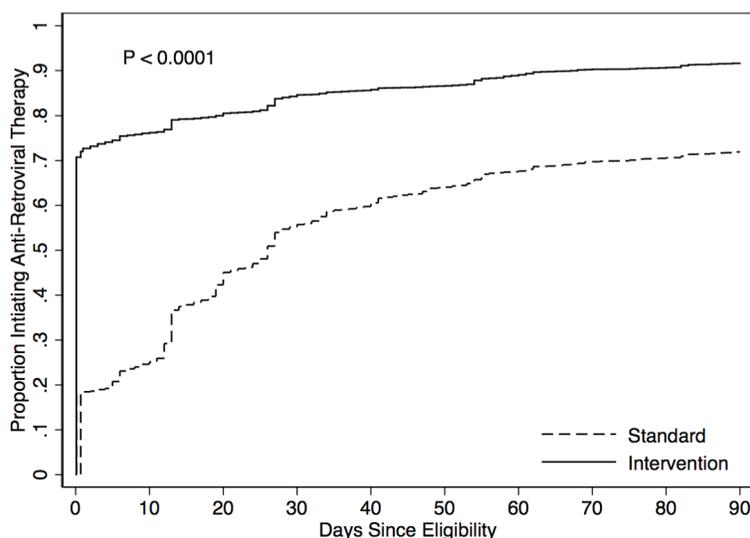
Gideon Amanyire<sup>1</sup>; Fred Collins Semitala<sup>1</sup>; Jennifer Namusobya<sup>1</sup>; Richard Katuramu<sup>2</sup>; Leatitia Kampiire<sup>2</sup>; Jeanna Wallenta<sup>3</sup>; David V. Glidden<sup>3</sup>; Moses R. Kamya; Diane V. Havlir<sup>3</sup>; **Elvin H. Geng**<sup>3</sup>  
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**Background:** In Africa, uptake of antiretroviral therapy (ART), even among clinically-eligible patients presenting for care, is often sub-optimal. Common reasons include sluggish diffusion of contemporary evidence about risks of delay (especially among symptomatic patients); overnight CD4 processing and therefore inefficient determination of treatment eligibility; and multiple pre-treatment adherence counseling norms.

**Methods:** We designed an intervention targeting prevalent barriers to ART uptake comprised of opinion-leader led teaching and coaching about risks of delayed initiation; introduction of a point-of-care CD4 testing platform (Alere PIMA); a revised counseling approach without mandatory multiple pre-initiation sessions and quantitative clinic feedback on ART initiation rates. We randomized twenty clinics in southwestern Uganda operated by the Ministry of Health and supported by a Ugandan NGO to the intervention in groups of five every six months. We evaluated all treatment eligible adults for ART initiation and a random sample for HIV RNA suppression one year after ART eligibility (defined as < 200 copies/mL). Mixed-effects logistic regression with a normal random effect for site and a fixed effect for intervention was used to estimate probability of ART initiation.

**Results:** Among 12,024 treatment eligible patients with a median CD4 level of 310/ $\mu$ l (IQR: 179-424), 79.6% met the primary outcome of ART initiation two weeks after eligibility in the intervention condition vs. 37.7% in the control condition (risk difference (RD): 41.9%, 95% CI: 40.1%-43.8%,  $p < 0.0001$ ) (Figure). Same-day ART initiation rose from 18.3% to 70.8% (RD: 52.5%, 95% CI: 50.7%-54.3%,  $p < 0.0001$ ). Among 414 patients randomly selected for HIV RNA measurement, when missing HIV RNA were counted as failure, 65.6% in the intervention were suppressed vs. 57.7% in the control (RD: 7.9%, 95% CI: -4.2% to 20.0%,  $p = 0.20$ ). Among the 335 patients (81%) in whom HIV RNA was successfully obtained, suppression was 86.2% in intervention and 70.6% in control condition (RD=15.6%, 95% CI: 4.4%-26.7%,  $p = 0.0078$ ).

**Conclusions:** A multi-component intervention targeting health care worker behavior doubled the probability of ART initiation 14 days after eligibility and improved HIV RNA suppression among those successfully measured. Implementation interventions can achieve rapid gains in the effectiveness and efficiency of real-world ART delivery systems and close gaps in the cascade of care.



**113LB A Randomized Trial to Accelerate HIV Care and ART Initiation Following HIV Diagnosis**

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**Background:** There is substantial attrition from HIV testing to initiation of care and antiretroviral therapy (ART). We tested three strategies to accelerate entry-into-care and ART initiation after testing positive at mobile HIV counselling and testing (HCT) units deployed in communities and workplaces in South Africa.

**Methods:** We conducted an unmasked individually randomized pragmatic trial. Following enrollment, participants were allocated equally into four arms: standard of care (SOC), point-of-care CD4 (POC CD4), POC CD4 plus strengths based care facilitation (CF), or POC CD4 plus transport reimbursement. Randomization was stratified by urban/rural status. POC CD4 count testing was accompanied by standardized counseling. CF consisted of five standardized sessions along with text messaging and ad hoc communication. Transport reimbursement was provided via cell phone transfer or at grocery stores. We assessed outcomes by self-report and by clinical documentation and calculated hazard ratios using Cox regression adjusted for randomization stratum. Here we present final results for the primary outcome of 90 day entry-into-care and a secondary outcome of 180 day ART initiation.

**Results:** We enrolled 2,558 participants, of whom 160 were excluded after randomization. Of the remaining 2398 participants, 1497 (62%) were women, the median age was 33 (IQR: 27, 41) years, and the median CD4<sup>+</sup> T-cell count (in arms offering POC CD4) was 427 cells/mm<sup>3</sup> (IQR: 287, 595). During the first 90 days following enrollment, 1,236 (52%) participants self-reported entry into care, with no difference by arm (Table). Overall, 764 (32%) participants had documented entry-into-care within 90 days to any of 90 clinics, and 371 (15%) had documented ART initiation within 180 days, with the POC CD4 + CF arm showing significant improvement relative to SOC (HR 1.4,  $p = 0.002$  and HR 1.4,  $p = 0.02$  for 90 day entry and 180 day ART, respectively).

**Conclusions:** POC CD4, with or without transport reimbursement or care facilitation, did not improve self-reported 90-day entry into HIV care. POC CD4 with strengths-based care facilitation did increase the secondary outcomes of clinically documented entry-into-care and ART initiation by 40%. While care facilitation could improve the HIV care continuum in South Africa, community and clinic-level strategies are likely also needed to achieve substantial increases in initiation of care and ART.

	SOC (n=591)	POC CD4 count (n=614)	POC CD4 + CF (n=603)	POC CD4 + transport (n=590)
<b>Entry-into-care by 90 days, self-report</b>				
n (%)	298 (50)	316 (51)	331 (55)	49 (49)
HR (95% CI)	1	1.0 (0.88, 1.2)	1.1 (0.94, 1.3)	1.0 (0.88, 1.2)
<b>Entry-into-care by 90 days, documented</b>				
n (%)	171 (29)	186 (30)	226 (38)	181 (31)
HR (95% CI)	1	1.0 (0.86, 1.3)	1.4 (1.1, 1.7)	1.1 (0.89, 1.3)
<b>ART initiation by 180 days, documented</b>				
n (%)	77 (13)	96 (16)	108 (18)	90 (15)
HR (95% CI)	1	1.2 (0.88, 1.6)	1.4 (1.1, 1.9)	1.2 (0.90, 1.6)

**114 Towards the Second UNAIDS Target: Population-Level ART Coverage in HPTN 071 (PopART)**

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**Background:** In 2014 UNAIDS set aspirational global targets for ART coverage for people living with HIV. Many countries currently fall below this target. HPTN 071 (PopART), a community randomised trial in Zambia and South Africa (SA), tests the impact on HIV incidence of a household-based combination HIV prevention approach provided by community-HIV-care-providers (ChiPs). ChiPs deliver universal testing; and for HIV-positive (HIV+) individuals support linkage to, and retention in, HIV care. ART is delivered through routine health care services. We present ART coverage after Round 1 of the intervention in 7 trial communities in which ART is offered to all HIV+ adults irrespective of CD4 count.

**Methods:** The first round of intervention was from November 2013 to mid-2015. Among adults who consented to participate, those included in analysis are all who either self-reported they were HIV+ or they were newly diagnosed by ChiPs with a rapid HIV test. Among these adults who are known by ChiPs to be HIV+, our main outcome is the number and percentage on ART by the end of Round 1, among those still resident in the community at the time of the last ChiP follow-up visit. As part of understanding how this outcome was achieved, we used "time to event" methods to estimate the time to start ART after first referral to HIV care by ChiPs.

**Results:** In Zambia 12,840, and in SA 3,300, adults were known by ChiPs to be HIV+ during Round 1. At the time of the first ChiPs' visit, in Zambia 6,249 (49%) and in SA 1,712 (52%) reported they were taking ART; 5,108 (40%) and 1,242 (38%) respectively were newly diagnosed with HIV. Among those not taking ART, in Zambia 6,197 and in SA 1,385 were referred to HIV care; from a time to event analysis, by 12 months later 58% and 53% respectively had initiated ART. At the end of Round 1, in Zambia 80% and in SA 83% of all the adults who were known to be HIV+ were still resident in the same area of the community; 1-2% died and others had moved within (~7%) or outside (~10%) the community. Among those still resident, in Zambia 71% of men and 72% of women, and in SA 58% of men and 69% of women, were taking ART by the end of Round 1.

**Conclusions:** Delivery of a home-based combination HIV intervention package conferred a substantial increase in population level ART coverage by the end of Round 1. The increase may be ongoing, as more adults link to HIV care, and as health promotion messages of universal treatment become more widely understood and accepted.

**115 Optimising South Africa's HIV Response: Results of the HIV and TB Investment Case**

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**Background:** South Africa's burden of disease due to HIV and TB is large, as is the public sector response. We were tasked by the South African government, the main funder of the HIV and TB programmes, to recommend the optimal mix of interventions to reach national and global HIV and TB targets under limited funding.

**Methods:** After a detailed review process, we selected 27 interventions, 9 factors increasing their technical efficiency, and 13 structural enablers impacting on HIV and/or TB, and parameterised an integrated suite of models including Thembisa, a local HIV transmission model, TIME Impact, a Spectrum-based TB transmission model, and a cost model. For HIV, we analysed the cost effectiveness of the programme at baseline and at current government targets, and developed novel optimisation methodology to identify the most cost-effective combination of interventions under two scenarios: a) the current budget envelope and b) the UNAIDS 90-90-90 targets. We combined each of these with two different TB scenarios: a) baseline and b) the TB 90-90-90 targets. Cost effectiveness was measured as cost per life-year saved over 20 years.

**Results:** Current government policy is relatively efficient but can be further improved (see Table). Under the current budget, the HIV programme could be optimised by scaling up cost-saving interventions (increasing condom availability and access to male medical circumcision and implementing social and behavioural change communication programmes that focus on increasing HIV testing uptake and discouraging multiple sexual partners) and spending the money thus saved on further scaling up ART. None of the technical efficiency factors except adherence clubs and home-based ART provision were found to be cost saving, and none of the examined structural enablers were able to compete with the other interventions on the basis of HIV endpoints- though both might be needed to reach the 90-90-90 targets. Results differ by province and district. Regarding the TB programme, targets will not be reached with HIV prevention and treatment alone; for this, a comprehensive package of TB and HIV prevention, intensified case finding, diagnosis and high quality treatment is required.

**Conclusions:** Overall, the total cost of the HIV programme will increase regardless of the choice of interventions, but this cost could decrease in the next 10 to 15 years. The total cost of the TB programme however could be reduced after only 5 years of high investments in both the HIV and TB programmes.

Result by scenario (HIV programme only)	Baseline	Government targets	Optimisation under	
			current budget	90/90/90 targets
<b>Life years saved [millions] (% change on baseline)</b>				
- 2015-2019	-	3.48 (16%)	2.14 (10%)	8.07 (37%)
- 2015-2034	-	-	10.8 (18%)	26.8 (45%)
<b>Total cost [billion 2014 USD]</b>				
- 2015-2019	10.6	11.4	11.2	13.7
- 2015-2034	59.8	-	58.7	64.6
<b>Incremental cost [billion 2014 USD] (% change on baseline)</b>				
- 2015-2019	-	0.9 (8%)	0.6 (6%)	3 (29%)
- 2015-2034	-	-	-1.2 (-2%)	4.8 (8%)
<b>Incremental cost [USD] per life year saved</b>				
- 2015-2019	-	238	283	364
- 2015-2034	-	-	Cost saving	179

**116 Virologic Efficacy of ART Begun at High CD4+ Counts via Streamlined Care in East Africa**

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<sup>1</sup>Makerere Univ-Univ of California San Francisco Rsr Collab, Kampala, Uganda; <sup>2</sup>Univ of California San Francisco, San Francisco, CA, USA; <sup>3</sup>Infectious Diseases Rsr Collab, Kampala, Uganda; <sup>4</sup>Makerere Univ Coll of Hlth Scis, Kampala, Uganda; <sup>5</sup>Kenya Med Rsr Inst, Kisumu, Kenya; <sup>6</sup>Univ of California Berkeley, Berkeley, CA, USA

**Background:** Overwhelming evidence supports ART for persons with high CD4 cell counts. However, treatment outcomes for adults with high CD4 counts in rural Africa have not been studied in large geographically diverse populations. We determined 48 week safety, retention in care, and viral suppression in adults with CD4≥350 in rural clinics in Uganda and Kenya using a nurse-driven streamlined ART delivery system.

**Methods:** At 16 rural Ugandan and Kenyan clinics (SEARCH Study: NCT01864603) all HIV+ individuals were offered ART (TDF/FTC+EFV) regardless of CD4+ count. We studied adults (≥15 years) with CD4≥350 who initiated ART from June 2013-June 2014. Streamlined care included: (1) nurse-driven triage and visits focused on symptom-based ART toxicity screening, (2) on-site nurse referral of complex cases to a physician, (3) a patient-centered care system, fostering a welcoming/supportive environment, (4) viral load (VL)

measurement and structured VL counseling, (5) provision of 3 months' ART refills, and (6) appointment reminders and patient tracking. Patients had visits at baseline, week 4 and 12, then every 12 weeks. VL was assessed at baseline, 24 and 48 weeks, along with basic safety laboratory tests, and 48-week virologic suppression was calculated as the % of persons with a measured week 48 VL  $\leq 500$  copies/mL (complete case analysis).

**Results:** Overall, 964 HIV+ adults with CD4  $\geq 350$  initiated ART. Median age was 33 years, 66% were female, median baseline CD4 was 607 cells/uL (IQR, 486-787/uL), and median baseline VL was 3.80 log copies/mL. At week 48, 874/964 (91%) of patients were retained (2 died, 28 moved, 8 withdrew, and 52/964 [5%] were lost to follow-up). Viral suppression was achieved in 748/800 (94%) with measured VL. Overall, grade III/IV clinical or laboratory adverse events (AE) were rare: 79 occurred in 63/964 (6.5%) patients. Top clinical AEs were fever (n=7) and dizziness (n=4). The most common laboratory AE was neutropenia (n=16, all asymptomatic). Overall, 9/964 (0.9%) persons switched ART from EFV to RTV/LPV (n=2 for dizziness, n=2 for gynecomastia, and n=5 for other reasons).

**Conclusions:** We found high viral suppression, retention in care and safety of ART delivered via a streamlined care system in East African adults with high CD4+ cell counts. These results amplify growing evidence that ongoing expansion of ART to asymptomatic adults with high CD4+ counts can be accomplished in rural resource-limited Sub-Saharan clinics using efficient, nurse-driven care models.

**117 HIV Mortality by Care Cascade Stage and Implications for Universal ART Eligibility**

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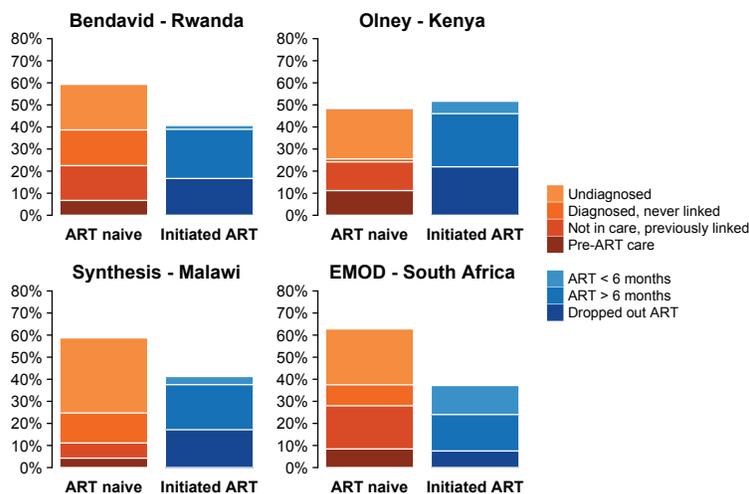
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**Background:** A decade after the scale up of ART in southern and eastern Africa, mortality rates among HIV-positive adults remain 3-6 times higher than in HIV-negative adults. Prioritising interventions for improving HIV care requires information about the care stages where most deaths arise, and thus where the greatest gains could be made. Immediate ART eligibility may fundamentally reshape the care cascade by removing the 'pre-ART care' stage where high dropout has been documented.

**Methods:** We reviewed empirical data and mathematical modelling estimates about mortality across stages of HIV care. Empirical estimates came from linked clinical and vital registration data from Western Cape, South Africa, and population cohorts in Uganda, Malawi, and South Africa. We used four mathematical models calibrated to HIV epidemics and care and treatment utilization in Rwanda, Kenya, Malawi, and South Africa. Models estimated the distribution of HIV deaths occurring at each stage of care and projected this over the next decade assuming continuation of current patterns of HIV care uptake and retention. Three models simulated the effects of changing guidelines to immediate ART initiation for all patients linked to care, assuming that retention would be similar to current levels.

**Results:** Only 10-30% of HIV-related deaths are estimated to occur among patients who are continuously on ART for 6 months or more. At present, the majority of HIV deaths occur among patients who did not initiate ART (Figure). Patients disengaging from ART have a high mortality rate, and models show that this will account for an increasing and substantial share of HIV deaths (21-44% in 2025). Assuming continuation of current care patterns, models projected that between 9% and 22% of HIV deaths from 2016-2025 would occur among patients who had linked to care but never initiated ART. Immediate ART initiation could reduce HIV deaths by between 6-14% over 2016-2025, mostly due to removing the opportunities to disengage before treatment initiation.

**Conclusions:** Even in settings with high ART coverage, the majority of HIV-related deaths are likely to continue to be among patients who are not on ART, rather than patients who are stable on ART. Programmes should continue to prioritise interventions to link and retain patients to and on ART. Universal eligibility for ART initiation may bring substantial benefits through the simplification in the care cascade.



**118 Trends in ART Discontinuation by Age in Malawi, 2004-2014**

**Catrina A. Mugglin<sup>1</sup>**; Andreas Haas<sup>1</sup>; Joep van Oosterhout<sup>2</sup>; Frank Chimbwandira<sup>3</sup>; Lyson Tenthani<sup>1</sup>; Malango Msukwa<sup>4</sup>; Oliver Gadabu<sup>5</sup>; Janne Estill<sup>1</sup>; Matthias Egger<sup>6</sup>; Olivia Keiser<sup>1</sup> <sup>1</sup>Inst of Social and Preventive Med, Univ of Bern, Bern, Switzerland; <sup>2</sup>Dignitas Intl, Zomba, Malawi; <sup>3</sup>Malawi Ministry of Hlth, Lilongwe, Malawi; <sup>4</sup>Inst of Social and Preventive Med, Univ of Bern, Lilongwe, Malawi; <sup>5</sup>Baobab Hlth Trust, Lilongwe, Malawi; <sup>6</sup>Univ Hosp Bern, Bern, Switzerland

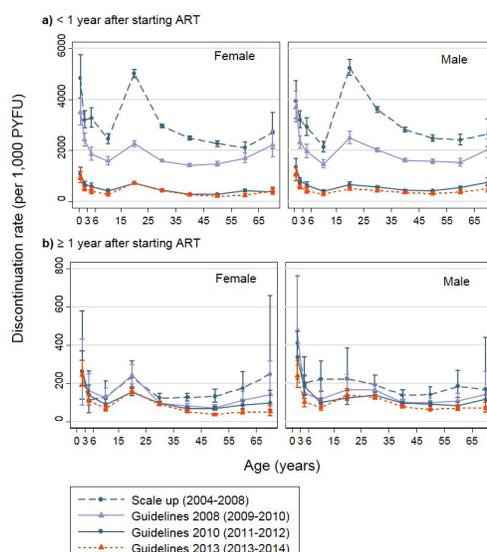
**Background:** In Malawi, the number of people alive on antiretroviral therapy (ART) increased from 10,761 in 2004 to 521,319 by the end of 2014. Long term outcomes remain incompletely understood. We analysed time trends of ART discontinuation rates across all age groups.

**Methods:** Our prospective cohort analysis of individual patient data from 20 large health care facilities with an electronic medical record (EMR) system in central and southern Malawi included data from all patients who started ART for the first time between 2004 and 2014. Follow-up time was split by age (10 groups, see Figure), calendar period according to changes in ART guidelines (4 periods, see Figure), and time since start of ART (<1 year,  $\geq 1$  year). We calculated time from ART initiation to discontinuation due to death, loss to follow-up (LTFU) or any other reason, or to database closure, whichever came first. LTFU was defined as patients not seen for >150 days after the first missed appointment. Patients with documented transfer out were excluded. Discontinuation rates and 95% Poisson confidence intervals were calculated per 1,000 person-years of follow-up (PYFU).

**Results:** Of 127,316 individuals included in the analysis 62% were female and the median age was 32 years. During 161,112 PYFU there were 75,472 discontinuation events (6,660 deaths, 68,685 LTFU, 127 other). Median follow up time for the  $\geq 1$  year analysis was 2.7 years (range 1-8 years). The Figure shows discontinuation rates by age and time period.

Rates were much higher (202–5234 events/1,000 PYFU) in the first year of ART than later on (35–475/1,000 PYFU). Discontinuation rates improved over calendar periods and the implementation of new ART guidelines, particularly during the first year of ART. Rates were highest for adolescents (15–24 years of age) and children (0–3 years). Although the peak in adolescence was evident in all calendar periods, it decreased over time. Rates were similar for female and male patients across all age groups. After excluding women on Option B+, the peak in female adolescents discontinuing treatment in the first year of ART almost disappeared (data not shown).

**Conclusions:** Discontinuation rates have improved dramatically over time but remain high in children and adolescents, particularly during the first year of ART. Future interventions should focus on retention of children and women on Option B+ in the first year of ART.



**Figure 1** Rates of discontinuation (95% CI) of ART by age group, calendar period and gender a) within one year of starting ART b) ≥ 1 year of starting ART (note different scale)

**119 Option B+: A Stepping Stone to Universal Treatment**

**Andreas Jahn**, Ministry of Hlth, Lilongwe, Malawi

Malawi has an estimated 1.1 million HIV infected population. Extreme financial and health system constraints have catalysed the development of highly standardized and simplified national guidelines and monitoring tools for HIV management. The program has relied on nurses for clinical staging and ART initiation since 2005. Quarterly supervision for all PMTCT/ART sites has been key for safe implementation of task shifting and decentralization to peripheral health centres.

In 2010, improved program data systems revealed low PMTCT coverage, attributed to unreliable CD4 count access, complex protocols and erratic supply of test kits and PMTCT ARVs. Option B+ was developed to address these challenges in the face of high pediatric HIV burden, short birth intervals and long breastfeeding. Implementation began in July 2011. Over 5000 health worker were retrained and the number of PMTCT/ART sites doubled to 595 within 9 months. The test & start policy for all HIV infected pregnant and breastfeeding women has led to a 47% increase in new ART initiations each quarter. By the end of 2015, 85,000 pregnant and 30,000 breastfeeding women had begun ART. ART population coverage among women increased rapidly, and half of all HIV infected women getting pregnant were already on ART.

Option B+ uptake and retention varies greatly between sites. Routine program data probably misclassify a considerable proportion of transferring patients as lost to follow-up. Operational studies from Malawi suggest that good public education and individual patient preparation/support are key for high uptake and retention among asymptomatic patients who may not see immediate health benefits from ART. ART start on the day of diagnosis has been successfully implemented under these conditions.

Malawi has refocused its HIV testing program and added several quality assurance mechanisms to reduce the risk of starting lifelong ART based on an inaccurate HIV rapid test result.

The routine system for quantification, ordering and distribution of essential medicines remains too weak and unresponsive to ensure uninterrupted supplies and to account for high value drugs such as ARVs. Malawi has built a highly efficient central supply management system for HIV commodities based on site level stock and service data verified during supervision.

Based on lessons learnt, Malawi will start a universal test & start policy for all PLHIV in 2016 and treatment coverage will approach the 90-90-90 target by 2020.

**120 Missing But in Action: Where Are the Men?**

**Helen Ayles**, London Sch of Hygiene & Trop Med, London, UK

In Sub-Saharan Africa men are under-represented at all stages in the cascade of HIV care. Men have a lower uptake of HIV testing, access care at later stages than women and have higher mortality than women. To realise the global targets of 90:90:90 we need to do much more to find men. In addition unless we reach near universal access we cannot hope to have any impact on reducing HIV incidence and ultimately stopping the HIV epidemic. From published and unpublished literature the gaps where men are failing to attain the 90:90:90 targets will be described and possible reasons for this will be discussed. In particular data will be presented from the HPTN 071 trial of combination prevention and universal test and treat where population level estimates of coverage in men is available in 21 communities in Zambia and South Africa. Various initiatives have been tested to improve the involvement of men at each stage of the treatment and prevention cascades. Some of these will be reviewed and the evidence of effect analysed. In order to reach universal coverage of HIV interventions, in generalised epidemics such as in sub-Sharan Africa, much more attention must be paid to men and their specific needs. If we fail to understand the barriers to accessing HIV care for men we will fail in our goal of ending the HIV epidemic.

**121 Antiretroviral Therapy for Life: Understanding and Improving Retention**

**Elvin H. Geng**, Univ of California San Francisco, San Francisco, CA, USA

Retention in HIV care can be thought of as the dynamic fit between the demand for treatment, as determined by a patient’s psychological, social, and economic characteristics, on the one hand and the accessibility, quality, and efficiency of health systems supplying HIV services on the other. Although recognition of the critical role of retention has led to rapid growth of research in this area, further insights depend on addressing both practical and conceptual barriers. First, while numerous studies have shown high rates of missed visits and loss to follow-up, greater clarity about retention requires both better data and different data. Documenting outcomes among patients ostensibly “lost” in

clinic-based administrative records, for example, can reveal the extent to which undocumented deaths (including deaths among patients who were well engaged) as well as “silent transfers” (undocumented movement across clinic settings) contribute to attrition in a particular setting. In addition, patient-provider communication and quality of care itself represent critical but under-explored dimensions of retention. Second, diverse barriers to retention have been documented in the literature and include depression, stigma, work demands, and long waiting times at clinic among many others. Application of qualitative and causal methodologies can elucidate the causal relationships between these factors, retention, and downstream outcomes such as productivity and HIV RNA suppression. Insight into this complex behavioral “anatomy” of retention can inform “retention 2.0” interventions. For example, exciting approaches seek to enhance patient activation, leverage social capital, and apply behavioral economics to create greater and more consistent demand for treatment. Health systems are evolving to use community based delivery strategies, mHealth and peers to enhance the accessibility, efficiency and quality of the supply of care. In addition, adaptive approaches offer the promise of “personalized public health.” Adaptive approaches seek to minimize expenditures where less is sufficient (optimizing efficiency), but intensify where more is needed (optimizing effectiveness). Insights from implementation science such as standardization of intervention specification, routine use of mixed methods, attention to implementation outcomes, and elucidating mechanisms to inform transportability can strengthen the next generation of research on retention in HIV care.

## 122 Innovations in Antiretroviral Therapy Delivery

**Anna Grimsrud**, *IAS, Cape Town, South Africa*

In September 2015, WHO announced “Treat all” recommending antiretroviral viral therapy (ART) for everyone living with HIV. The most recent UNAIDS estimates suggest that 15.8 million people are accessing ART and 36.9 million people globally are living with HIV. If we are to reach the ambitious “90-90-90” targets and more than double the treatment cohort, innovations in ART delivery are needed. There are a growing number of examples of differentiated models of ART delivery that provide quality, patient-centred care and in turn, free up the capacity of the health care system to support those most in need. Examples from the field will be presented, alongside data of their effectiveness, evidence of their broader implementation and some insights on what may be needed to support expansion and inclusion in national guidelines. While the principles of differentiation have primarily focused on simplifying models of care for the “stable” patient, the case will be made for their application across other groups of patients, in different contexts and for all sub-populations. Global funders, normative agencies, implementers and civil society acknowledge that innovations to service delivery are urgently required. However, questions remain about how to ensure quality care to the millions of people living with HIV, to what extent differentiated models lead to efficiency gains, and how models of ART delivery can support the HIV continuum of care and other chronic diseases.

## 123 Visceral Adiposity in the Modern HIV Treatment Era

**Grace A. McComsey**, *Case Western Reserve Univ, Cleveland, OH, USA*

Lipohypertrophy or gains in fat depots, the most concerning of which being visceral abdominal and ectopic fat, occur commonly after the initiation of treatment for chronic illnesses including HIV infection. Although incidence of lipodystrophy or peripheral fat loss was drastically attenuated by the avoidance of thymidine NRTI agents, a different picture is surfacing for lipohypertrophy. Recent data revealed that accumulation of visceral fat occur occurring with contemporary antiretroviral regimens perceived to be metabolically-friendly. These central fat changes are clinically important as they can predispose to cardiovascular disease and could have devastating consequences on quality of life, and importantly have been shown to predict mortality. There are limited data on the pathogenesis of central fat accumulation, and the advent of these changes with all antiretroviral classes should make us question the importance of HIV itself, directly or indirectly thru inflammation and immune activation. Associations with viral-specific factors will be therefore emphasized, and discussions of potential mechanisms will include specific dietary factors that could have major impact on gut barrier integrity. Finally, strategies to prevent or treat visceral fat accumulation will be discussed.

## 124 NAFLD and NASH in HIV Infection

**Elizabeth C. Verna**, *Columbia Univ, New York, NY, USA*

Liver disease is a leading cause of morbidity and mortality among persons with HIV, and in this era of safer and more effective hepatitis C therapy, non-alcoholic fatty liver disease (NAFLD) may emerge as the most common liver disease in this population. For a variety of reasons, NAFLD is extremely common in patients with HIV, and may be more likely to progress to its more ominous forms, including steatohepatitis (NASH) and NAFLD-related fibrosis or cirrhosis. Significant work has been done to identify mechanisms of NAFLD formation, including the influence of intestinal permeability as well as metabolic and immunologic factors, all which may be of particular importance in patients with HIV. Current treatment strategies are largely based upon modification of NAFLD risk factors, however several drugs are now being studied to assess both reduction in hepatic steatosis as well as reversal of fibrosis. Thus it is increasingly important for clinicians to accurately identify and stage patients with NAFLD in order to determine prognosis and evaluate them for available and future treatments.

## 125 Immunopathogenesis of Metabolic Complications in Treated HIV Infection

**Suzanne M. Crowe**, *Burnet Inst, Melbourne, Australia*

Despite effective antiretroviral therapy (ART), HIV-infected individuals remain at higher risk than the general population for several morbidities including metabolic complications. While innate immune activation declines during suppressive ART, it also persists in many individuals and may contribute to these metabolic complications. HIV persistence, microbial translocation, and co-infections may all contribute to persistent macrophage activation in tissues as well as both local and systemic inflammation. These immunologic perturbations may contribute to the risk of metabolic diseases as described in the other talks of this session. In some cases, metabolic derangements including oxidized lipoproteins may further contribute to immune activation and the inflammatory state. These processes may highlight several potential interventional targets to decrease the risk of metabolic complications in treated HIV infection.

## 126 What Exactly Does Antiretroviral Therapy Do to Bone?

**Patrick Mallon**; for the UCD HIV Molecular Research Group, *Univ Coll Dublin, Dublin, Ireland*

Both low bone mineral density (BMD) and fractures are prevalent in people living with HIV, with HIV an independent risk factor for low BMD in some studies. In addition, there is a well established association between exposure to antiretroviral therapy (ART) and loss of BMD, largely limited to the first year after initiation of either first-line or second line ART in viraemic patients. Although the extent of BMD loss varies depending on the ART regimen used, that the effect occurs with all ART suggests a role for both ART and host response to ART in its pathogenesis, with a temporary uncoupling of bone metabolism leading to discreet losses of BMD as a viable underlying explanation.

The pathogenesis underlying development of low BMD and osteoporosis in HIV is complex, with systemic effects from immunological and virological changes, disruptions to vitamin D metabolism and subclinical renal dysfunction all potentially implicated. Once the initial bone loss with ART initiation has occurred, the relative contribution of specific ART when compared to other factors to ongoing bone health remains debated, with significant gaps remaining in our knowledge of the natural history of changes in BMD, changes in underlying bone quality and ultrastructure and the association between these factors and fracture prevalence, type and severity, particularly in the setting of a persistently high bone turnover state.

Addressing these gaps should be a major research focus in an ageing population to prevent fractures, with innovative interventions focused on preserving bone health through optimising vitamin D status and exploratory use of bisphosphonates at ART initiation currently underway.

**127 Visualizing the Early Events of Antigen Recognition by B Cells****Facundo Batista**, *Francis Crick Inst, London, UK*

B lymphocytes form an integral part of the immune system via the production of specific antibodies and by establishing immunological memory enabling a swift and effective response to pathogenic assault. To fully understand the processes whereby this is achieved, it is essential to gain a comprehensive understanding of the events involved in B cell activation and antibody production. This is initiated by the encounter with and acquisition of cognate antigen by the B cell receptor (BCR); processes occurring primarily in secondary lymph nodes (SLOs) such as the lymph node (LN) and which are regulated by complex systems. The functionality of these systems is strictly dependent on maintaining the architecture of the SLOs. During this talk I will describe how the LN structure enables the early encounter of B cells with antigen. Antigenic properties such as the size and nature of the pathogen affect the specialized lymph node antigen delivery systems that exist in readiness to deliver pathogen-derived antigen to B cells. This variability in the types of encounter enables the most appropriate response for that particular antigen to occur, giving the maximal protection to the host. Lastly, the consequences of the disruption of the LN architecture on the immune response will also be described. Its critical role in the immune response is evident following infection, which causes a temporary disruption in the cellular organization of lymphoid organs leading to a reduction in immune responses against subsequent challenge until such time as the LN architecture is restored.

**128 Are Follicular Dendritic Cells a Reservoir for HIV?****Michael C. Carroll**, *Harvard Univ, Boston, MA, USA*

Lymph nodes (LN) which are the sentinels of the immune system, are the site where lymphocytes engage cognate antigen and become activated. How antigen localizes to the lymph node and is "seen" by B and T cells is still not entirely clear; although it is apparent that follicular dendritic cells (FDC) are essential for retention of B cell antigens in germinal centers. Recently, we identified a novel mechanism whereby FDC take-up complement opsonized immune complexes (IC) via the CD21 receptor and cycle the complexes in a non-degradative endosomal compartment (*Heesters et al 2013 Immunity*). Early studies identified Human Immunodeficiency Virus (HIV) viral particles associated with FDC in the B cell follicles; where they are bound via complement receptor 2 (CD21) and/or Fc Receptors; but are not thought to become productively infected. Whether FDC act as a reservoir for infectious virus has been debated in the literature for over two decades. We propose that FDC retain infectious HIV virus similar to that identified for complement opsonized IC. Despite the success of antiretroviral therapy (ART), it does not cure HIV as discontinuation of treatment results in viral rebound. We found that human FDC isolated from LNs of patients on ART retain infectious HIV within a non-degradative cycling compartment and transmit infectious virus to uninfected CD4+ T cells *in vitro*. Importantly, treatment of the HIV+ FDC with soluble complement receptor 2 Ig fusion protein (sCD21-Ig) purges the FDC of HIV virions and prevents viral transmission *in vitro*. Our results provide an explanation for how FDC can retain infectious HIV for extended periods and suggest a therapeutic strategy to limit this potential viral reservoir in HIV+ individuals.

**129 Imaging Lymphoid Tissues to Understand Viral Persistence and Impaired Function****Jacob D. Estes**, *Frederick Natl Lab, Leidos Biomed Rsr, Frederick, MD, USA*

A primary obstacle to curing HIV infection is the early establishment of long-lived viral reservoirs from which infection rebounds if antiretroviral therapy (ART) is interrupted. There is currently considerable effort directed to devising strategies to eliminate or greatly reduce these reservoirs so it would be possible to discontinue ART for extended or indefinite periods of time, referred to as a functional cure. To date, these strategies have largely been assessed by monitoring changes in T cell HIV reservoirs from peripheral blood (PB), but the lymphoid tissues (LT) are the major tissue compartments where latently and persistently infected cells reside and infectious virus persists on the follicular dendritic cell network (FDCn) in lymphoid follicles. Because HIV is primarily a disease of lymphoid tissues, a detailed understanding of HIV reservoir establishment and persistence is particularly difficult to study in humans but can be readily studied in SIV nonhuman primate (NHP) models. These NHP models have been invaluable in understanding the timing of viral seeding, viral dynamics and compartmentalization, and cell populations involved in viral persistence within LTs. These studies have highlighted B cell follicles in viral persistence both before and during ART, with infected T<sub>H</sub> cells and viral particles bound to the FDCn as potentially important cells harboring infectious virus. Novel strategies to purge virus from these tissues may be hindered by the pathologic damage to these immune organs induced by persistent chronic inflammation and immune activation, which leads to a profound impairment of normal lymph node function. Adjunctive strategies to reverse LT damage will likely need to be considered to realize the full potential of HIV cure therapeutic approaches, which will need full access to these important immune organs where most HIV reservoirs reside.

**130 The Lymph Node, Cytotoxic T cell, and HIV/SIV Infections****Elizabeth Connick**, *Univ of Colorado, Denver, CO, USA*

HIV-specific CTL partially, but incompletely suppress virus replication in most infected individuals. Multiple mechanisms have been invoked to explain the failure of CTL to fully suppress virus replication including numerical and functional deficiencies. Whether there is an immune privileged site that is impervious to CTL has not been fully explored. In asymptomatic disease, HIV replication is concentrated in T follicular helper cells (TFH) located within B cell follicles (F) of secondary lymphoid tissues including lymph nodes, spleen, and GALT. In lymph nodes from untreated people, HIV-specific CTL fail to accumulate within F. In the SIV-infected rhesus macaque model, virus replication during chronic, asymptomatic disease is similarly concentrated in F of all lymphoid tissues and SIV-specific CTL fail to accumulate in large numbers. Compartmentalization of virus replication within lymphoid tissues appears related to CTL distribution. Frequencies of virus-specific CTL in F and extrafollicular (EF) compartments of lymph node and spleen of SIV-infected macaques predict SIV RNA+ cells within these compartments. In simian AIDS, when CTL are often numerically deficient, the follicular concentration of virus replication is attenuated. In acute infection, prior to when CTL exert substantial effects on virus replication, as well as in chronically infected animals after CD8 depletion, SIV RNA+ cells are wide spread and not concentrated in F. Thus, EF cells are capable of replicating virus, but virus replication is efficiently suppressed in them when CTL are present. Substantial differences in perforin or granzyme B expression are not evident between F and EF CTL in chronic SIV infection. Frequencies of SIV RNA+ cells increase modestly within F after CD8 depletion, suggesting that the few CTL present in F exert antiviral activity. Few SIV-specific CTL express the follicular homing molecule CXCR5 in the absence of the extrafollicular retention molecule CCR7, which likely accounts for their failure to home to B cell follicles and suppress virus replication. Thus, B cell follicles are immune privileged sites that shield TFH from CTL killing and are exploited by HIV and SIV, and likely other infections and malignancies. Strategies to induce migration of virus-specific CTL into B cell follicles, such as CTL transduction with CXCR5, could lead to improved viral control and possibly a functional cure for HIV.

**131 Tuberculosis: Why Do I Have to Take So Many Pills?****Eric J. Rubin**, *Harvard Sch of PH, Boston, MA, USA*

Tuberculosis has two characteristics that are central to the therapy of disease and the prevention of spread. One, latency, or infection without clinically apparent disease, creates a large reservoir from which new cases of TB can appear and spread. The other, the slow response of active disease to treatment, creates a need for a large and expensive infrastructure to ensure completion of drug therapy. While both of these issues have long been recognized, new insights suggest possible underlying mechanisms and, perhaps, new strategies to subvert each of these problems.

**132 The Evolving Epidemiology of HIV Infection in Persons Who Inject Drugs: Indiana 2015****John T. Brooks**, *CDC, Atlanta, GA, USA*

This session is directed to clinicians and scientists interested in the evolving epidemiology of HIV infection among persons who inject drugs. It is assumed participants are familiar with the principles of HIV transmission, its control, and the epidemiology of HIV infection in the United States. At completion of the session, participants will understand how to prevent potential outbreaks of HIV infection among new populations of persons who inject drugs, and to recognize and respond to an outbreak should one occur.

HIV infections attributable to injection drug use in the U.S. have declined steadily since the early 1990s and have accounted for less than 8% of all diagnosed infections since 2010. In early 2015, public health workers serving a small rural Indiana community (adult population approximately 3,100) detected an outbreak of HIV infections within a network of persons who injected drugs. The ensuing investigation identified over 180 new HIV infections that had mostly occurred since mid-2014. Controlling the outbreak demanded a large and coordinated response by county, state and federal partners working closely with the community and other stakeholders. Fueled by the growing national epidemic of opioid drug abuse, injection drug use – heralded by viral hepatitis C infections – is spreading among populations not previously considered to be at high risk of HIV infection. Clinicians and public health authorities will need to work together with policy makers and at-risk communities to prevent similar future HIV outbreaks as the context of injection drug use in the U.S. evolves.

### 133 Type 1 Interferon Resistance Is a Hallmark of Mucosally Transmitted HIV-1

**Frederic Bibollet-Ruche**<sup>1</sup>; Shilpa Iyer<sup>1</sup>; Ronnie Russell<sup>1</sup>; Andrew G. Smith<sup>1</sup>; Christiana M. Shaw<sup>1</sup>; Yingying Li<sup>1</sup>; Timothy Decker<sup>1</sup>; George M. Shaw<sup>2</sup>; Persephone Borrow<sup>3</sup>; Beatrice Hahn<sup>1</sup>  
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**Background:** Mucosally transmitted founder (TF) viruses are more resistant to the antiviral effects of type 1 interferons (IFNs) than HIV-1 strains that predominate during chronic infection. To determine whether IFN-resistant viruses are specifically selected during the HIV-1 transmission process, we generated viral isolates by limiting dilution of both plasma and genital fluids of epidemiologically linked transmission pairs and examined their relative IFN resistance.

**Methods:** Plasma was collected from the chronically infected donor (CD) and acute recipient (AR) of transmission pairs (n=7); donor genital secretion samples (cervico-vaginal lavage or semen) were available for 3 of these pairs. Plasma and the non-cellular fraction of genital secretions were incubated with activated CD4+T-cells to generate limiting-dilution isolates. The infectivity of each virus isolate was determined in the TZM-bl assay. IFN $\alpha$ 2 sensitivity of each isolate was analyzed by determining the IC<sub>50</sub> in primary CD4+T-cells. Env content was determined using a modified ELISA to quantify gp120.

**Results:** We obtained a total of 283 single-genome derived isolates, 191 from CDs and 92 from ARs. Plasma isolates from CDs were significantly more sensitive to type I IFNs than isolates derived from the genital secretions (mean IC<sub>50</sub> 45 U/ml and 92 U/ml respectively, p<0.0001). In all transmission pairs, isolates derived from ARs were significantly more IFN resistant (mean IC<sub>50</sub> 215 U/ml, p<0.0001) than isolates derived from plasma or genital secretion of CDs. AR isolates replicated to higher titers than CD isolates in the absence of IFN (p<0.0001), and unlike most donor isolates, were not fully inhibited at maximal IFN $\alpha$ 2 concentrations. In CD, the IFN $\alpha$ 2 IC<sub>50</sub> of isolates positively correlated with virus particle infectivity (p<0.0001) and negatively correlated with the replication potential in CD4+T-cells (p<0.0001). Additionally, AR isolates had significantly higher Env content per virus particle compared to CD isolates (p<0.0001) yet Env content was not correlated with IFN $\alpha$ 2 IC<sub>50</sub> (p=0.09).

**Conclusions:** Viruses circulating in plasma and sexual secretions of chronically infected individuals exhibit a wide range of IFN resistance, and are generally more IFN sensitive than TF viruses. IFN-resistant viruses are specifically selected from a pool of biologically diverse viruses during mucosal HIV-1 transmission.

### 134 Expression and Potency of IFN $\alpha$ Subtypes in an Ex Vivo Model of Acute HIV-1 Infection

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<sup>1</sup>Univ of Colorado Anschutz Med Campus, Aurora, CO, USA; <sup>2</sup>Univ of Duisburg-Essen, Essen, Germany; <sup>3</sup>NIAID, NIH, Hamilton, MT, USA; <sup>4</sup>Univ of Colorado Hosp, Aurora, CO, USA

**Background:** HIV-1 is transmitted primarily across mucosal surfaces and rapidly spreads within the intestinal mucosa during acute infection. The type I interferons (IFNs) likely serve as a first line of defense, but the relative expression, antiviral properties and effector mechanisms utilized by the 12 IFN $\alpha$  subtypes against HIV-1 infection of mucosal tissues remain unknown.

**Methods:** We evaluated the expression of all IFN $\alpha$  subtypes in HIV-1-exposed plasmacytoid dendritic cells by next-generation sequencing (NGS). The relative antiviral potency of each IFN $\alpha$  subtype against HIV-1 (BaL) and Transmitted/Founder HIV-1 strains CH40, CH58 and CH470 were determined *ex vivo* using the human intestinal Lamina Propria Aggregate Culture (LPAC) model (Steele et al, Retrovirology 2014). Gene expression levels of restriction factors Mx2, tetherin and APOBEC3 were evaluated in purified LP CD4 T cells. Virion infectivities were determined by computing the ratio of infectious titer using the TZM-bl assay and virus particle levels by p24 ELISA. G-to-A mutation rates were evaluated by NGS.

**Results:** IFN $\alpha$  subtype transcripts from the centromeric half of the *IFNA* gene complex, particularly IFN $\alpha$ 1, 5, 8, 14 and 2, were highly expressed in pDCs following HIV-1 exposure. There was an inverse relationship between *IFNA* subtype expression and potency. IFN $\alpha$ 8, IFN $\alpha$ 6 and IFN $\alpha$ 14 were the most potent in restricting HIV-1 infection. IFN $\alpha$ 2, the clinically-approved subtype, and IFN $\alpha$ 1 were both highly expressed but exhibited relatively weak antiviral activity. The relative potencies correlated with binding affinity to the type I IFN receptor and the induction levels of HIV-1 restriction factors Mx2 and Tetherin/BST-2 but not APOBEC3G, D and F. However, despite the lack of APOBEC3 transcriptional induction, the higher relative potency of IFN $\alpha$ 8 and IFN $\alpha$ 14 correlated with stronger inhibition of virion infectivity, which is linked to deaminase-independent APOBEC3 restriction activity. By contrast, both potent (IFN $\alpha$ 8) and weak (IFN $\alpha$ 1) subtypes significantly induced HIV-1 GG-to-AG hypermutation.

**Conclusions:** The results unravel skewed gene expression and non-redundant functions of the IFN $\alpha$  subtypes against HIV-1 infection, with strong implications for HIV-1 mucosal immunity, viral evolution and IFN $\alpha$ -based functional cure strategies.

### 135 Novel Mechanism of Interferon Restriction of HIV-1 in Humans

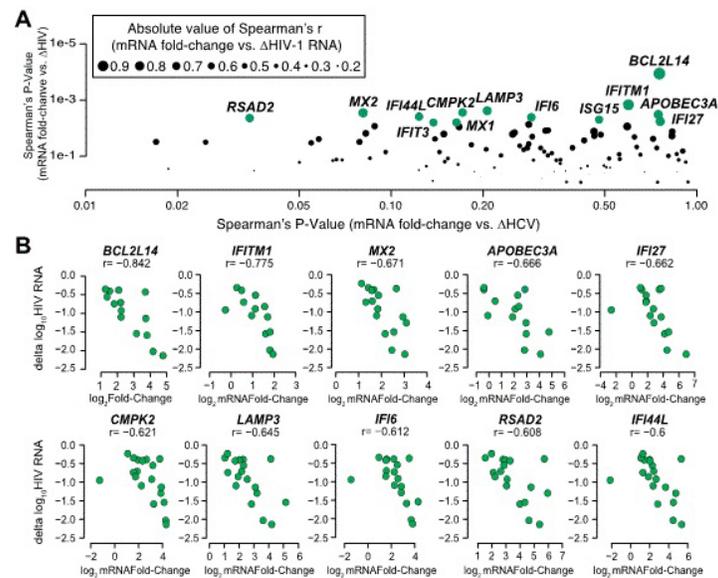
**Ramy El-Diwanly**<sup>1</sup>; Michael Chattergoon<sup>1</sup>; Justin R. Bailey<sup>1</sup>; Stuart C. Ray<sup>1</sup>; Sarah J. Wheelan<sup>1</sup>; Joel N. Blankson<sup>1</sup>; Robert Siliciano<sup>2</sup>; David L. Thomas<sup>1</sup>; Ashwin Balagopal<sup>1</sup>  
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**Background:** Type 1 interferons are critical to control of SIV and HIV-1. However, with several notable exceptions such as MX2, we do not know which of the hundreds of interferon-stimulated genes (ISGs) restrict HIV-1 replication. We hypothesized that the administration of interferon alpha to HIV-1 infected humans would reduce HIV-1 RNA by inducing expression of restriction factors in activated T cells, where HIV-1 chiefly replicates.

**Methods:** HIV-1 kinetics were measured in plasma from 19 human subjects with untreated HIV-1 infection after administration of weight-based peginterferon alpha 2b (IFN). We purified HIV-1 permissive cells (CD38+/HLA-DR+/CD4+/CD3+ lymphocytes, or activated CD4+ T-Cells) from peripheral blood mononuclear cells by FACS sorting before and after IFN. mRNA abundance was quantified using paired end RNAseq. Alignments were performed with RSEM and differential expression was calculated using EBseq. Genes were discarded that had uncertain probabilities of equal expression (PPEE<0.95) or differential expression (PPDE<0.95) in the majority of subjects, after false discovery rate correction (FDR<0.05). ISGs were identified individually from the remaining genes by paired t test if they showed significant induction with IFN, adjusting for multiple comparisons. Spearman rank-correlations were performed between fold-changes of newly defined ISGs and HIV-1 RNA decline at 72 hours. Genes whose induction correlated with plasma HIV-1 RNA decline and that had not been previously described for HIV-1 were validated *in vitro*.

**Results:** There were 99 ISGs differentially expressed in the activated CD4+ T cells of  $\geq 11/19$  participants. Of those 99, 13 were also strongly correlated with the resultant reduction in plasma HIV-1 RNA. Included were expected ISGs (e.g.s, MX2, APOBEC3A). In addition, we identified several novel candidate HIV-1 restriction factors (**Figure**) and used principal components analyses to select among these for ISGs that independently restrict HIV-1 replication. CMPK2 and BCL2L14 were selected for further study. In cell culture, both CMPK2 and BCL2L14 (and MX2, as control) were confirmed to be induced by IFN. With all three ISGs, restriction of HIV-1 production was attenuated by blocking expression of the gene using RNA interference.

**Conclusions:** These studies identify CMPK2 and BCL2L14 as novel HIV restriction factors and may provide new insights into how interferon restricts the replication of HIV-1 and possibly other chronic viral infections.



### 136 SERINC3 and SERINC5 Are Novel Antiviral Proteins Antagonized by HIV-1 Nef

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**Background:** HIV-1 Nef is required for efficient virus replication *in vivo*. One function of Nef is to enhance the infectivity of progeny virions. Recent studies shown that the glycoGag protein of Moloney murine leukemia virus mimics the effect of Nef on HIV-1 infectivity. This suggests that Nef and glycoGag target a common antiviral factor.

**Methods:** Progeny virions produced by T lymphoid cells were purified in OptiPrep gradients, and virus-associated host proteins were identified by mass spectrometry. The incorporation of tagged SERINC3 and SERINC5 into virions was examined by immunoblotting. The impact of SERINC3 on HIV-1 infectivity was examined by siRNA-mediated depletion. T lymphoid cells lacking SERINC3, SERINC5, or both were generated using the CRISPR-Cas9 knockout approach. The infectivity of progeny virions was examined by measuring beta-galactosidase activity in infected TZM-bl indicator cells, or by infecting primary cells with HIV-GFP. HIV-1 replication was monitored by p24 ELISA. Virus entry was examined with the Blam-Vpr fusion assay. SERINC3 and SERINC5 expression levels were examined by RNAseq and qRT-PCR.

**Results:** Our proteomic analysis of HIV-1 virions indicated that Nef and glycoGag inhibit the incorporation of the multipass transmembrane protein SERINC3 into virions. A biochemical analysis confirmed this finding, and showed that Nef and glycoGag also inhibit the incorporation of SERINC5. The simultaneous knockdown of SERINC3 and SERINC5 precisely phenocopied the effects of Nef and glycoGag on HIV infectivity. Furthermore, the infectivity of Nef-deficient HIV-1 virions increased more than 100-fold when produced in double-knockout CD4+ T cells lacking SERINC3 and SERINC5, and re-expression of SERINC3 and SERINC5 confirmed that their absence accounted for the infectivity enhancement. SERINC3 and SERINC5 together also restricted HIV-1 replication, and this restriction was counteracted by Nef.

**Conclusions:** We identified the plasma membrane proteins SERINC3 and SERINC5 as novel restriction factors that are incorporated into progeny virions in the absence of Nef and inhibit HIV-1 infectivity. Nef and glycoGag counteract the SERINC3 by inducing their endocytosis. Since SERINC3 and SERINC5 are highly expressed in primary human HIV-1 target cells, preventing their downregulation by Nef is a potential anti-HIV strategy.

### 137 The PPIP122-125 Motif in HIV-1 CA Is an Essential Assembly and Maturation Element

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**Background:** During HIV-1 particle assembly, Gag molecules bind to the plasma membrane and form a hexameric protein lattice, an assembly process driven largely by the capsid (CA) domain of Gag. Upon cleavage of the Gag polyprotein by the viral protease, the released CA proteins form a cone-shaped capsid core within mature virions. In this study, we focused on characterizing the role of a highly conserved PPIP motif (CA residues 122-125) in the loop connecting helices 6 and 7 (H6-7) in assembly, release, and maturation of HIV-1 particles.

**Methods:** We performed alanine-scanning mutagenesis of the PPIP122-125 motif and characterized assembly, production, replication, and infectivity of mutant viral particles. Structural properties of mutant CA proteins and virions were analyzed by EM and x-ray crystallography. Compensatory mutations that rescued the defects imposed by changes in the PPIP motif were also selected through prolonged viral passage in multiple T-cell lines.

**Results:** We show that mutations P122A and I124A significantly decrease production of mature particles and severely inhibit viral infectivity. In addition, both mutants are highly impaired in their ability to replicate in T-cell lines. In contrast, mutation of P123 and P125 residues does not significantly affect viral replication. EM analysis of P122A and I124A mutants revealed abnormal virion structures containing a discontinuous immature Gag lattice. We show that suppressor mutations V111/T58A are able to rescue the replication defects of both P122A and I124A mutants. Another combination of compensatory mutations, T58S/T107I, was found to rescue the P122A mutant. We demonstrate that the T58A/T107I/P122A mutant is also more sensitive to the CA-based inhibitor PF74. We are currently characterizing the properties of mature viral cores containing P122A or I124A substitutions along with corresponding compensatory mutations. Structural characteristics of the mutant and revertant CA proteins are being investigated by x-ray crystallography.

**Conclusions:** We identified and characterized a novel structural element, the PPIP122-125 motif, which is important for HIV-1 assembly and maturation. Based on the analysis of the Gag lattice structure in the immature virion (Schur *et al.*, Nature 2015), we suggest that the H6-7 loop of the HIV-1 CA domain plays a role in formation of interhexamer contacts in the immature Gag lattice and also plays an important role in CA-CA contacts in the mature CA lattice.

**138 Characterization of 2 CCR5 Tropic HIV-1 Viruses Exhibiting High Env Content**Sean P. O'Brien<sup>1</sup>; Terra M. Ireland<sup>1</sup>; Julian W. Bess<sup>1</sup>; James D. Roser<sup>1</sup>; Elena N. Chertova<sup>1</sup>; Gregory Q. Del Prete<sup>2</sup>; Brandon Keele<sup>2</sup>; Jeffrey Lifson<sup>2</sup><sup>1</sup>Leidos Biomed Rsr, Inc, Frederick, MD, USA; <sup>2</sup>Frederick Natl Lab, Frederick, MD, USA

**Background:** Generating high Env content HIV-1 virions with trimers in their native state would be useful for Env characterization and as a potential immunogen source for various vaccine strategies. While a truncation in the tail of the simian immunodeficiency virus greatly increases Env incorporation, attempts to transfer this approach to HIV have failed. Here we used a long-term culture approach to generate and characterize high Env HIV from the HIV-1<sub>Bal</sub> and HIV-1<sub>ADA</sub> viral lineages.

**Methods:** The A66-R5 T-cell line was infected with HIV-1<sub>Bal</sub> and HIV-1<sub>ADA</sub>. Limiting dilution biological clones were generated at various time points post infection and virions derived from these clones were characterized for Env content. Gag and Env sequence analysis of the biological clones were used to identify potential molecular correlates of high-Env expression/incorporation. An infectious molecular clone (IMC) was generated for HIV-1<sub>Bal</sub>, which was used to identify viral determinants of Env incorporation.

**Results:** We isolated a biological clone of HIV-1<sub>ADA</sub> constitutively producing virus with ~52 Env trimers per virion. Sequence analysis revealed a frame shift mutation causing a premature stop codon just past the membrane spanning domain of gp41. We also isolated a biological clone of HIV-1<sub>Bal</sub> constitutively expressing virus with ~56 Env trimers per virion. Sequence analysis revealed an intact and full length Envelope but with three selected mutations in Gag (R15K, P66S, I75L). An infectious molecular clone (IMC) of this virus was generated and transfection produced virus was used to infect the A66-R5 cell line. Twenty-five limiting dilution biological clones were generated from the infected culture. Env content of the clones was highly heterogeneous ranging from ~4 to ~64 trimers per virion and was stable over time in culture.

**Conclusions:** We were able to develop two productively infected cell-lines producing CCR5 tropic HIV-1<sub>Bal</sub> and HIV-1<sub>ADA</sub> with levels of virion Env incorporation 4-8 fold higher than typical HIV-1 strains. Tail-truncation and possible Gag-Env interactions could explain the viral contributions to high-Env. Subsequent biological clones derived from cultures infected with an IMC of HIV-1<sub>Bal</sub> exhibited a wide range in average trimers per virion, suggesting cellular parameters combine with viral genetics to significantly impact Env content.

**139 Nuclear Entry of HIV Requires Reshaping of Integrase Multimers**Frauke Christ<sup>1</sup>; Lieve Dirix<sup>2</sup>; Doortje Borrenberghs<sup>2</sup>; Flore De Wit<sup>1</sup>; Jolien Blokken<sup>1</sup>; Susana Rocha<sup>1</sup>; Jelle Hendrix<sup>2</sup>; Zeger Debysers<sup>1</sup>; Johan Hofkens<sup>2</sup><sup>1</sup>Katholieke Universiteit Leuven, Leuven, Belgium; <sup>2</sup>Katholieke Universiteit Leuven, Heverlee, Belgium

**Background:** The detailed mechanism of HIV nuclear entry is only partially understood. Studies are typically limited to averaged information, whereas the heterogeneous pool of infecting virus particles requires single virus analysis. Technological limitations and the complexity of the process are the main obstacles to elucidate the nuclear import of viral particles. Using HIV trans-incorporating fluorescently labeled integrase (IN) we established complementary microscopy methods to visualize the dynamics of IN during nuclear entry of single PICs.

**Methods:** We established state-of-the-art technology to accurately determine the fluorescence intensity of the single IN complex in the infected cell. The intensity directly relates to the number of IN molecules; hence the stoichiometry of each single complex can be studied in the context of its relative location in the cell. Furthermore, we used a complementary method that employs FRET via acceptor photobleaching to quantitatively study fluorescently labeled IN oligomers. The obtained FRET ratio is a measure of the interaction between the IN subunits and provides information about conformational changes. Through combination of both methods we investigated the dynamic interplay between the different IN subunits during HIV nuclear import.

**Results:** Nuclear entry was associated with a significant reduction in the number of IN molecules in the PIC in HeLaP4 cells, a T cell line as well as in primary CD4+ T cells implying a general mechanism. Upon nuclear entry, but before chromatin tethering, the interaction with the host factor LEDGF/p75 induced an increase in the FRET ratio of the IN multimer. Addition of LEDGINS, small molecule inhibitors of the IN-LEDGF/p75 interaction, during virus production, prematurely increased the FRET ratio in the virions. Upon infection these pretreated viral particles were refractory to a reduction in the number of IN molecules per PIC and defective for nuclear entry.

**Conclusions:** The composition of the PIC undergoes dynamic changes in both IN stoichiometry and affinity of IN-IN interaction. Our data indicate a role of the nuclear pore as a molecular filter, only tolerating PICs with a specific composition/size to pass through. Upon nuclear entry LEDGF/p75 induces rearrangements in the PIC complex allowing integration. In conclusion, the ability to study PIC composition at the single virus level provides the long-sought approach to unravel nuclear import of HIV-1 and to study the MOA of inhibitors such as LEDGINS.

**140 1970s HIV-1 Genomes Reveal the Early History of the North American HIV/AIDS Epidemic**Michael Worobey<sup>1</sup>; Thomas D. Watts<sup>1</sup>; Richard A. McKay<sup>2</sup>; Timothy Granade<sup>3</sup>; Beryl A. Koblin<sup>4</sup>; Walid Heneine<sup>3</sup>; Harold W. Jaffe<sup>3</sup><sup>1</sup>Univ of Arizona, Tucson, AZ, USA; <sup>2</sup>Univ of Cambridge, Cambridge, UK; <sup>3</sup>CDC, Atlanta, GA, USA; <sup>4</sup>New York Blood Cntr, New York, NY, USA

**Background:** The precise origins of the western hemispheric HIV/AIDS pandemic remain contentious. Phylogenetic studies based upon HIV-1 sequences sampled after (in most cases long after) the recognition of AIDS in 1981 have suggested a lengthy cryptic period of circulation in the U.S. and Canada throughout the 1970s, and an even older presence in the Caribbean. However, no comprehensive evolutionary genomic analysis of HIV-1 in North America closer to the putative emergence of the virus in the 1970s has been possible since no non-African HIV-1 complete genomes from that era have been sequenced to date.

**Methods:** Here, we combine approaches from molecular biology, phylogenetics, and historical analysis to investigate the timing and geography of the emergence of HIV-1 group M subtype B in the U.S. We designed an approach to overcome the challenge of recovering HIV-1 genetic material from older serum specimens in which viral RNA template material is highly fragmented and at low concentration. We recovered near-full length HIV-1 genome sequences from eight U.S. patient serum samples from 1978-79 (comprising eight of the nine earliest HIV-1 group M genomes sequenced worldwide to date) and also from the individual known as 'Patient O'. We then used maximum likelihood and Bayesian 'relaxed molecular clock' phylogenetic and phylogenomic methods to infer timing and geography.

**Results:** This early genomic 'snapshot' of HIV-1 reveals that by 1978-79 the HIV-1 epidemic in the U.S. already exhibited extensive genetic diversity – particularly in New York City (NYC) – having emerged around 1970 from a founder virus drawn from an older and more diverse subtype B epidemic in the Caribbean. Moreover, there is neither biological nor historical evidence that Patient O was the primary case in the U.S. or for subtype B as a whole.

**Conclusions:** Our findings reveal a series of key founder events in the genesis of the subtype B epidemic, with a single virus moving from the African epicenter of HIV-1 group M to the Caribbean by 1967 [1964-1970], a single virus moving from the Caribbean to establish an epidemic in NYC by 1971 [1969-73], and yet another single virus moving from there to San Francisco by ~1975 (but with extensive geographical mixing in the U.S. and beyond shortly thereafter). We discuss, in the context of these insights into the early spread of HIV/AIDS in North America, the genesis and persistence of beliefs about 'Patient O' that are unsupported by scientific data.

**141 SIV-Infected Brain Macrophages Leave the Central Nervous System**Xavier Alvarez<sup>1</sup>; Cecily Midkiff<sup>1</sup>; Andrew Lackner<sup>1</sup>; Kenneth C. Williams<sup>2</sup><sup>1</sup>Tulane Natl Primate Rsr Cntr, Covington, LA, USA; <sup>2</sup>Boston Coll, Chestnut Hill, MA, USA

**Background:** The primary cells in the Central Nervous System (CNS) infected with SIV and HIV are macrophages/microglia. It has been hypothesized that these cells serve as a reservoir of SIV and HIV infection within the CNS, but recent data suggest they can leave the CNS and join lymphatics in some case with virus. Understanding the biology of these cells and their traffic out of the CNS is critical to assess this hypothesis.

**Methods:** To assess the fate of brain macrophages we introduced fluorescent Super Paramagnetic Iron Oxide Nanoparticles (SPIONs) into the cisterna magna which are taken up by phagocytic cells in the brain. This was done in six SIV infected and two normal macaques. We also trace labeled the CSF with dextran-red for short periods. We introduced the SPIONs and follow their fate detecting also their point of exit from the CNS.

**Results:** We found, one hour after SPIONs were instilled at the cisterna magna, they were present throughout the perivascular space mainly as extracellular single beads with some being phagocytized by perivascular cells (PVCs). By 24 hours post inoculation all the SPIONs were intracellular and over the next 28 days the frequency of cells containing SPIONs fell throughout the CNS with the exception of the cranial nerves. There were larger numbers of SPIONs in the CNS of animals with SIV infection than non infected animals. Beginning at 7 days after intracisternal instillation of SPIONs we found SPION containing cells outside of the CNS with the greatest number in the cervical lymph node 7 days after SPION administration. This timing was consistent in both SIV-infected and control animals. We determined that SPIONs are carried out by cells (PVCs) and not in the fluid phase traced with dextran red. In a normal animal the cervical lymph node, with a volume of 0.5 ml, the number of SPIONs carrying cells is a million showing that there is a relative large CNS efflux of cells. We identified several points for emigration out of the CNS as those at the cribriform plate, the cranial nerves, along the neural cord at the ganglia at the brachial plexus and the sacral cord.

**Conclusions:** The SPION+ cells were consistently CD163+ indicating that they were monocyte/macrophage lineage cells. Interestingly, rare SPION+CD163+ cells were also SIV+. This experiment shows for the first time that brain perivascular cells (macrophage lineage) leave the CNS into the periphery and may carry virus with the possibility of reseeding the body with potentially new viral clones.

#### 142 CSF Lymphocyte and Monocyte Activation and Trafficking in Primary HIV Infection

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**Background:** Trafficking of immune cells to the central nervous system is hypothesized to facilitate HIV entry and immune-induced neuronal injury, and is mediated by surface proteins such as chemokine receptors and  $\alpha 4$  integrin. We longitudinally assessed immune cell activation and surface marker expression in cerebrospinal fluid (CSF) and blood and their relationship with CSF HIV RNA during primary HIV infection (PHI) before and after combination antiretroviral therapy (cART).

**Methods:** Longitudinal paired blood and CSF were obtained in initially cART-naïve PHI (<12 mo since infection) participants; some subjects independently initiated cART during follow up. Multiparameter flow cytometry on fresh samples was used to determine activation (% CD38+HLADR+) and chemokine receptor expression (% CCR5+CXCR3+) on CD4+ and CD8+ T cells, and activation (% CD14+CD16+) and  $\alpha 4$  integrin expression (% and mean fluorescence intensity (MFI) of CD49d+) on monocytes. CSF chemokines (IP-10 and MCP-1) were quantified by ELISA. Analyses employed Spearman correlation, within-subject correlation, and linear mixed models.

**Results:** 51 participants enrolled at a median 3.3 mo post infection had 168 total visits (113 untreated, 55 on cART) with median 7 mo follow up (range 0–40). Baseline CSF IP-10 but not MCP-1 correlated with CD4+ and CD8+ T cell activation and HIV RNA in CSF ( $r=0.48, 0.59, 0.77$ ; all  $p<0.05$ ). Pre-cART, rates of increase in T cell activation were 4 times higher in CSF than blood. In unadjusted longitudinal analysis, CSF CD4+ and CD8+ T cell activation correlated with CSF HIV RNA (all  $p\leq 0.01$ ); in multivariate analysis CSF CD4+ but not CD8+ T cell activation was an independent predictor of CSF HIV RNA. CSF monocyte activation and  $\alpha 4$  expression did not correlate with CSF HIV RNA. In blood but not CSF of untreated participants, monocyte  $\alpha 4$  MFI correlated with CD4+ and CD8+ T cell activation (all  $p<0.05$ ). During follow up on cART, blood but not CSF T cell activation declined with days on treatment (slope=-0.06,  $p=0.001$ ). During cART, blood monocyte  $\alpha 4$  MFI correlated with blood T cell activation, and CSF monocyte  $\alpha 4$  MFI correlated with CSF CD4+ T cell activation (all  $p<0.05$ ).

**Conclusions:** In untreated PHI, T cell activation increases faster in CSF than blood, and CSF CD4+ T cell activation but not monocyte activation correlates with CSF HIV RNA. Intrathecal T cell activation does not decline during early follow up on cART. The role of  $\alpha 4$  integrin in trafficking leukocytes to the CSF warrants further exploration.

#### 143 Compartmentalized HIV DNA Populations Persist in CSF Despite Suppressive ART

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**Background:** Persistence of HIV DNA in the central nervous system likely contributes to inflammation, brain damage and neurocognitive (NC) impairment during antiretroviral therapy (ART). The effects of early ART initiation on these parameters are still unknown.

**Methods:** Paired blood and 40 mL cerebrospinal fluid (CSF) samples were collected from 16 HIV+ individuals on suppressive ART (<50 copies/ml): 9 subjects started ART  $\leq 4$  months from estimated date of infection (EDI) and 7 started ART >14 months from EDI. NC functioning was measured by Global Deficit Score (GDS). HIV DNA levels were measured in peripheral blood mononuclear cells (PBMC) and CSF cells by droplet digital PCR; soluble inflammatory markers (sCD163, IL-6, MCP-1, TNF- $\alpha$ ) and marker of neuronal damage (neurofilament chain [NFL]) were measured in blood plasma and CSF supernatant by immunoassays. Next generation sequencing (NGS) data by Roche 454 were successfully generated for HIV *env* from 8 paired blood and CSF cell pellets (3 with early and 5 with late ART). Viral compartmentalization analysis via Fst statistics was performed using NGS data and repeated using representative haplotypes to guard against possible skewing of allelic frequencies due to PCR amplification and other biases. Cross-sectional comparisons between groups (early versus later ART) were performed using non-parametric statistical analysis.

**Results:** In these suppressed HIV+ individuals (median duration on ART: 2.6 years), HIV DNA was detected in 62.5% (10/16) of CSF cell pellets and 93.8% (15/16) of PBMCs. Early initiation of ART was associated with lower CSF levels of IL-6 ( $p=0.03$ ) and TNF- $\alpha$  ( $p=0.02$ ), but no difference in GDS, NFL, or HIV DNA as compared to later ART group. Significant compartmentalization of HIV DNA populations between blood and CSF were detected in 7 out of 8 subjects, and these findings were congruent between the two approaches mentioned above. Phylogenetic analysis confirmed presence of monophyletic HIV DNA populations within the CSF for each participant (aRT > 0.9), and their persistence over time in the two participants with longitudinal sampling (2 and 5 months between time points).

**Conclusions:** A compartmentalized HIV DNA population in CSF was detectable in the majority of HIV+ individuals despite long-term suppressive ART and even when ART was started during early HIV-infection. Also, early ART start was associated with lower inflammation in CSF (IL-6 and TNF- $\alpha$ ), but no difference in GDS compared to later start of ART.

#### 144 Mitochondrial DNA Copy Number and Neurocognitive Impairment in HIV-Infected Persons

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**Background:** Mitochondrial DNA (mtDNA) content in peripheral blood mononuclear cells (PBMC) declines with age and has been associated with neurocognitive function in non-HIV-infected persons. Low mtDNA copy number may indicate disordered mtDNA replication and high copy number may indicate a cellular response to mitochondrial dysfunction. Extracellular (or “free”) mtDNA is a TLR-9 ligand affecting the innate immune response. We tested relationships between both PBMC cellular mtDNA content and cerebrospinal fluid (CSF) cell-free mtDNA levels and neurocognitive impairment in the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) study.

**Methods:** PBMC mtDNA content was measured in 1011 CHARTER participants. The effect of PBMC mtDNA content on global deficit score (GDS), GDS impairment (GDS $\geq 0.5$ ), and HIV-Associated Neurocognitive Disorder (HAND) were tested by logistic regression, adjusting for platelet count, age, gender, genetic ancestry, comorbidity (incidental or contributing to neurocognitive impairment), nadir CD4 and HIV RNA in plasma. CSF free mtDNA was assessed by droplet digital PCR in a subset of 335 participants. Cell-free mtDNA associations with CSF inflammation and iron-related biomarkers CXCL10, IL-6, IL-8, TNF- $\alpha$ , transferrin (TF), ceruloplasmin (CP), and vascular endothelial growth factor (VEGF), HIV viral load (VL), and GDS were evaluated.

**Results:** Lower PBMC mtDNA copy number per cell was associated with lower platelet count ( $p=3e-7$ ), older age ( $p=0.002$ ) and longer ART duration ( $p=0.0008$ ). PBMC mtDNA content was associated with GDS ( $p=0.02$ ), GDS impairment ( $p=0.0003$ ) and HAND ( $p=0.009$ ). CSF free mtDNA levels were positively associated with CSF CXCL10 ( $p<0.001$ ) and TNF- $\alpha$  ( $p<0.05$ ). After adjusting for CSF WBC and VL, free mtDNA levels were associated with CSF inflammation- and iron-related biomarkers TF ( $p<0.05$ ) and CP ( $p<0.05$ ). With correction for ART, free mtDNA was associated with CSF VEGF ( $<0.05$ ) and IL-6 ( $p=0.05$ ). Unlike PBMC mtDNA, CSF free mtDNA was not associated with age or NCI.

**Conclusions:** Higher PBMC mtDNA content was associated with worse neurocognitive performance in HIV+ adults, although PBMC mtDNA content was lower with older age. Higher mtDNA content can be caused by increased replication in response to mitochondrial dysfunction, indicating a connection between neurocognitive impairment and a systemic decrease of mitochondrial function observable in PBMCs. Associations with CSF biomarkers indicate that CSF free mtDNA may mediate neuroinflammation during HIV infection.

**145 Lipid Profiles and APOE4 Allele Impact Midlife Cognitive Decline in HIV+ Men on ART**

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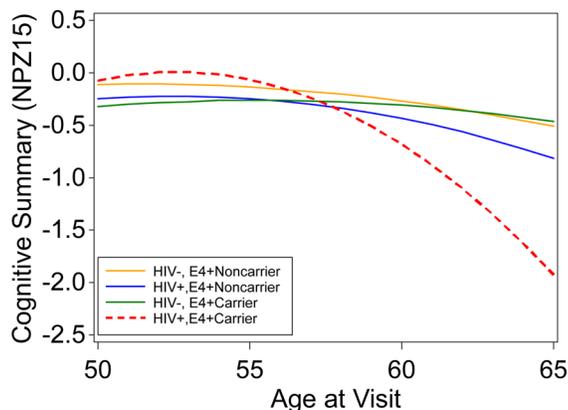
<sup>1</sup>Massachusetts General Hosp, Boston, MA, USA; <sup>2</sup>Dana-Farber Cancer Inst, Boston, MA, USA; <sup>3</sup>Dana-Farber Cancer Inst, Brookline, MA, USA; <sup>4</sup>Northwestern Univ, Feinberg Sch of Med, Chicago, IL, USA

**Background:** Dyslipidemia and the ApolipoproteinE4 (APOε4) allele are recognized risk factors for cognitive decline in the general population, but how these risks are modified by HIV serostatus is unclear. This longitudinal study examined relationships between lipid profiles, APOε4 allele, and cognitive decline in a cohort of ART-treated HIV+ men over age 50.

**Methods:** In a nested case-control study from the Multicenter AIDS Cohort Study (MACS) public data, 273 HIV+ men (50-65 years, without active heavy drug use, ≥2 visits with neurocognitive testing between 1996-2010, baseline VL<400 copies/ml, and continuous ART use ≥95% visits) were matched by baseline age, race, education, smoking, and alcohol use to 516 HIV- controls using R MatchIt. A composite measure of global cognition was created from 15 cognitive tests in 6 domains. The association between HIV serostatus, time-varying lipid markers (total cholesterol, LDL, HDL, and triglycerides), APOE genotype (n=344), and cognitive decline was examined using multivariable mixed effects models, adjusting for baseline age, CD4 count, IQ, CES-D, and smoking.

**Results:** The median baseline age of participants was 51 years (IQR: 50-54); 81% white, 89% had education >12 years; heavy smoking (21% vs 17%; p=0.3) and alcohol use (17% vs 11%; p=0.2) were similar between HIV+ and HIV- men, respectively. HIV+ men had baseline median CD4 count of 514 cells/uL, and 70% were virally suppressed in study (<50 copies/ml with ≤2 blips, blip <400 copies/ml). Higher total cholesterol, LDL, and triglycerides, and lower HDL levels were associated with faster rate of cognitive decline in HIV+ men in mixed effects models (p<0.05). There was no significant relationship between HIV serostatus and baseline cognitive score, suggesting that cognitive decline diverged after study entry. In mixed models adjusted for the same terms, APOε4 allele was associated with accelerated cognitive decline in HIV+ men (p=0.01). In all models, CES-D ≥ 16 and older baseline age were associated with lower cognitive scores; IQ was associated with higher cognitive scores.

**Conclusions:** Measures of lipid markers were associated with faster rates of cognitive decline among ART-treated HIV+ men over the age of 50. HIV+ APOε4 carriers are at substantial risk for accelerated midlife cognitive decline, and are predicted to decline at younger ages than HIV- carriers. These findings suggest pathways affecting lipid metabolism may accelerate cognitive aging among older HIV+ individuals.



**146 Paroxetine and Fluconazole Therapy for HAND: A Double-Blind, Placebo-Controlled Trial**

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**Background:** Paroxetine and fluconazole have potent neuroprotective effects in an *in vitro* model of mitochondrial stress and HIV-protein mediated neural damage. We studied the safety, tolerability, and efficacy of both paroxetine and fluconazole for the treatment of HIV-associated neurocognitive disorder (HAND).

**Methods:** HIV+ individuals with cognitive impairment were enrolled in a 24 week randomized double-blind, placebo-controlled 2x2 factorial design study. Participants were randomly assigned to 1 of 4 groups: 1) paroxetine 20mg/day orally, 2) fluconazole 100mg every 12 hours orally, 3) paroxetine 20mg/day and fluconazole 100mg every 12 hours, or 4) placebo. Safety, tolerability, and efficacy, including neuropsychological testing and cerebrospinal fluid (CSF) lipid markers of oxidative stress, were evaluated. Change in neuropsychological test performance and oxidative stress markers was modeled using a general linear regression model.

**Results:** 45 HIV+ individuals were enrolled. There was no difference in adverse events among the treatment arms. HIV+ individuals in the paroxetine arms (alone or in combination with fluconazole), compared to the no paroxetine arms (placebo or fluconazole alone) showed a benefit in cognitive performance in the NPZ8 summary measure of 8 neuropsychological tests (NPZ8 mean change = 0.15 vs -0.33, p=0.010), and a computerized test of executive function (Cal Cap sequential mean change = 0.46 vs 0.06, p=0.002), after adjusting for depression symptomatology and baseline NPZ8 in as-treated analyses. There was no difference in change in depression symptomatology or CSF lipid markers of oxidative stress between paroxetine and no-paroxetine arms. HIV+ individuals in the fluconazole arms (alone or in combination with paroxetine) compared to the no fluconazole arms (placebo or paroxetine alone) did not show a benefit in cognitive performance, but fluconazole was associated with a decrease in CSF ceramide 18:0 (p=0.039) after adjustment for baseline ceramide level in intent-to-treat analyses.

**Conclusions:** Paroxetine and fluconazole in HIV+ individuals were safe and well tolerated. Paroxetine may be associated with cognitive improvement, even after adjusting for depression symptoms. Fluconazole was associated with a decrease in a lipid marker of oxidative stress. Studies of additional outcome measures including other CSF markers of oxidative stress and functional neuroimaging are underway to examine specific mechanisms of neuroprotection.

**147 MVC and TDF Reduce Neurocognitive Impairment in Initial ART: ACTG A5303**

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**Background:** HIV associated neurocognitive impairment remains problematic despite suppressive antiretroviral therapy (ART). Potential mechanisms include persistent low level HIV infection in the central nervous system (CNS) which sustains inflammation that causes neuronal damage. The neurocognitive effects of maraviroc (MVC), a CCR5 coreceptor inhibitor with uncertain immune modulatory effects and potentially better CNS penetration relative to tenofovir disoproxil fumarate (TDF), are unknown. We investigated neurocognitive changes with MVC- vs TDF-containing ART in ACTG A5303 where both regimens were shown to have similar efficacy in suppressing plasma HIV viremia.

**Methods:** ACTG A5303 was a double-blind, placebo-controlled, multicenter, 48 week study conducted in the US. ART naïve participants with CCR5 tropic HIV-1 and viral load (VL) >1000 copies/mL were randomized 1:1 to MVC 150mg QD + TDF placebo or TDF 300mg QD + MVC placebo (plus DRV+ RTV+FTC); stratified by VL < / ≥100,000 c/mL and age < / ≥30 yrs. The neuropsychological (NP) battery of 15 tests done at baseline, week 24 and week 48 assessed Language, Attention, Executive, Learning, Memory, Speed of Processing, and Fine motor domains, and were standardized into z scores then converted into deficit scores (DS) and a global deficit score (GDS). The 48-week changes from baseline in the NP scores and the GDS were compared by Wilcoxon or Kruskal-Wallis test between arms, and among baseline impairment groups (classified as normal, mild (2 DS ≥1) and moderate (2 DS ≥2)).

**Results:** Of the 230 baseline participants (MVC=119, TDF=111), the arms were comparable for: 9% were female; median age was 33 yrs, 44% White, 31% Black, 22% Hispanic; >12 years education 70%; median VL was 4.5 log<sub>10</sub> c/mL and CD4 count was 390 cells/mm<sup>3</sup>. There were no significant differences in GDS between the arms at baseline (median (IQR) MVC 0.33 (0.07, 0.73); TDF 0.33 (0.13, 0.64)). There were no significant differences between arms for the 48-week changes in standardized NP scores and the GDS (p>0.05; Figure 1A). Those with moderate impairment at baseline experienced greater ART-mediated declines in GDS (median (IQR) -0.4 (-0.7, -0.2)) than those with mild (-0.2 (-0.3, -0.1)) or no impairment (0 (-0.1, 0.1), p<0.001, Figure 1B).

**Conclusions:** Neurocognitive functioning improved with effective ART, and was comparable in those treated with MVC or TDF in combination with DRV/RTV+FTC. The higher the baseline impairment, the greater the neurocognitive improvement and response to ART.

Figure 1.A: Median (IQR) 48-week change in GDS by arm

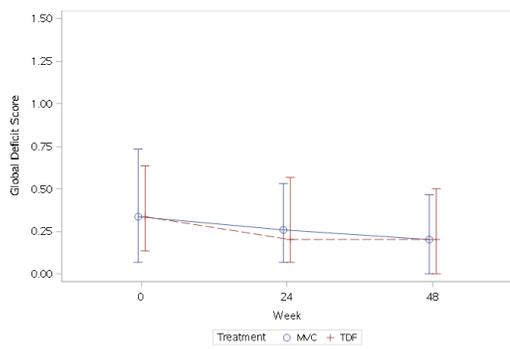
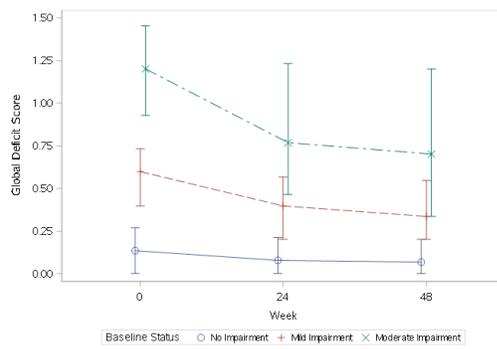


Figure 1.B: Median (IQR) 48-week change in GDS by baseline impairment groups



**148 Brain MRI Changes Associated With Poorer Cognitive Function in Treated HIV Infection**

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**Background:** Grey and white matter (GM and WM) abnormalities have been reported in HIV-positive subjects. However, data are sparse in virologically suppressed cohorts compared with appropriate control populations.

**Methods:** We assessed cognitive function across 6 domains, brain tissue volumes (T1 structural MRI) and WM microstructure (diffusion weighted MRI) in 134 HIV-positive individuals on suppressive cART (median (range) 56 (45-82) years) and 79 demographically comparable HIV-uninfected subjects (57 (47-80) years).

To delineate localised differences in brain structure between HIV-positive subjects and controls, whole brain (voxelwise) analyses of GM volume (GMV), WM volume (WMV), fractional anisotropy (FA), mean, axial and radial diffusivity (MD, AD and RD respectively) were performed using permutation testing, adjusting for age, intracranial volume and scanner type.

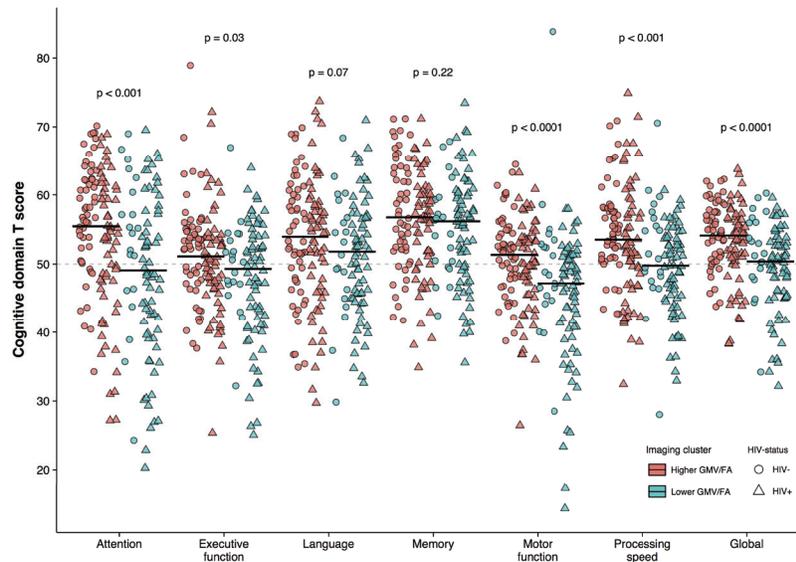
To relate imaging findings to cognitive measures, GMV, FA and cognitive domain T scores were integrated using the kmeans clustering statistical method.

**Results:** Significantly lower T-scores, representing poorer cognitive function, were evident in 4/6 cognitive domains. Median T scores in HIV-positive subjects vs. controls were: attention: 50.2 vs. 56.8; executive function: 49.3 vs. 52.3; motor function: 48.5 vs. 51.2; processing speed: 51.2 vs. 54.6 (p<0.01 for all); language: 52.6 vs. 53.2 and memory: 56.7 vs. 56.2 (p=0.42 for both).

Voxelwise analyses demonstrated significantly lower GMV, principally in the intra- and supracalcarine cortices and the lingual gyrus (35%, 27% and 13% voxels affected), in HIV-positive subjects vs. controls. Additionally, widespread abnormalities in white matter microstructure (FA, MD, RD but not AD) but not WMV were evident in HIV-positive individuals vs. controls.

Subjects optimally clustered into a higher GMV/FA cluster (n=116, cluster means: GMV 701 mL, FA 0.492) and a lower GMV/FA cluster (n=88, cluster means: GMV 614 mL, FA 0.467). HIV-positive subjects were more likely to be members of the lower GMV/FA cluster compared with controls, 52.3% vs. 27.0% (X<sup>2</sup>=11.3, p<0.001). This cluster also exhibited poorer cognitive function (figure 1).

**Conclusions:** Grey matter atrophy and white matter microstructural abnormalities suggestive of demyelination were evident in HIV-positive subjects compared to controls. These changes were in spite of fully suppressive cART and were associated with poorer cognitive function. Future work to understand the underlying pathogenesis of these imaging changes is warranted.



149 **Networks of HCV Transmissions Among Persons Who Inject Drugs: Indiana, 2015**

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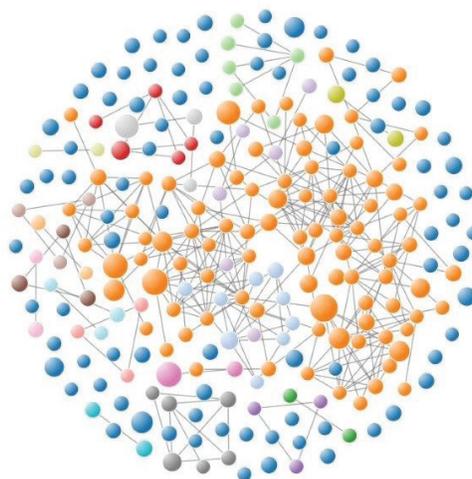
**Background:** Early in 2015, an outbreak of HIV-1 infection associated with injection of the prescription opioid oxycodone was identified in Scott County, Indiana. The investigation revealed that the affected community had a high prevalence of hepatitis C virus (HCV) infection. To characterize the circulating HCV strains, molecular analysis of HCV from 492 specimens was conducted.

**Methods:** HCV strains were genotyped using phylogenetic analysis of NS5b sequences. To sample intra-host HCV variants (quasispecies), next generation sequencing (NGS) of the HCV hypervariable region 1 (HVR1) was performed. NGS HVR1 sequences were analyzed using Global Hepatitis Outbreak and Surveillance Technology (GHST), a recently developed bioinformatics toolkit for inferring genetic relationships among HCV strains and graphically presenting HCV transmission networks.

**Results:** Of the 492 HCV antibody reactive specimens, 334 (67.9%) were HCV-RNA positive, and the distribution of HCV genotypes included: 1a (69.8%), 1b (2.4%), 2b (5.1%), 3a (22.8%). Among the cases of HCV infection, 23% were co-infected with HIV. Phylogenetic analysis of the NS5b sequences identified one major cluster of genotype 1a strains (n=104) with >93% homology, and 2 clusters of the genotype 3a strains (n=46; n=8) with >94% and 95% homology, respectively. Of the 240 cases tested by GHST, 70 (29%) were infected with >1 HCV strain, and 46 (19%) were infected with >1 HCV genotype. One person was infected with 5 strains of genotypes 1a, 1b, 3 and 6. NGS analyses identified a total of about 335 HCV strains, with 65% of the strains organized into 19 transmission clusters (median cluster size = 3). One major transmission cluster involved 92 cases (Fig. 1: Orange nodes).

Fig.1: Network of transmission clusters identified by Global Hepatitis Outbreak and Surveillance Technology (GHST). Clusters are color-coded; each node represents a case; size is proportional to HCV HVR1 heterogeneity. Links join cases with genetic distance below the relatedness threshold.

**Conclusions:** Co-circulation of a large number of HCV strains indicates multiple independent introductions of HCV to the community over a long period of time. Identification of a third of the tested cases infected with >1 HCV strain highlights longstanding endemicity of HCV infection in the community. A complex transmission network genetically detected by GHST reveals continuous HCV dissemination, and provides a novel tool to supplement public health intervention efforts for identification and disruption



**150 Hepatitis B and C, Alcohol, and CD4 Drive End-Stage Liver Disease in HIV+ Adults**

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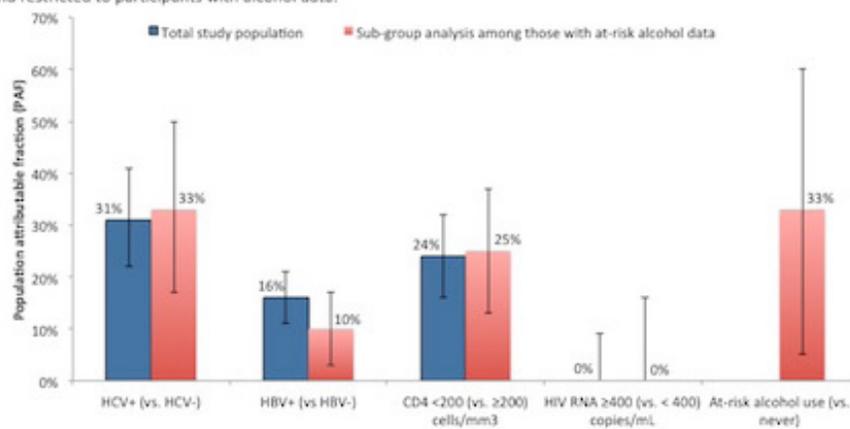
**Background:** The increased burden of end-stage liver disease (ESLD) in HIV-infected adults is likely driven by both HIV-related and ESLD risk factors. Our objective was to estimate the population attributable fractions (PAF) for hepatitis C (HCV) and B (HBV) infection, at-risk alcohol use, and other ESLD risk factors, interpreted as the proportion of ESLD that could be avoided in HIV-infected adults if all were *unexposed* to the modifiable risk factor of interest.

**Methods:** We included adults (>=18 years) participating in one of 11 contributing cohorts to the NA-ACCORD with validated ESLD diagnosis from 1 Jan 2000 to 31 Dec 2009 were included. HCV, HBV, smoking, CD4 <200 cells/mm<sup>3</sup>, HIV RNA >=400 copies/mL, and a past clinical AIDS diagnosis were examined for their relationship with ESLD. HCV and HBV were measured at study entry; all other variables were time-dependent. A sub-group analysis was conducted restricting to participants with alcohol data; at-risk alcohol use was measured at study entry, defined as >=3 drinks on any day or >=7 drinks per week for females and >=4 drinks on any day or >=14 drinks per week males. Cox proportional hazard models with piecewise constant baseline hazard functions were used to estimate adjusted hazard ratios, which were used to determine adjusted PAFs for the modifiable risk factors of interest.

**Results:** 35,044 adults contributed 134,315 person-years and 387 incident ESLD diagnoses; median follow up was 3.1 years. At baseline, participants who developed ESLD were older, more likely to be male and white, and had greater proportions with HCV, HBV, at-risk alcohol use, smoking, a CD4 <200 cells/mm<sup>3</sup>, and a past AIDS diagnosis compared to those who did not develop ESLD. The PAFs from the primary and sub-group analyses are shown in Figure 1. In the sub-group analysis of 12,158 adults with alcohol data, the PAF for at-risk alcohol use was 33%; there were no substantial differences in PAF estimates for other risk factors.

**Conclusions:** Preventing HCV and HBV could avoid up to 31% and 16% of ESLD in HIV-infected adults, respectively. HCV treatment provision with newly available direct-acting agents may have a notable effect on preventing ESLD in HIV-infected patients. Prevention programs for at-risk alcohol use could avoid up to 33% of ESLD. A quarter of ESLD events may be averted with strong immune function further supporting early and consistent ART use for non-HIV disease prevention.

**Figure 1:** Population attributable fractions and 95% confidence intervals for hepatitis C (HCV), hepatitis B (HBV), at-risk alcohol use, other traditional risk factors and HIV-related risk factors for end-stage liver disease, among all participants and restricted to participants with alcohol data.



Footnote: Population attributable fractions have been adjusted for all the risk factors in the figure, as well as age, sex, race, HIV transmission risk, smoking, and clinical AIDS diagnosis.

**151 Antiretroviral Therapy Reduces Intrahepatic Hepatitis C in HIV-1/HCV Coinfection**

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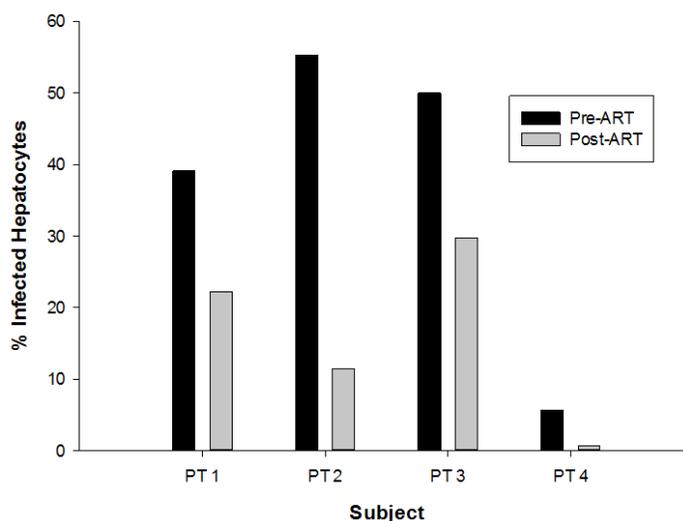
**Background:** HIV-1 worsens hepatitis C virus (HCV) replication in co-infected patients, and we and others have recently reported that antiretroviral therapy (ART) initiation reduces plasma HCV RNA levels (Sherman, *Sci Trans Med*, 2014; Balagopal, *Hepatology*, 2014). We now test the hypothesis that HIV-1 control with ART decreases intrahepatic HCV replication.

**Methods:** We obtained paired plasma samples and liver biopsies before and >12 weeks after ART initiation in four HIV-1/HCV co-infected persons who had high pre-ART plasma HIV-1 RNA levels. Liver biopsies were snap-frozen in liquid N<sub>2</sub> until processing, then lightly fixed on polyethylene naphthalate membrane slides. Single hepatocytes were isolated by laser capture microdissection as we have done previously (Kandathil, *Gastroenterology*, 2013); RNA was extracted from each cell separately, and HCV RNA was quantified by realtime PCR and compared to levels of 7sI, an abundant intracellular molecule. Plasma HIV-1 and HCV RNA levels were separately quantified in batch. Results are presented as median (range).

**Results:** The median age was 53.7 (43.7-60.6) years, 1/4 (25%) subjects were female, and 3/4 (75%) were black. The median pre-ART plasma HIV-1 levels and CD4+ T cell count were 5.06 (4.69-5.21) log<sub>10</sub> cp/mL and 246 (203-302) cells/μL, respectively. The median interval between liver biopsies was 196 (168-217) days. CD4+ T cell counts increased by a median (range) of 84 (-35 - 243) cells/μL. HCV RNA levels decreased from a median of 7.06 (6.05-7.29) IU/mL to 6.01 (4.45-6.51) IU/mL.

Four hundred separate hepatocytes were laser captured from each liver biopsy, obtained before and after ART, for a total of 2473 separate cells. The proportion of HCV-infected hepatocytes declined from a median of 44.5% (5.7%-55.3%) pre-ART to 16.9% (0.6%-29.7%) after HIV-1 suppression (p<0.05; Figure).

**Conclusions:** In four intensively studied HIV-1/HCV co-infected persons who initiated ART, decreases in plasma HCV RNA levels were associated with a reduction in the proportion of HCV-infected hepatocytes. These results provide rationale for the recommendation to treatment of HIV infection prior to the initiation of HCV therapy in persons with HIV/HCV coinfection.



### 152 Broadly Neutralizing Antibodies Avert HCV Reinfection or Subsequent Chronic Infection

**Sabrina J. Merat**<sup>1</sup>; Richard Molenkamp<sup>2</sup>; Dorien van de Berg<sup>1</sup>; Camille Bru<sup>1</sup>; Sylvie M. Koekkoek<sup>2</sup>; Neeltje A. Kootstra<sup>2</sup>; Maria Prins<sup>3</sup>; Arjen Q. Bakker<sup>1</sup>; Tim Beaumont<sup>1</sup>; Janke Schinkel<sup>2</sup>  
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**Background:** Understanding of protective immunity against hepatitis C virus (HCV) chronic infection is needed for designing an effective vaccine. Development of a broad neutralizing antibody response against the HCV E1E2 glycoproteins early in infection has been associated with clearance of infection. However, little is known about the characteristics of protective antibodies.

**Methods:** Therefore, we analyzed the antibody repertoire of twelve participants from the Amsterdam Cohort Study among drug users with median follow-up of 16.8 years. Five subjects became chronically infected; 3 following primary infection, and 2 cleared a primary infection but became chronically infected following reinfection. Seven subjects never became chronically infected following spontaneous clearance, although 4 out of 7 subjects had documented reinfections. From each subject CD27+IgG+ memory B cells, collected 1 year after initial HCV infection, were immortalized by transduction with BCL6 and BCL-XL. To characterize the breadth of the antibody repertoire, 10,000 B cells were analyzed for binding to E1E2 from genotype 1 to 6 using a multiplex flow cytometry-based assay.

**Results:** In 10 of 12 subjects, E1E2 specific B cell cultures were detected with a median frequency of 0.14%. Interestingly, in subjects who had prolonged risk behavior but never became chronically infected, the antibody repertoire was broader compared to subjects who became chronically infected. In 5 of 7 subjects who never became chronically infected, 0.02 to 0.37% of the B cells recognized at least 5 HCV genotypes, whereas in none of the 5 chronically infected subjects such B cells were detected ( $p = 0.03$ ). From 3 clearers with B cells recognizing at least 5 genotypes, 75 cultures were selected to identify the epitope of these antibodies. All selected cultures recognized to a non-linear epitope. Using alanine mutagenesis scanning, we determined that the major epitope targeted by these antibodies was an epitope combining epitope II and domain B. In addition, 14 cultures from 2 of these 3 clearers bound to the AR4 epitope. Since these epitopes are the targets of broadly neutralizing antibodies, B cell clones were isolated to confirm the neutralization capacity of these antibodies.

**Conclusions:** These data strongly suggest that broadly neutralizing antibodies protect against primary and subsequent HCV chronic infections.

### 153 High Response Rate in HCV-Genotype 4 Patients Treated With Ravidasvir and Sofosbuvir

Gamal Esmat<sup>1</sup>; Maissa El Raziky<sup>2</sup>; Asmaa Goma<sup>3</sup>; Tamer Elbaz<sup>4</sup>; Mahmoud Abouelkhair<sup>2</sup>; Alyaa Sabry<sup>3</sup>; Hadeel Gamal Eldeen<sup>4</sup>; Mohammed Karim<sup>2</sup>; Mohammed Abdel-Hamid<sup>5</sup>; Ola Nada<sup>5</sup>; Sherine Helmy<sup>6</sup>; Hanaa Abdel-Maguid<sup>6</sup>; Richard Colonno<sup>7</sup>; Nathaniel Brown<sup>7</sup>; Eric Ruby<sup>7</sup>; Pamela Vig<sup>7</sup>; **Imam Waked**<sup>3</sup>

<sup>1</sup>Cairo Univ, Cairo, Egypt; <sup>2</sup>Cairo Fatemic Hosp, Cairo, Egypt; <sup>3</sup>Natl Liver Inst, Shebeen El Kom, Egypt; <sup>4</sup>Kasr Alaini Viral Hepatitis Cntr, Cairo, Egypt; <sup>5</sup>MyLab Lab, Cairo, Egypt; <sup>6</sup>European Egyptian Pharmaceutical Industries, Alexandria, Egypt; <sup>7</sup>Presidio Pharmaceuticals, San Francisco, CA, USA

**Background:** Egypt has the highest prevalence of hepatitis C infection in the world, of which 90% is due to HCV genotype-4 (gt-4). Increasing HCV-related morbidity in Egypt presents an urgent need for highly curative, safe and affordable therapies. We report results from a Phase 3 registrational trial in Egyptian HCV gt-4 patients, assessing the combination of ravidasvir (RDV), a pan-genotypic HCV NSSA inhibitor, and sofosbuvir (SOF), a nucleotide HCV NS5B polymerase inhibitor, with or without ribavirin (RBV).

**Methods:** Key inclusion criteria were age 18-65 yr, HCV gt-4 infection, serum HCV RNA  $>4 \log_{10}$  IU/mL, and absence of decompensated cirrhosis or other causes of liver disease. Patients were enrolled into 3 groups: treatment-naïve non-cirrhotic and cirrhotic, by Fibrosan & FIB-4 score (Group 1); interferon (IFN)-experienced non-cirrhotic (Group 2); and IFN-experienced cirrhotic (Group 3). Groups 1 and 2 were treated with RDV 200 mg QD + SOF 400 mg QD for 12 wk, randomized 1:1 to additional RBV (weight-based) or no RBV. Group 3 patients all received RDV+SOF+RBV and were randomized 1:1 to 12 wk vs. 16 wk of treatment. The primary endpoint is sustained virologic response (SVR), defined as serum HCV RNA below the lower limit of detection (LLD  $<12$  IU/mL by the Abbott Real-Time™ PCR assay) at 12 wk post-treatment (SVR12).

**Results:** This study is fully enrolled with 300 patients (150 in Group 1, 80 in Group 2, and 70 in Group 3); 284 patients had completed treatment at the time of this abstract. Study treatment has been generally well tolerated, with one serious adverse event possibly attributed to study drug (transient episode of symptomatic bradycardia). The most common adverse events are headache and fatigue. HCV RNA decreased by ~6 logs in all groups by Wk 1, with 94% of patients HCV RNA undetectable by Wk 4. Of the 265 patients who have reached 4 wk post-treatment, 262 (99%) had RNA

**Conclusions:** To our knowledge, this is the largest IFN-free clinical trial in HCV gt-4 patients. Treatment with RDV+SOF±RBV has been well-tolerated and shows high sustained response rates in a large population of Egyptian patients, regardless of previous treatment status or underlying cirrhosis.

**154LB Ledipasvir/Sofosbuvir for 6 Weeks in HIV-Infected Patients With Acute HCV Infection**

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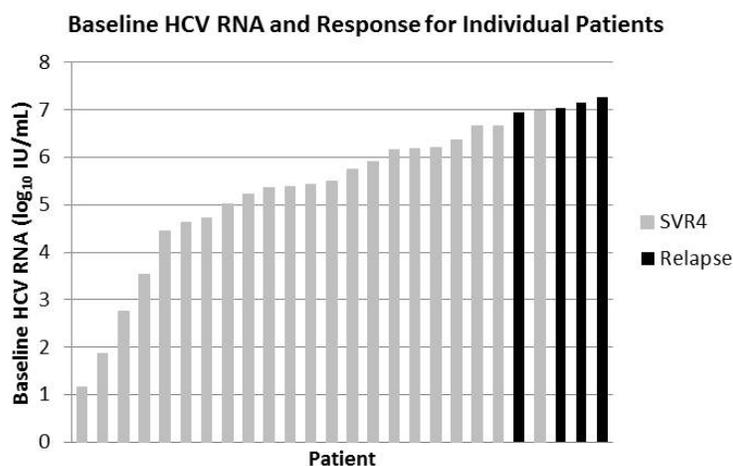
<sup>1</sup>Medizinische Uniklinik, Bonn, Germany; <sup>2</sup>Royal Free Hosp, London, UK; <sup>3</sup>Gilead Scis, Inc, Foster City, CA, USA; <sup>4</sup>Cntr for Infectiology, Berlin, Germany; <sup>5</sup>Infektiologikum, Frankfurt/Main, Frankfurt, Germany; <sup>6</sup>Chelsea and Westminster Hosp NHS Fdn Trust, London, UK

**Background:** There is no currently approved treatment for acute HCV infection. Guidelines recommend 24 weeks of therapy with interferon (IFN) and ribavirin in HIV coinfected individuals who are diagnosed with acute HCV. Shorter duration therapy with all-oral agents may offer a better-tolerated more efficacious alternative. Here we evaluated the safety, tolerability and efficacy of ledipasvir (LDV)/sofosbuvir (SOF) fixed dose combination for 6 weeks in genotype 1 or 4 HIV-infected patients with acute HCV infection.

**Methods:** Patients with an acute HCV infection of <24 weeks duration as per NEAT AHC guidelines were included. Patients were required to either be receiving HIV antiretroviral (ARV) therapy with HIV RNA <200 copies/mL, or not be receiving any treatment for HIV with no plans to start therapy. Enrollment of patients with active illicit drug use was permitted. Patients with acute opportunistic infections or HBV co-infection were excluded. The primary endpoint was sustained viral response defined as HCV RNA.

**Results:** Twenty-six patients were enrolled. All were male, the majority were Caucasian (92%), IL28B non-CC (54%), and receiving ARV therapy (96%). The median baseline HCV RNA was 5.4 log<sub>10</sub> IU/mL. Nineteen (73%) patients had HCV genotype 1a infection and 7 (27%) had genotype 4 infection. All patients completed therapy. 22/26 (85%) achieved SVR4. Four (15%) patients relapsed. There was a strong relationship between baseline HCV RNA and treatment outcome (Figure). All patients (21/21) with baseline HCV RNA <9 million IU/mL achieved SVR4. Treatment was safe and well tolerated. Twenty two of 26 (85%) patients had an adverse event; the majority being mild or moderate. One patient had serious adverse events related to a motor vehicle accident and illicit drug use. No patients discontinued, died or experienced HIV rebound. Post treatment week 12 data will be presented.

**Conclusions:** LDV/SOF for 6 weeks is effective and well tolerated in HIV-infected patients with acute HCV infection who have a baseline HCV RNA <9 million. Acutely HCV-infected patients with a higher viral load should be considered for longer duration of therapy.

**155 Empirical TB Treatment in Advanced HIV Disease: Results of the TB Fast Track Trial**

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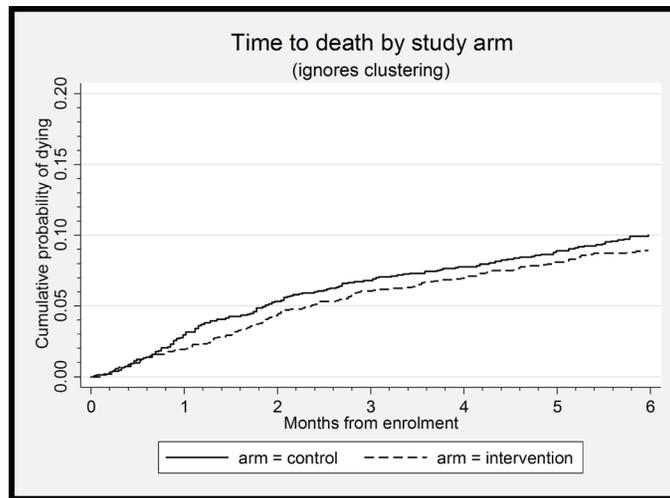
<sup>1</sup>London Sch of Hygiene & Trop Med, London, UK; <sup>2</sup>The Aurum Inst, Johannesburg, South Africa; <sup>3</sup>Fndn for Professional Develop, Pretoria, South Africa; <sup>4</sup>Johns Hopkins Univ Sch of Med, Baltimore, MD, USA

**Background:** Early mortality remains high among HIV-positive people starting ART; TB is the leading cause of death. We hypothesised that an algorithm designed for primary care nurses, using point-of-care tools to identify HIV+ people at high risk of TB, with rapid initiation of TB treatment, then ART, could reduce early mortality. We tested this management strategy in an open cluster-randomised trial, where clusters were South African primary care clinics.

**Methods:** 24 primary care clinics were randomised 1:1 to intervention or control arms. We enrolled HIV+ adults with CD4 ≤ 150, not taking ART or TB treatment. In the intervention arm, study nurses categorised participants' TB probability as high (any of Hb <10g/dl, body mass index ≤ 18.5 or a positive lateral flow assay for urinary lipoarabinomannan), medium (any TB symptom, no high probability criteria) or low (no TB symptoms or high probability criteria). If high probability, participants started TB treatment immediately, then ART two weeks later; if medium probability, further TB investigations were arranged, with re-assessment within a week; if low probability, ART was initiated. The primary outcome was all-cause mortality at 6 months; secondary outcomes included the 6-month risk of hospitalisation. Analyses used methods appropriate for cluster-randomised trials with a small number of clusters. The study is complete: the primary outcome results are final.

**Results:** 3030 participants (55% female, median age 37 years, median CD4 72 cell/mm<sup>3</sup>) were included in the analysis (intervention: 1508; control: 1522). In the intervention arm, 45.7%, 31.5% and 22.8% were categorised as high, medium and low priority respectively. At 6 months, 98.6% (intervention) vs 97.7% (control) had known vital status: mortality rate was 19.0 (intervention) vs. 21.5 (control) per 100 pyrs; adjusted rate ratio 0.87 (95% CI 0.61, 1.24). By 6 months, 13.9% (intervention) vs 10.8% (control) participants had been hospitalised at least once (adjusted risk ratio [aRR] 1.14, 95% CI 0.74, 1.74), and 16.6% (intervention) vs 15.1% (control) participants had been hospitalised and/or had died (aRR 1.01 (95%CI 0.80, 1.28)).

**Conclusions:** A management strategy prioritising TB treatment for ambulatory HIV+ individuals at high risk of TB did not reduce mortality or the risk of hospitalisation by 6 months. Further work is needed to identify strategies to reduce early mortality among HIV+ people presenting for ART with low CD4 counts.



**156 Acceptability of Large-Scale Household-Based TB Screening: HPTN 071 (PopART) Trial**

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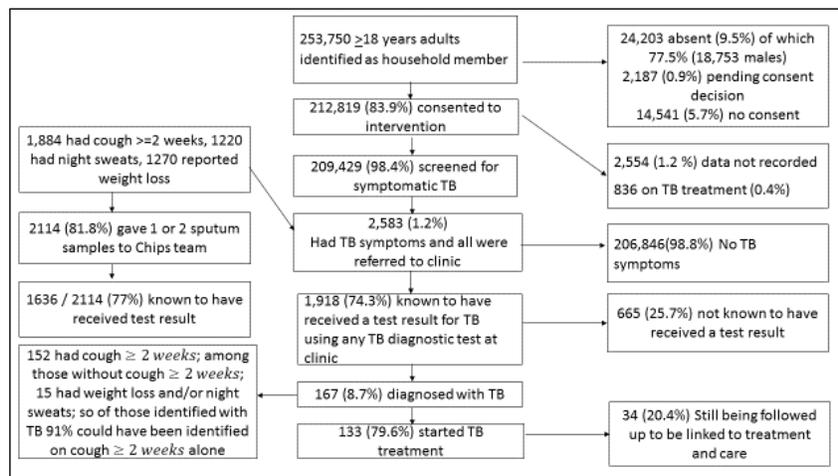
**Background:** HPTN 071 (PopART) is a 3-arm community-randomised trial in 21 communities, 12 in Zambia and 9 in South Africa, which aims to determine the impact of a combination prevention intervention on HIV incidence. Community HIV care providers (ChiPs) are responsible for the delivery of the intervention and linkage to care. A combination HIV prevention package that includes door-to-door HIV testing and TB screening is offered in 8 of 12 communities in Zambia (total adult population of approximately 214,000).

**Methods:** Individuals gave verbal consent to take part in the study intervention..A standard symptom questionnaire (screening tool) delivered by the ChiPs was used to detect any three of the most common signs or symptoms of TB (weight loss, cough for  $\geq 2$  weeks, and/or night sweats). For adults with  $\geq 1$  of these three most common symptoms, the ChiP teams collected two sputum samples and delivered them to the clinic for diagnosis using either smear microscopy or Gene X-pert. TB positive adults were started on TB treatment at the clinic. The household intervention is delivered in annual rounds, and data was analyzed from the first annual round or the period November 2013 up to June 2015 for Zambia only

**Results:** During this period 102, 511 households in the 8 communities were visited, 97% of households consented to enumeration of household members (99,129), and 84% of adult household members consented to participate (212,819 of 253,750 members). 123,272 were males and median age was 29 among adults who consented to participate, 98.4% (209,429/212,819) were screened for TB. Of those screened, 1.2% (2,583/209,429) were symptomatic for TB and of those symptomatic 74.3% (1,918/2,583) were followed up and received a test result for TB. Among adults who received a test result, 8.7% (167/1,918) had a positive test result and 79.6% (133/167) were started on TB treatment (see figure 1). Overall TB notification rate was 650/100,000 (167/209,429 x 100,000 = 650). Adults with a positive TB test result who are not known to have started TB treatment are being followed up to ensure that they are all linked to care.

**Conclusions:** We found 9% new TB cases among people with signs and symptoms of TB that would not otherwise have been identified. Screening large numbers of individuals for TB using a simple screening tool as part of a large, community-based household HIV intervention is feasible and can contribute towards active case finding and linkage to care.

**Figure 1: Flow chart showing uptake of TB screening and linkage to care**



## 157 HIV-Associated XDR TB Is Transmitted in Households and Hospitals in South Africa

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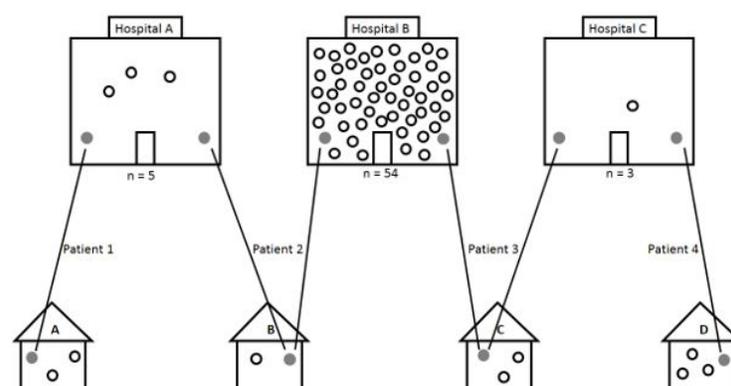
**Background:** Transmission of drug-resistant TB threatens gains in global TB and HIV control, particularly in high-burden settings such as South Africa. Recent data suggests the majority of drug-resistant cases worldwide arise due to transmission, however, studies are needed to characterize the nature (e.g., location, timing) of transmission. In KwaZulu-Natal province, South Africa, we have demonstrated that up to 83% of XDR TB cases are genotypically-clustered, using IS6110 RFLP and targeted gene sequencing, suggesting person-to-person transmission is driving the XDR TB epidemic in this high-HIV prevalence setting. In the current study, we employed social network analysis to further characterize patterns of transmission.

**Methods:** We enrolled patients diagnosed with XDR TB by culture and drug-susceptibility testing in KwaZulu-Natal from 2010–2014. Patients were interviewed at the time of diagnosis about their social networks at home, work, and other community locations, as well as about hospitalizations in the five years preceding their XDR TB diagnosis. An epidemiologic link was defined as two participants having either a social network connection or overlapping admission at the same hospital.

**Results:** Among 404 patients with XDR TB, the median age was 34 (IQR 28–43) and 58% were female. 311 (77%) patients were HIV-infected, with a median CD4 count of 255 cells/mm<sup>3</sup> (IQR 117–431); 177 (57%) were on ART at the time of their XDR TB diagnosis and 155 (88%) had an undetectable viral load. Epidemiologic links were identified for 287 (71%) patients. 83 (21%) patients were linked as social network contacts; of these, 92% lived in the same home, 4% worked together, and 4% spent time together in a congregate setting (e.g., church, bar). 267 (66%) patients overlapped with other XDR TB patients in the hospital, 66 of whom were hospitalized at more than one hospital. There were 63 (16%) patients with both hospital and social network links to other XDR TB patients, four of whom are depicted in Figure 1.

**Conclusions:** The XDR TB epidemic in this high HIV prevalence setting in South Africa is being driven by direct transmission of drug-resistant TB strains in both hospitals and households. Social network analysis has provided valuable insights into the multitude of interactions associated with transmission. This more comprehensive understanding is important for designing interventions that both limit exposure in hospitals and focus contact tracing efforts to households where the majority of transmission is occurring.

Figure 1. Hospital- and community-based XDR TB linkages.



Epidemiologic network demonstrating linkages between community (e.g., homes) and hospitals. Each circle represents a XDR TB case enrolled in the study. The four patients represented by shaded circles were hospitalized at least once with other study patients, and also shared a living space with study patients.

## 158LB Beta-Lactams Against TB: Teaching a New Trick to an Old Dog

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**Background:** It is generally accepted that  $\beta$ -lactam antibiotics are ineffective against *Mycobacterium tuberculosis*. Carbapenems are relatively resistant to mycobacterial  $\beta$ -lactamases *in vitro*. We investigated whether the carbapenems meropenem and faropenem combined with amoxicillin and clavulanic acid (A/CA) have antituberculosis activity in humans.

**Methods:** Groups of 15 patients with newly diagnosed, smear-positive, drug-sensitive pulmonary tuberculosis received one of three treatments for 14 days: meropenem 2g intravenously or faropenem 600mg orally, both combined with oral A/CA 500mg/125mg, all given three times per day, or standard antituberculosis treatment as control. We collected sputum overnight for colony forming unit (CFU) counting on solid agar plates and for assessing time to culture positivity (TTP) in liquid medium. Full pharmacokinetic profiling was performed on day 14. Patients were hospitalised for daily safety assessments.

**Results:** The viable mycobacterial sputum load was significantly reduced with standard treatment (validating the laboratory assays) and with meropenem A/CA but not with faropenem A/CA (Table). Meropenem exposures were much higher than faropenem exposures (Table). AUC and  $C_{max}$  were significantly associated with meropenem activity measured by CFU ( $r^2=0.62$ ;  $p=0.002$  and  $r^2=0.49$ ;  $p=0.066$ , respectively). Mild intermittent diarrhea was reported by >50% of patients in both carbapenem groups and was probably related to A/CA.

**Conclusions:** The combination of intravenous meropenem and oral A/CA has antituberculosis activity in humans of similar magnitude as previously reported for rifampin (10mg/kg), pyrazinamide, bedaquiline and PA-824 over the first 2 weeks of treatment. This establishes  $\beta$ -lactams as a class with clinical antituberculosis activity. Oral faropenem combined with A/CA was ineffective owing to insufficient exposure. Both treatments were safe. Meropenem A/CA should be considered for all patients with highly resistant tuberculosis in whom intravenous treatment is feasible.

	Meropenem A/CA	Faropenem A/CA	Standard treatment
Mean fall of log <sub>10</sub> CFU/ml sputum per day (95%CI)	0.11 (0.07 to 0.15) p<0.001	0.00 (-0.04 to 0.04) p=0.976	0.17 (0.13 to 0.21) p<0.001
Mean % increase of TTP per day (95%CI), hours	3.51 (2.35 to 4.68) p<0.001	-0.39 (-1.51 to 0.74) p=0.346	6.48 (5.27 to 7.71) p<0.001
Mean C <sub>max</sub> (range), µg/mL	285 (142-669)	2.398 (1.073-4.326)	n/a
Mean AUC (range), µg*h/mL	304 (225-553)	4.018 (1.517-8.382)	n/a
Mean T>MIC (range), %	77 (60-95)	14 (0-32)	n/a

A/CA=amoxicillin/clavulanic acid, AUC=area under the concentration curve, CFU=colony forming units, CI=confidence interval, C<sub>max</sub>=maximum serum concentration, p=compared to no effect (statistical model), T>MIC=time of the dosing interval above minimum inhibitory concentration, TTP=time to culture positivity. MIC meropenem 2.5 µg/mL. MIC faropenem: 1.25 µg/mL

**159 Pre-ART Cryptococcal Antigen Titer Associated With Preemptive Fluconazole Failure**

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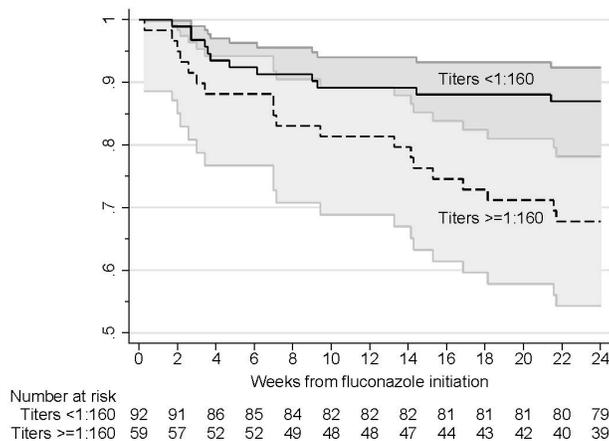
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**Background:** In low and middle-income countries, the prevalence of cryptococcal antigenemia (CrAg+) averages 7.2% (95% confidence interval (CI), 6.8-7.6%) among persons with CD4<100 cells/µL. CrAg+ persons are at elevated risk of mortality compared to persons without cryptococcal antigenemia (CrAg-) who are HIV-infected. Although preemptive fluconazole therapy can halt the progression from asymptomatic antigenemia to symptomatic cryptococcal meningitis and death, risk factors for progression to cryptococcal meningitis are unknown.

**Methods:** We assessed 6-month survival of CrAg+ persons who received preemptive fluconazole monotherapy of 800mg/day for 2 weeks and then 400mg/day for 8 weeks, as part of a stepped-wedge randomized trial evaluating CrAg screening at 18 Ugandan clinics. During the intervention, CrAg screening was reflexively performed at the time of CD4 result, when CD4<100 cells/µL. ART was initiated 2 weeks after fluconazole therapy initiation. We assessed 6-month breakthrough cryptococcal meningitis rates, and survival with respect to baseline CrAg titers and CD4 counts.

**Results:** From 2,135 persons screened with CD4<100 cells/µL, the CrAg+ prevalence was 7.1% (95%CI: 6.1-8.2). 152 asymptomatic CrAg+ HIV-infected, ART-naïve adults received fluconazole. Among 151 CrAg+ persons with baseline titers, 39% (n=59) had a pre-ART CrAg titer of ≥1:160 and 61% (n=92) of <1:160. 11 of the 151 participants went on to develop cryptococcal meningitis within 6 months; and patients with titers ≥1:160 were at elevated risk versus those with titers <1:160 (Hazard Ratio (HR)=5.4; 95%CI: 1.1-27.3; P=.04). Overall 6-month survival was 78%. Patients with CrAg titer ≥1:160 had 2.5-fold higher 6-month mortality risk versus those with titers <1:160 when receiving preemptive therapy (HR=2.5; 95%CI: 1.2-5.2; P=.01). When stratified by CD4 (>50 vs. 50 cells/µL), only those with low CD4 and titers ≥1:160 were at elevated risk for failure (versus high CD4, <1:160 titer patients) (HR=3.2; 95%CI: 1.1-9.5; P=.04). All patients with titers ≥1:1000 were at elevated risk for failure, irrespective of CD4 (HR=2.3; 95%CI: 1.0-4.9; P=.04).

**Conclusions:** Among CrAg+ persons with baseline CrAg titer of ≥1:160, 32% failed preemptive fluconazole therapy and died despite receiving recommended preemptive therapy. Developing semi-quantitative rapid CrAg tests may be warranted to stratify those at risk of death in whom more aggressive antifungal preemptive therapy may be necessary, especially among patients with extremely low CD4 values.



**160 Immediate ART Initiation Reduces Risk of Infection-Related Cancer in HIV Infection**

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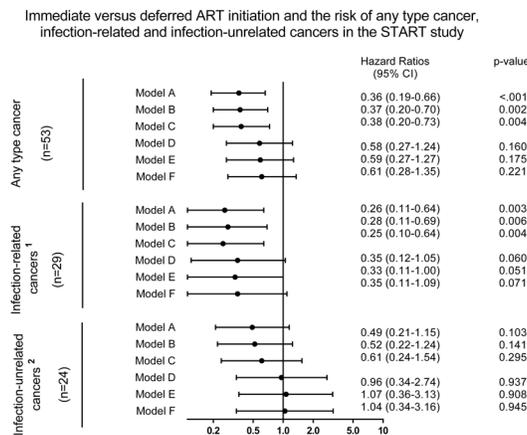
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**Background:** In the START study, immediate antiretroviral therapy (ART) initiation reduced the overall risk of cancer by 64%. We hypothesized that the reduction in cancer risk was higher for infection-related vs infection-unrelated cancer and mainly determined by differences in CD4 counts and HIV RNA levels between the study arms.

**Methods:** Incident malignancies in START were categorized into any type, infection-related and infection-unrelated cancer. Infection-related cancer was defined as cancer driven by any infectious agent. Independent factors associated with each cancer category were assessed by multivariable Cox models. To investigate why immediate ART initiation reduced cancer risk we used sequential adjustment for baseline covariates, cancer risk factors and HIV-specific variables to fit Cox models with a study arm indicator.

**Results:** There were 14 cancers among persons randomized to the immediate ART arm (6 infection-related and 8 infection-unrelated) and 39 cancers in the deferred arm (23 infection-related and 16 infection-unrelated) (Hazard Ratios [HR]; 95% CI; of immediate vs deferred ART initiation were 0.26; 0.11-0.64; for infection-related and 0.49; 0.21-1.15; for infection-unrelated cancer). In adjusted analyses with both treatment groups combined, older age (adjusted HR; 95% CI: 1.85; 1.44-2.39 per 10y), white race (2.80; 1.13-6.92 vs black) and HIV RNA (1.57; 1.08-2.28 per 1log higher) were linked to risk of any type cancer. Independent predictors of infection-related cancer were older age (1.42; 0.99-2.02 per 10y), higher BMI (1.08; 1.01-1.16 per Kg/m<sup>2</sup>), low income region (0.32, 0.14-0.74 for high vs low income) and HIV RNA (2.32; 1.35-3.98 per 1log higher). Older age was the only independent predictor of infection-unrelated cancer (2.58; 1.75-3.81 per 10y). Adjustment for latest HIV RNA level, but not for CD4 count or cancer risk factors, attenuated the effect of immediate ART on any type of cancer and infection-unrelated cancer (Figure). Adjustment for latest HIV RNA level had little impact on the protective effect of immediate ART on infection-related cancer.

**Conclusions:** Immediate ART initiation significantly reduces the risk of infection-related cancer. Though limited by small sample size, this benefit doesn't appear to be solely attributable to HIV RNA suppression and may be also mediated by other mechanisms.



Hazard ratios for cancer events: immediate versus deferred ART initiation START (N=4,685)

1 infection-related cancers: Human Herpesvirus 8 (Kaposi sarcoma), Epstein-Barr virus (non-Hodgkin lymphoma, Hodgkin lymphoma), Human Papilloma virus (anal cancer, cervical cancer)

2 infection-unrelated cancers: prostate cancer, lung cancer, testis cancer, plasma cell myeloma, fibrosarcoma, breast cancer, bladder cancer, ureteric cancer, malignant melanoma, myeloid leukemia, thyroid cancer, leiomyosarcoma, squamous cell carcinoma of the head and neck, squamous cell cancer, gastric adenocarcinoma, liver cancer

A: univariable, estimated in a Cox proportional hazards model with a single treatment indicator

B: adjusted for baseline covariates: age, gender, race, geographical region, smoking, BMI, hepatitis B/C, CD4 cell count and log<sub>10</sub> HIV RNA

C: adjusted for latest CD4 cell count, modelled as a continuous variable

D: adjusted for latest HIV RNA, modelled as <200 copies/mL vs HIV RNA >200 copies/mL

E: adjusted for latest CD4 cell count and latest HIV RNA

F: as in (B) with further adjustment for latest CD4 cell count and latest HIV RNA

161 ACTG A5298: A Phase 3 Trial of the Quadrivalent HPV Vaccine in Older HIV+ Adults

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**Background:** HIV-infected individuals are at increased risk for human papillomavirus (HPV)-associated cancers. Quadrivalent HPV vaccine (qHPV) licensure studies were conducted in HIV-uninfected populations ≤26 yrs old with low exposure to HPV; qHPV is safe and immunogenic in HIV infected adults.

**Methods:** Phase 3, randomized, double-blind, placebo-controlled trial of qHPV in HIV-infected adults age ≥27 years and older with no previous HPV-associated cancer. Men were required to have a recent history of receptive anal sex. Participants had baseline assessments for anal and oral HPV (2 timepoints), anal cytology, and high resolution anoscopy (HRA). Participants received qHPV (or placebo) at entry, weeks 8 and 24. Anal HPV, cytology and oral HPV testing were obtained every 6 months. Treatment of HSIL and follow-up HRA was according to local standard of care. The primary endpoint was persistent anal HPV infection (or single detection at the final visit) in those without the infection at baseline. We hypothesized a 65% reduction with qHPV. The study was conducted at 23 US sites and 1 Brazil site. 99.9% confidence intervals are presented since the data are from a pre-specified DSMB review employing a Haybittle-Peto boundary of 0.001.

**Results:** 575 participants were enrolled (472 (82%) male, 103 (18%) women), 262 (46%) were white/non-Hispanic, 179 (31%) were black/non-Hispanic, 117 (20%) were Hispanic. Median age was 47 years [IQR 41-53], median CD4 602/μL [IQR 436-767], 83% had HIV RNA <50 copies/mL, 558 (97%) completed vaccination. With 2.1 years median follow-up, persistent anal HPV infection with qHPV types was 30% lower than hypothesized. Conditional power to show a significant difference in the primary endpoint was 7% should the study complete with similar event rates. Conditional power for detecting a difference in anal HSIL was <1%. The DSMB recommended stopping the trial due to futility. The qHPV was safe and well tolerated.

**Conclusions:** This study does not support HPV vaccination in older HIV-infected adults for prevention of new anal HPV infections or to improve HSIL treatment outcomes, but does suggest a trend for protection against oral infection. The role of qHPV for prevention of oral HPV infections should be further investigated. These results underscore the need for HPV vaccination prior to HPV exposure.

	qHPV (n=288)	Placebo (n=287)	Hazard ratio † (99.9% CI)
Persistent anal HPV infection or single detection at final visit	23/286 (8.0%)	28/283 (9.9%)	0.78 (0.31, 1.97)
Persistent anal HPV infection	9/286 (3.1%)	13/283 (4.6%)	0.66 (0.16, 2.75)
Persistent oral HPV infection or single detection at final visit	3/288 (1.0%)	8/286 (2.8%)	0.36 (0.04, 3.33)
Persistent oral HPV infection	0/288 (0%)	6/286 (2.1%)	0.00 (not able to calculate)
Anal HSIL on histology: week 52 and beyond	38/288 (13.2%)	36/286 (12.5%)	1.03* (0.52, 2.05)
Abnormal cytology at baseline	182/285 (64%)	188/284 (66%)	0.96* (0.79, 1.16)
Abnormal cytology at week 52	123/231 (53%)	121/229 (53%)	1.00* (0.75, 1.33)
Abnormal cytology at week 104	94/191 (49%)	103/187 (55%)	0.90* (0.65, 1.24)

Randomization was stratified based on sex and the presence of anal HSIL. † Hazard ratio estimate from the Cox model. \*Mantel-Haenszel relative risk estimate adjusting for both stratification factors.

**162 Is Intensive Cervical Cancer Screening Justified in Immunosuppressed Women?**

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**Background:** Current cervical cancer screening guidelines recommend more intensive screening of immunosuppressed women, including HIV+ women and those on immunosuppressive agents (e.g., calcineurin inhibitors, corticosteroids). Whether this approach is justified in settings with uniform access to comprehensive care, including combination antiretroviral therapy and cervical cancer screening, is unclear.

**Methods:** Among adult members of a large integrated healthcare system, we performed a nested case-control study of 20,157 incident cases of cervical intraepithelial neoplasia (CIN) 2, 3 or cancer (CIN 2+) and 5:1 selected controls (N=100,780), matched on age, diagnosis date (+/-1 year), years in the health plan (+/-1 year), and date of first health plan Pap test (+/-1 year). A primary predictor was HIV status, both overall comparing HIV+ and HIV- women, and among HIV+ women stratified by recent CD4 T-cells/μL (<200, 200-499, ≥500 cells/μL). A second predictor of interest was recent (<18 months) prescription of immunosuppressive agents. Odds ratios (OR) from conditional logistic regression models were adjusted for race/ethnicity, smoking (ever/never), and recent hormonal prescriptions (e.g., oral contraceptives, hormone replacement therapy).

**Results:** The mean age of CIN 2+ cases and controls was 36 years. 52% of cases were white, 19% were Hispanic, and 8% were black; race/ethnicity was similar in controls. Cases were more likely than controls to be current smokers (20% vs. 13%) and to have been prescribed hormones (49% vs. 44%). The prevalence of HIV was 0.18% in cases and 0.08% in controls, with 47% of HIV+ women having CD4 counts ≥500 cells/μL. As shown in the Table, the adjusted OR for CIN 2+ for HIV+ women compared with HIV- women was 2.2 (95% CI: 1.5-3.3). In the model stratifying HIV+ women by CD4 count, adjusted ORs (HIV- reference) were 0.9 (0.4-1.9), 3.5 (1.9-6.5), and 5.3 (1.8-15.2), for women with CD4≥500, 200-499, and <200 cells/μL, respectively. The prevalence of immunosuppressive agents was 6.8% in CIN 2+ cases and 6.4% in controls, with an adjusted OR of 1.05 (0.99-1.12). Results were similar for the outcome of CIN 3+ (data not shown).

**Conclusions:** The increased risk of CIN 2+ among HIV+ women appears to be limited to those with CD4 counts <500 cells/μL. Immunosuppressive agents do not appear to confer significant increased risk of CIN 2+. More intensive screening of HIV+ women may be justified based on CD4 cell counts, but not for women prescribed immunosuppressive agents.

**Table. Odds ratios (95% CI) for HIV status and immunosuppressive agents as risk factors for CIN2+**

	Unadjusted	Adjusted*
HIV status		
HIV+	2.3 (1.5-3.4)	2.2 (1.5-3.3)
HIV- (ref)	1.0	1.0
HIV status, CD4 cells/μL		
HIV+, CD4≥500	1.0 (0.5-2.1)	0.9 (0.4-1.9)
HIV+, CD4 200-499	3.5 (1.9-6.3)	3.5 (1.9-6.5)
HIV+, CD4<200	5.0 (1.8-14.3)	5.3 (1.8-15.2)
HIV- (ref)	1.0	1.0
Immunosuppressive agents		
Recent use	1.07 (1.01-1.14)	1.05 (0.99-1.12)
No recent use (ref)	1.0	1.0

\*Adjusted for HIV status (overall or stratified by CD4 count), immunosuppressive agents, race/ethnicity, smoking, and recent hormonal prescriptions

**163 WITHDRAWN**

**164 Isolated Seminal HIV-1 RNA Shedding in African Men With Uninfected Women Partners**

**Andrew Mujugira**<sup>1</sup>; Connie M. Celum<sup>1</sup>; Allan Ronald<sup>2</sup>; Nelly R. Mugo<sup>3</sup>; Robert W. Coombs<sup>1</sup>; Jared M. Baeten<sup>1</sup>; for the Partners PrEP Study Team  
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**Background:** Eliminating HIV-1 transmission using antiretroviral therapy (ART) requires suppressing HIV-1 RNA in plasma and genital secretions. However, intermittent seminal shedding occurs despite effective ART and suppressed plasma HIV-1 RNA concentrations. Given the need to counsel HIV-1 infected men initiating ART about their transmission risk, information about timing and likelihood of achieving HIV-1 RNA suppression in semen and male-to-female HIV-1 transmission is needed.

**Methods:** We used data from a prospective study of heterosexual HIV-1 serodiscordant African couples (Partners PrEP Study) to assess the frequency and magnitude of seminal HIV-1 RNA shedding after 0-3, 4-6 and >6 months of ART. HIV-1 RNA was quantified in semen and blood plasma using the Abbott m2000 Real-Time HIV-1 assay (Abbott

Diagnostics). The primary outcome was seminal HIV-1 RNA concentration >80 copies/mL. Secondary biologic outcomes were pregnancy incidence, as an indicator of unprotected sex, and HIV-1 acquisition in initially uninfected women whose male partners initiated ART during follow-up.

**Results:** We followed 1772 HIV-1 infected men for 4554 person-years. Of these, 755 initiated ART during follow up, and semen HIV-1 RNA results were available for 231 (31%). Median time from ART initiation to semen HIV-1 RNA quantitation was 2.96 months. Seminal HIV-1 RNA was detected in 20% (31/155), 10% (5/49) and 9% (6/70) of samples after 0-3, 4-6 and >6 months of ART, respectively. Among men with suppressed plasma HIV-1 RNA concentrations (<80 copies/ml) [N=192], the frequency of semen HIV-1 RNA detection was 7% (6/88), 3% (1/40) and 3% (2/64) in samples collected 0-3, 4-6 and >6 months after ART initiation. The median quantity of seminal HIV-1 RNA among those with suppressed plasma HIV-1 RNA was 2.97 log<sub>10</sub> copies/ml (range, 2.53-3.41). Plasma and semen HIV-1 RNA concentrations were correlated (Spearman's r=0.58, p<0.001). After >6 months ART, pregnancy incidence was 13.7 per 100 person-years (9 pregnancies/66 person-years) after excluding time when pregnant or using contraception, and there were no male-to-female HIV-1 transmission events in the 231 ART-exposed couples.

**Conclusions:** Seminal HIV-1 RNA shedding was rare and low quantity among heterosexual HIV-1 infected men who achieved plasma HIV-1 RNA suppression. No HIV-1 infections were observed among HIV-1 uninfected women partners, despite high pregnancy incidence, indicating no HIV-1 transmission in the context of unprotected sex.

**165 Exploring the Effectiveness of Traditional Circumcision Practices in Preventing HIV**

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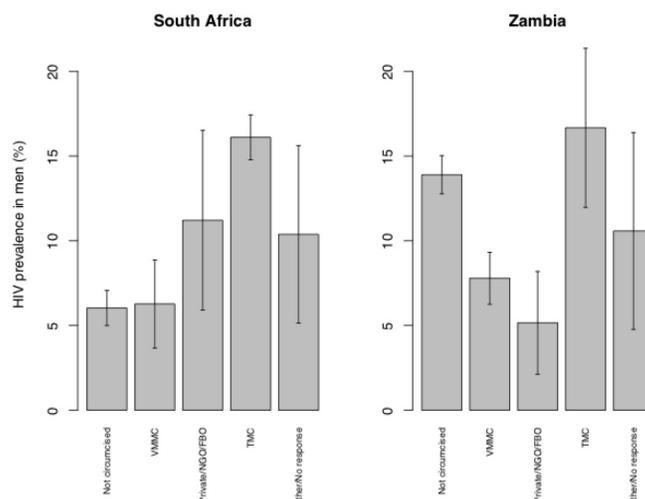
<sup>1</sup>Imperial Coll London, London, UK; <sup>2</sup>London Sch of Hygiene & Trop Med, London, UK; <sup>3</sup>Univ of Stellenbosch, Tygerberg, South Africa; <sup>4</sup>Univ of Stellenbosch, Cape Town, South Africa

**Background:** Traditional male circumcision (TMC) is widespread across different cultures and religions, and is highly prevalent in sub-Saharan Africa. Given variability between cultures in the traditional procedure in terms of foreskin removal, it is unknown if TMC is always as protective against HIV as voluntary male medical circumcision (VMMC) regardless of the traditional procedure used.

**Methods:** HPTN 071 (PopART) is a large-scale combination prevention trial underway in South Africa and Zambia, for which a population cohort of adults aged 18-44 has been randomly recruited. We use logistic regression on data from the baseline survey of the population cohort to examine associations between measured HIV serostatus and self-reported circumcision status, adjusting for potential covariates. We divide individuals into those who have not been circumcised, and those who have undergone one of the following: TMC in Zambia; TMC in South Africa; VMMC; and those who report being circumcised by a practitioner other than a traditional practitioner or government health worker, or report being circumcised but do not specify by who. We differentiate TMC by country to reflect that the traditional procedure used may differ.

**Results:** 5,301 out of the 11,231 men in the population cohort reported being circumcised. 60.6% of those circumcised reported being circumcised by a traditional practitioner, and 28.5% reported VMMC. 83.2% of circumcised men in South Africa (mostly Xhosa) were circumcised by a traditional practitioner, while only 14.0% of circumcised men in Zambia had undergone TMC. Figure 1 shows that in both countries HIV prevalence was higher amongst men who have undergone TMC (16.1% in South Africa; 16.7% in Zambia) than those who had undergone VMMC (6.3% and 7.8% respectively). After adjusting for age, education, marital status and number of lifetime partners, HIV prevalence remained significantly higher in men who underwent TMC in both South Africa (adjusted odds ratio=1.67, 95% confidence interval 1.32-2.14, P<0.001) and in Zambia (1.55, 95% confidence interval 1.00-2.36, P=0.046) compared to those who underwent VMMC.

**Conclusions:** Traditional male circumcision, as practiced by ethnic groups in South Africa and Zambia, is associated with higher HIV prevalence than voluntary male medical circumcision. Further work, designed to examine the extent to which traditional circumcision, as practiced in this and other regions, is protective against HIV acquisition, should be carried out.



**Figure 1: HIV prevalence (%) in men by country, divided into uncircumcised men and those who are circumcised by who carried out the circumcision. Private=private practitioner; NGO=non-governmental organization; FBO=faith-based organization. Error bars show exact 95% binomial confidence intervals for prevalence.**

**166 2013-14 Rwanda HIV Incidence Household Survey: Understanding HIV Epidemic in Rwanda**

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**Background:** In Rwanda since the scaling up of the antiretroviral therapy ten years ago, the HIV prevalence among adults aged 15 to 49 years has been stable at 3%. Although, the incidence is a more informative epidemiological measure of the HIV epidemic, until now Rwanda has relied on models and estimates of this measure. We conducted the first nationally representative HIV incidence household survey in Rwanda among adults aged 15 to 49 to better characterize its HIV epidemic.

**Methods:** A prospective HIV incidence population-based survey conducted in all five Rwandan provinces for the year period of 2013-2014 and using two-stage sampling. We randomly selected 492 villages in the first stage and 14 households in the second stage. We obtained a sample of 13,728 respondents from 6,796 households. Participants were surveyed on their HIV knowledge, risky behaviour and demographics, and they were HIV tested using rapid tests and ELISA. Those testing HIV negative were enrolled in the cohort and those testing HIV positive were only included in the baseline survey. The cohort was followed for a year, with no special intervention or education. After a year, the 12,686 (92%) participants who completed follow-up were HIV re-tested and re-surveyed.

**Results:** Among 12,686 completers, 6182 were male (47%) and the mean age was 30 years. The majority (89%) lived in rural areas, 38% were single and 57% were married or cohabitating. In total, 24% of males was circumcised and 6.7% of women were pregnant. Over the year, 33 cohort members sero-converted resulting in an HIV incidence of 0.27% (95% confidence interval: 0.18-0.35%); 0.21% among men and 0.32% among women. The distribution of HIV cases was indicative of multiple breakouts, with 3 villages and 2 households having multiple sero-conversions. Incidence was highest in adults aged 46-55 years (0.38%); however the most cases (12) occurred among those aged 16-25 years. Similarly, incidence was highest among the widowed (1.30%), single (0.35%) and divorced (0.38%). HIV incidence showed urban rural variation with higher incidence (0.65%) in urban areas.

**Conclusions:** The HIV incidence in Rwanda was slightly higher than model estimates used in the past. Moreover, this household survey demonstrates that young adults and singles are the among largest contributors to HIV incidence in Rwanda and that outbreak contribute to the ongoing epidemic. The national HIV program should plan for HIV preventive interventions tailored to those populations.

**167 Antiretroviral Therapy and HIV Acquisition in a South African Population-Based Cohort**

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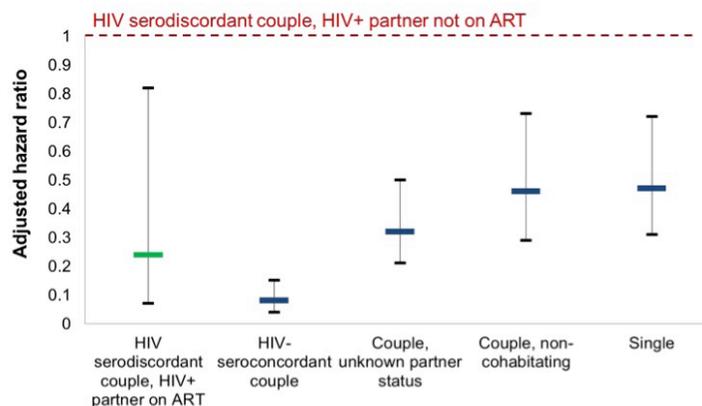
<sup>1</sup>Harvard Sch of PH, Boston, MA, USA; <sup>2</sup>Africa Cntr for Hlth and Pop Studies, Mtubatuba, South Africa; <sup>3</sup>Brown Univ Sch of PH, Providence, RI, USA; <sup>4</sup>The Fenway Inst, Fenway Hlth, Boston, MA, USA

**Background:** Antiretroviral therapy (ART) is highly efficacious in preventing HIV in randomized trials of volunteer serodiscordant couples with full serostatus awareness. However, evidence of the effectiveness of ART in preventing HIV in population-based samples, where serostatus may not be known to the individual or their partner, is lacking. Here, we assess the effect of ART on HIV acquisition in a population-based cohort in rural KwaZulu-Natal, South Africa. In this cohort, couples are linked via surveillance rather than via clinics, allowing for estimation of the effect of ART in a “real-world” setting where individuals are not necessarily aware of their HIV status or the status of their partner.

**Methods:** All HIV-uninfected individuals present between January 2005 and December 2013 (n=17,016) with at least two HIV tests were included, regardless of whether or not they had a cohabitating partner. Individuals with cohabitating partners were categorized as having an HIV-uninfected partner, an HIV-infected partner who was not on ART, or an HIV-infected partner who was on ART. ART status of HIV-infected partners was determined via public-sector ART clinic data. Interval-censored time-varying parametric proportional hazards regression was used to assess how the partner’s ART status affected HIV acquisition risk.

**Results:** Of the 17,016 individuals, 1,846 had an HIV-uninfected and 196 had an HIV-infected partner over the follow-up period, of whom 76 initiated ART during follow-up. HIV incidence was 0.3 per 100 person-years among individuals with an HIV-uninfected partner (95% confidence interval [CI] 0.2-0.5), compared to 5.6 per 100 person-years (95% CI 3.5-8.4) among individuals with an HIV-infected partner who was not on ART, and 1.4 per 100 person-years (95% CI 0.4-3.5) among individuals with an HIV-infected partner who was on ART. In an adjusted model, HIV-infected partner’s use of ART was associated with a 77% decrease in HIV acquisition risk amongst serodiscordant couples (aHR=0.23, 95% CI 0.07-0.80).

**Conclusions:** ART initiation was associated with substantially reduced HIV incidence, but less than has been seen in more controlled settings. Achieving effective population control of HIV incidence, and thus elimination of the epidemic, may not be possible only through ART provision to seropositive persons. A combination of additional interventions is likely to be necessary.



**Prospective cohort study.** N = 17,016 individuals; 1,846 with an HIV+ partner. Interval-censored survival analysis with time-varying ART exposure, education, multiple partners and condom use, as well as age and sex.

**168LB Option B+ in Malawi: Have 4 Years of “Treat All” Shown That 90-90-90 Is Achievable?**

Beth Tippett Barr<sup>1</sup>; **Andreas Jahn<sup>2</sup>**; Sundeep K. Gupta<sup>3</sup>; Alice Maida<sup>3</sup>; Frank Chimbwandra<sup>3</sup>

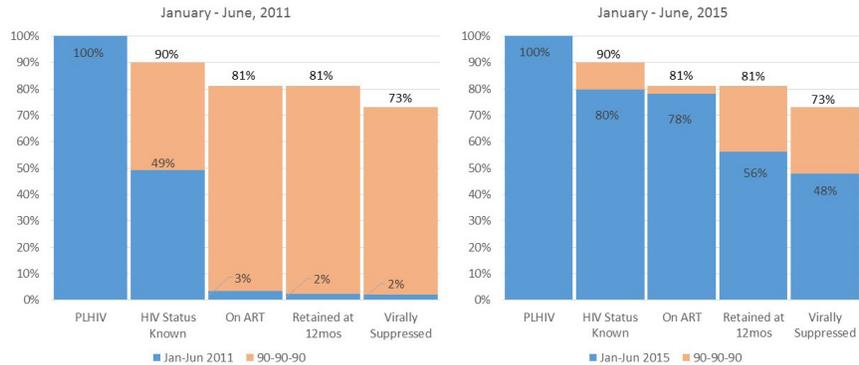
<sup>1</sup>CDC, Harare, Zimbabwe; <sup>2</sup>Ministry of Hlth, Lilongwe, Malawi; <sup>3</sup>CDC, Lilongwe, Malawi

**Background:** In 2014, UNAIDS released the 90-90-90 objectives: 90% of persons living with HIV (PLHIV) are identified, 90% of known PLHIV (or 82% of all) are on sustained ART, of whom 90% (or 73% of all) are virally suppressed by 2020. In September 2015, WHO released guidance endorsing ‘Treat All’ as a critical strategy for epidemic control. Reaching 73% viral suppression by 2020 implicitly assumes that ‘Start All’ will facilitate a rapid increase in the identification of PLHIV to reach the first step in the cascade. We reviewed Malawi’s national data on PMTCT Option B+ as an early ‘Treat All’ model to understand progress towards 90-90-90 in a defined population.

**Methods:** The Malawi Ministry of Health collects, validates and publishes all HIV program data on a quarterly basis (www.hiv.health.gov.mw). Quarterly report data was analyzed to compare the six month period prior to implementation of Option B+ with the same six month period four years later. Comparisons on cascade coverage were made using the Spectrum model population denominator as the point of comparison for each 90. The contributions of initiation and retention were separated under the 2<sup>nd</sup> 90, and those lost to follow-up were considered not to be virally suppressed.

**Results:** Between 2011 and 2015, the proportion of all estimated pregnant PLHIV who knew their HIV status increased from 49% to 80%. The proportion of known positive and on ART increased from 6.5% to 98%, or 78% of all pregnant PLHIV nationally. Retention on ART at 12 months was 72% (56% of all), and of the 23% of ART patients who received a routine VL test, 85% were virally suppressed (or 48% of all), resulting in Malawi attaining an estimated 80-56-48 on the 90-90-90 cascade, compared to 49-3-2 in 2011 (Figure 1).

**Conclusions:** Use of the 90-90-90 cascade to monitor progress towards epidemic control provides clear programmatic insight. “Treat all” under Option B+ in Malawi resulted in rapid and demonstrable progress towards achieving the 90-90-90 objectives, both in ART initiation and viral suppression. However, even in this easy-to-access population with high ANC attendance, the first 90 was not reached. To accomplish 90-90-90 nationally, a proactive approach is required to reach the first 90, not reliance on existing testing coverage and service delivery models. Under the 2<sup>nd</sup> 90 it is important to ensure both ART initiation and ART retention are monitored, in order to ensure appropriate interventions to increase coverage are implemented.



**169LB Measuring the Impact of Test & Treat on the HIV Cascade: The Challenge of Mobility**

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<sup>1</sup>Université Paris Descartes, Paris, France; <sup>2</sup>Africa Cntr for Population Hlth, Mtubatuba, South Africa; <sup>3</sup>INSERM, Bordeaux, France; <sup>4</sup>Univ of Southampton, Southampton, UK; <sup>5</sup>London Sch of Hygiene & Trop Med, London, UK; <sup>6</sup>Harvard Sch of PH, Boston, MA, USA; <sup>7</sup>INSERM U897, ISPED, Univ de Bordeaux, Bordeaux, France; <sup>8</sup>Africa Cntr for Hlth and Pop Studies, Mtubatuba, South Africa

**Background:** Universal test and treat (UTT) could substantially improve the HIV care cascade at population level (i.e. the proportion of all HIV-infected people being diagnosed, on ART and virally suppressed at a given date) and thus reduce HIV incidence. Several trials are currently exploring this hypothesis.

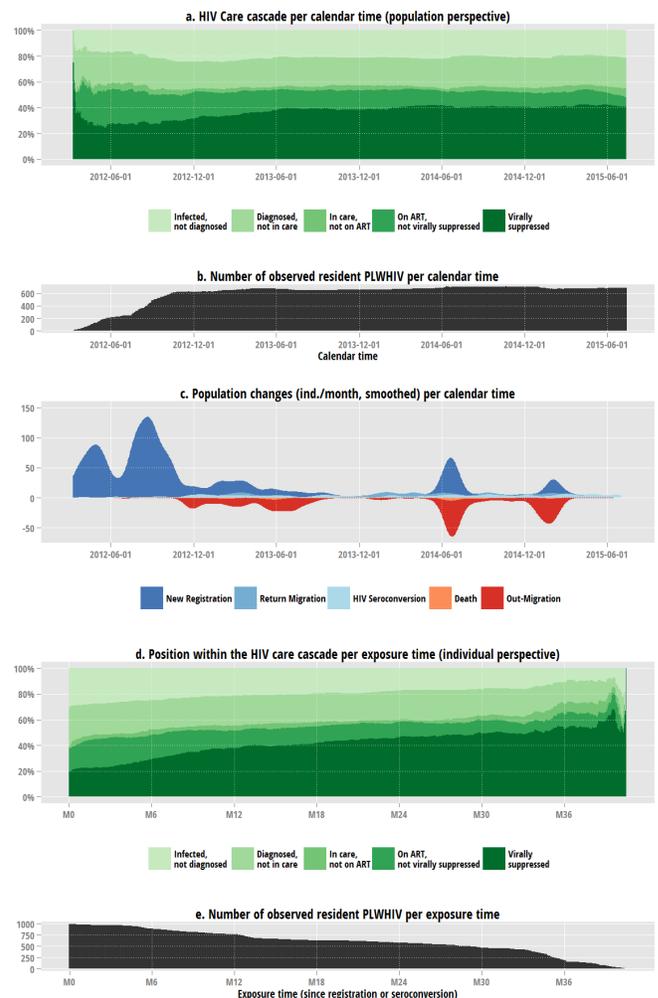
Due to demographic change, the study population of HIV-infected individuals is composed of people with various degrees of exposure to the trial interventions. This structural effect could potentially dilute the impact observed at population level of a UTT strategy. Here, we describe a dynamic cascade according to both calendar (population) and exposure (individual) time approaches, using preliminary data from the ANRS 12249 TasP cluster-randomized trial ongoing in rural KwaZulu-Natal (South Africa).

**Methods:** Analysis was conducted within a subgroup of 4 clusters with the longest follow-up time where five six-monthly rounds of home-based HIV testing had been conducted between March 2012 and July 2015. Resident members 16 years and above were offered rapid HIV testing and asked to provide dried blood spots (DBS) each time. Those ascertained HIV-positive were referred to local trial clinics for ART initiation and follow-up.

HIV tests results and information on clinic visits, ART prescription, viral load and CD4 count, migration and death were used to calculate residency status, HIV status and HIV care status for each individual on each calendar day. This calendar cascade was then compared to the exposure cascade, where each status was recalculated for individuals at any given date with exposure time defined as the duration since trial registration.

**Results:** According to calendar time, the overall cascade improved rapidly during the first 15 months of the trial (from 25 to 40% virally suppressed), but more slowly thereafter (Fig a). Although the target population size of HIV-infected people remained rather stable over time (~665 individuals, Fig b), population turnover was high (Fig c). According to exposure time, with a decreasing sample size over time (Fig e), the cascade improved continuously between M0 and M30, from ~20% to ~50% virally suppressed (Fig. d).

**Conclusions:** Population mobility dilutes the observed impact of UTT interventions on the cascade at population level. These preliminary findings also suggest that the impact of a UTT approach could be maximized as long as there is a coordination to facilitate continued access to care when people move.



**169aLB HIV-1 Infection With Multiclass Resistance Despite Preexposure Prophylaxis (PrEP)**

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**Background:** PrEP is reported to have nearly 100% prevention efficacy in men who have sex with men (MSM) when adherence is high. We report a 43 year old MSM who seroconverted to multi-class resistant HIV-1 (day 0; 4th generation Ag/Ab combo screen positive, p24 positive, Western Blot negative) after 24 months of successful PrEP, despite clinical and pharmacokinetic data suggesting long-term adherence to FTC/TDF.

**Methods:** Pharmacy dispensing records were obtained for FTC/TDF. Liquid chromatography–mass spectrometry (LC-MS) was performed on the untimed plasma sample from day 0 to determine TDF and FTC concentrations. Dried blood spots (DBS) were collected on day 16, while on FTC/TDF-based combination antiretroviral therapy, for determination of intracellular TVF–DP concentration. Standard population sequencing and deep sequencing to 2% of the viral population was completed on day 7 plasma (HIV RNA 28,326 copies/mL), as was phenotypic testing for resistance to integrase inhibitors. Phylogenetic analysis of the V3 loop of envelope protein gp120 was completed on day 7 plasma to characterize the founder virus.

**Results:** Pharmacy dispensing records demonstrated consistent prescription refills. DBS testing revealed TVF–DP of 2,297 fmol/punch indicating consistent dose-taking in the preceding 1-2 months, thus overlapping with the seroconversion time. LC-MS on day 0 plasma was inconclusive for TDF (calibration range 181-2,385 ng/ml) and FTC (range 736-50,200 ng/ml) due to the untimed nature of the sample relative to dosing and the high lower limit of quantification of the assay. Standard and deep sequencing of virus from day 7 revealed CCR5-tropic clade B HIV-1 with mutations conferring resistance to NRTIs (41L, 67G, 69D, 70R, 184V, 215E), NNRTIs (181C) and INSTIs (51Y, 92Q), suggesting transmitted rather than acquired resistance (table 1). Phenotypic drug resistance testing of the integrase class on day 7 plasma revealed reduced response to all integrase inhibitors (table 1). Phylogenetic analysis revealed a very narrow range of sequence diversity, consistent with infection from a single source.

**Conclusions:** This patient's clinical history, pharmacy records and DBS results consistent with long-term dosing of FTC/TDF suggest that HIV infection is possible despite adherence to daily oral PrEP when exposed to FTC/TDF-resistant virus. To our knowledge, this is the first reported case of breakthrough HIV infection with evidence of long-term adherence to FTC/TDF.

Antiretroviral Class	Standard consensus sequencing and deep sequencing mutations	Resistance Analysis (Estimated IC <sub>50</sub> fold change)*
NRTI	41L, 67G, 69D, 70R, 184V, 215E	Abacavir reduced response (1.9x) Lamivudine resistant (61x) Emtricitabine resistant (38x) Tenofovir reduced response (1.3x)
NNRTI	181C	Nevirapine resistant (43x)
PI	10I	Not significant
INSTI	51Y, 92Q	Raltegravir reduced response (2.7x) Elvitegravir resistant (>100x) Dolutegravir reduced response (9.6x)

**Table 1.** Baseline resistance testing 7 days after initial HIV detection. \*IC<sub>50</sub> fold change for INSTI class is reported from phenotypic drug resistance testing.

**169bLB Gel Applied as Anal Lube Without Applicator Provides Poor Rectal Mucosal HIV Coverage**

Eugenie Shieh<sup>1</sup>; Ethel Weld<sup>1</sup>; Edward J. Fuchs<sup>1</sup>; Karen W. Buckheit<sup>2</sup>; Robert W. Buckheit Jr<sup>2</sup>; Jennifer Breakey<sup>1</sup>; Craig Hendrix<sup>1</sup>

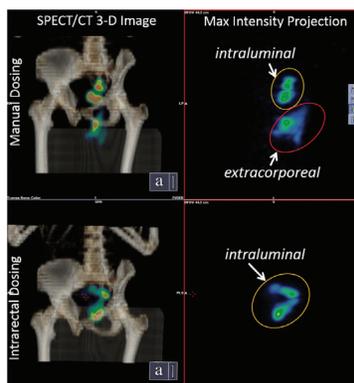
<sup>1</sup>Johns Hopkins Univ, Baltimore, MD, USA; <sup>2</sup>ImQuest Biosciences, Frederick, MD, USA

**Background:** With the rising incidence of HIV in men who have sex with men (MSM) and the proven efficacy of oral HIV pre-exposure prophylaxis, more behaviorally-congruent periodic dosing strategies, such as rectal HIV microbicides, are in demand. Rectal gel microbicide studies have utilized vaginal applicators to administer the gels, but these applicators have not been well received. Further, this doesn't mimic the real-world use of sexual lubricants, which usually involves application inside or on the anus and on the partner's penis. We examined the rectal distribution of gels when dosed as sexual lubricants compared to dosing with an applicator.

**Methods:** Five healthy, HIV-negative MSM were enrolled. 10 mL of 99mTc-DTPA-radiolabelled hydroxyethyl cellulose (HEC) gel universal placebo was administered intra-rectally with a 4 cm applicator attached to a syringe. After washout, the same participants were given 10 mL of 99mTc-DTPA-radiolabelled Wet® Original gel lubricant for self-administration to the anus with use of fingers and a phallic device as if applying a sexual lubricant in their usual manner with a sexual partner (manual dosing). Manual dosing was then followed by 5 minutes of simulated receptive anal intercourse with a phallic device. SPECT/CT was performed 4 hours after each gel administration.

**Results:** Gel administered by both methods was centered in the rectosigmoid, median 7.7 cm (interquartile range 5.2-13.4) from the anorectal junction. However, the manual dosing method was associated with more variable distribution, 12.5 cm (7.3-37.0), compared to a more uniform 18.1 cm (17.35-18.5) distribution after applicator dosing. When applying the gel manually as a sexual lubricant, participants utilized 2.7 mL (1.8, 6.6) of the 10 mL lubricant dose unit. Of the initial 10 mL dose intended for manual application, only 4% (1%, 27%) was retained within the colon after simulated sex. By contrast, 92% (91%, 93%) of the applicator dose was retained (paired t-test, p=0.01).

**Conclusions:** Manual application of a sexual lubricant gel delivers a small, variable fraction of the gel dose in a variable rectosigmoid distribution compared to the same volume dosed intra-rectally. These preliminary results raise concern that manually dosing a rectal microbicide gel as a sexual lubricant may not provide adequate, predictable mucosal coverage for HIV protection. Assessment of antiretroviral rectal microbicide products is needed to confirm these preliminary results.



**170 Location and Population: Response to the Who and Where in the HIV Epidemic****Peter Ghys**, UNAIDS, Geneva, Switzerland

The effectiveness of most elements of the AIDS response is well known, and applying that response can end the AIDS epidemic by 2030. A key element is reaching the right people with the right programmes and services. In the early years of the response populations that needed to be reached by programmes were characterised as general population, high risk groups, and bridge populations. Recent developments in data collection for key populations, in development and application of epidemiological models, in use of facility-based data and in mapping and visualisation techniques allow a much more precise picture of the location of populations that need to be reached with services. Currently available surveillance tools will be reviewed, including integrated bio-behavioural surveys for key populations, sentinel surveillance among pregnant women, national population-based surveys, and case-based surveillance. Current methods and data sources for programmatic monitoring and related data systems will be reviewed. Regarding epidemiological results, examples of local size estimates and estimates of HIV prevalence will be presented for men who have sex with men, sex workers, people who inject drugs; as well as sub-national gender and age-specific estimates of HIV prevalence and incidence. Regarding programme gaps, examples will be given of sub-national PLHIV's awareness gaps, ART gaps, male circumcision gaps, and condom programme gaps. Future developments include geospatial epidemiological models, more precise estimation of local programmatic gaps including for key population programmes, VMMC and condom programming.

**171 HIV in Fishing Communities: Prevalence, Incidence, Risk Factors, and Interventions****Zachary A. Kwena**, Lake Victoria Consortium for Health Research, Kenya Med Rsr Inst, Kisumu, Kenya

As the world coalesces around achieving WHO's 90-90-90 targets, initial steps are focused on finding and shutting down sources of new infections. We sought to describe the burden, risk factors and potential prevention interventions for HIV infections in fishing communities. This largely involves syntheses of published findings as well as findings from recent studies in the fishing communities. Some of the recent studies were conducted under the umbrella of Lake Victoria Consortium for Health Research which has a mission of improving the health of the fishing communities that inhabit the Lake Victoria shoreline and islands in Kenya, Tanzania and Uganda (<http://www.wavuvi.org/about-wavuvi-concern>). HIV prevalence is variable across fishing villages and gender. In Kenya, HIV prevalence range from 14.2% in Busia to 37.4% in Homa Bay. Women are more affected (37.5%) compared to men (29.4%). HIV prevalence in Uganda range from 22% to 29% with gender differences. HIV prevalence in women is reported to reach 40% in some communities. Overall HIV prevalence in Tanzania is 7.6% with gender and regional differences. The prevalence is higher in women (11%) compared to men (6.3%) and higher in Kagera region (12.5%) compared to Mwanza (7.3%) and Mara (6%). HIV incidence is also variable across different communities. In Uganda, incidence range from 3.39 to 4.9 and seem to increase with age and alcohol consumption. In Kenya, HIV incidence vary from 4.2 to 9.3% and seem to depict regional variations. Risk factors for HIV infection include: age at sexual debut, multiple and transactional sexual partnerships, alcohol consumption and low condom use. The risky behaviors are precipitated by high number of women in fishing villages, scarcity of fish, high cash flow, high fisherfolk mobility and culture of risk denial. Potential interventions include: reaching the fishermen with late night radio prevention messages, synchronizing opening and closing of health clinics with fisherfolk availability, targeting couples for interventions to reduce risky behaviors, and empowering women to own boats to reduce their interactions with men over fish. Fishing communities experience high HIV burden that exhibit gender and regional variations. The high burden results from personal, interpersonal and environmental factors. Identifying appropriate HIV interventions in these communities require thorough understanding of their high risk social environment.

**172 Sex, Stigmas, and Systems: Global Issues in HIV Among Young MSM****LaRon E. Nelson**, Univ of Rochester, Rochester, NY, USA

The burden of new HIV continues to disproportionately impact young MSM. In the United States and Canada MSM under age 25 represent the majority of new infections. Young Black MSM in the United States are overrepresented in the number of new cases and continue to be the group with the largest percent increases in new infections. In other regions of the world, such as sub-Saharan Africa, data is only now beginning to emerge regarding the scope of the HIV epidemic in MSM. More research is being conducted to better understand the mechanism by which stigmas contribute to age-disparities in HIV infection and HIV outcomes of MSM around the world. Various forms of stigma include HIV stigma, stigma against same-gender sexual practices and identities, and stigma directed towards MSM who do not conform to masculine gender norms. The sexualities of MSM may be further stigmatized for those who also engage in sexual practices with cisgender and/or transgender women. The intersections of these various stigmas have the potential to be very powerful barriers to moving young MSM along the HIV continuum of care, including diagnosis. Recent research also suggests that stigmas—conceptualized as a source of chronic environmental stress—may exert a physiological load on body systems and consequently have negative impacts on clinical outcomes for HIV-infected individuals. Moreover, the experiences of these stigmas are not limited to social situations but also manifest themselves in clinic and program environments where young MSM are expected to receive care and support. Health systems-level anti-stigma intervention approaches are needed that target healthcare providers and personnel to create healthcare environments that support the autonomy and human rights of youth who are MSM. There is also a need for models of care that reduce the frequency with which young MSM are exposed to environments that they experience as stigmatizing. Models of care that decentralize access to health care such as telehealth and mobile-app enabled platforms for care engagement and coordination have the potential to increase access and utilization of HIV prevention and treatment services for young MSM. These and other types of healthcare systems transformations are necessary in order to optimize the impact of biomedical prevention and treatment technologies for young MSM.

**173 HIV and Migrants****Julia Del Amo**, Inst de Salud Carlos III, Madrid, Spain

Migrants have been long identified as one of the populations with highest vulnerability to HIV infection and its consequences. Migrants encompass heterogeneous groups of persons with different migration drivers - economic, social, political, cultural and environmental - as well as with distinct risk-contexts for HIV infection. Some migrant groups have a disproportionate burden of HIV infection which often exhibits distinct gender patterns. The relative contribution of migrants to national epidemics varies across the world, the highest being in Europe and Asia. Between 2007 and 2012 nearly two-fifths of all HIV cases reported in the European Union/European Economic Area were of migrant origin; the commonest being Sub-Saharan Africa, other European countries and Latin-America & The Caribbean. The epidemiological patterns of HIV in these migrants resembles that of the countries of origin; with a highly feminized and fundamentally heterosexually acquired epidemic in migrants from Sub-Saharan Africa and a very high proportion of Men who have sex with Men (MSM) among the cases from Latin-America. HIV infection can be acquired in the pre and post-migration phases as well as in the migratory transit; circular migration is of relevance in some settings such as the US/Mexican border. There is increasing evidence of the higher risk of HIV acquisition among migrant MSM in the post-migration phase, highlighting the sexual diversity of the migrant population. Most migrant groups, particularly the undocumented, experience difficulties to access HIV testing and health care. Further, in most countries undocumented migrants are not entitled to antiretroviral treatment (ART). As a consequence, late HIV diagnosis and presentation are commoner among migrants who also exhibit lower CD4 cell counts at ART initiation and poorer immunological and virological responses. Access to ART is one of the pillars of the end of AIDS UNAIDS strategy. It has been firmly established how early HIV diagnosis and linkage to care are beneficial at individual and community levels but this all relies in universal and equitable access to ART. Finally, effective implementation of large-scale treatment programs cannot be achieved without a global commitment to guarantee access to ART for all persons living with HIV which does not leave a subset of the population behind.

**174 Global Epidemiology of HIV Infection in Adolescents****Annette H. Sohn**, TREAT Asia, Bangkok, Thailand

Global HIV surveillance estimates variably categorize adolescents (10-19 years) with children (<15 years), youth (15-24 years), and non-adults (<25 years). At the end of 2013, there were 2.1 million adolescents living with HIV (ALHIV), 220,000 of whom had been newly infected that year. In 2014, there were 3.9 million youth with HIV, of whom 2.8 million

were in sub-Saharan Africa. Almost two-thirds of all adolescents and youth with HIV are females. Capturing adolescent data is particularly complex because these data represent a combination of perinatally and behaviorally infected ALHIV – a factor that is critical to understanding clinical and programmatic outcomes, but which is not disaggregated in surveillance or program evaluation. These groups have substantially different social contexts and HIV disease experiences. Perinatally infected ALHIV are frequently orphaned, highly treatment experienced, and experiencing long-term complications of their disease after a lifetime of immunosuppression. Behaviorally infected ALHIV are coping with a relatively recent diagnosis, more often from key affected populations, have less severe disease and better immune function, and poorer treatment uptake. Adherence and retention research has shown that older adolescents, who are more likely to be behaviorally infected, are twice as likely to be lost to follow-up compared to younger adolescents and older adults. However, HIV-related mortality among ALHIV is more frequently due to deaths of the perinatally infected. The persistent increase in ALHIV death compared to a 41% decrease observed in adults since 2005 represents a serious failure of the global HIV response. When it was reported in 2014 that HIV was the second leading cause of death of adolescents worldwide and the most common cause of death in sub-Saharan Africa, policy makers and advocates began campaigning to promote investment into the continuum of prevention, testing, and care for adolescents. In order to achieve current targets for reduction of new infections and expansion of care and treatment, resources would have to increase by at least 45%. In addition, the quality of the strategic information used to monitor the ALHIV epidemic and the impact of programmatic interventions would need to improve by disaggregating surveillance data by age, sex, mode of exposure, and key population.

### 175 Blame It On the Brain

**Lisa K. Simons**, *Ann and Robert H. Lurie Children's Hosp of Chicago, Chicago, IL, USA*

Adolescence is understood to be a period of tremendous growth and development: physically, cognitively, emotionally and socially. While most adolescents experience their peak physical health during this time, morbidity and mortality during adolescence rise substantially. This paradoxical increase is largely related to an upsurge in risk-taking behaviors observed in adolescence. Over the past fifteen years, advancements in neuroimaging studies have offered enhanced understanding of structural and functional changes which occur in the brain during adolescence. One model of conceptualizing adolescent risk-taking behavior (proposed by developmental psychologists such as B.J. Casey and Laurence Steinberg) relies in part on the differential timing and rate of maturation of regions of the brain implicated in modulating reward sensitivity and exerting cognitive control. While these neurodevelopmental processes should not be presumed to be the singular cause for increased risk-taking behaviors during adolescence, they suggest some neural explanation for adolescents' propensity towards risk-taking behaviors. This expanded understanding of adolescent neurodevelopment has important implications for clinicians caring for adolescents living with HIV and for those developing interventions targeted at adolescents.

### 176 Finding and Engaging Adolescents With HIV in Low- and Middle-Income Countries

**Rashida A. Ferrand**, *London Sch of Hygiene & Trop Med, London, UK*

Adolescents are the only group in which HIV-associated mortality continues to rise, despite the remarkable global scale-up of antiretroviral therapy. This is mainly due to delayed diagnosis with less than a third aware of their HIV status globally. HIV testing strategies for adolescents to date have primarily been similar to those employed for adults and fail to take into account the specific legal, sociocultural and structural barriers to HIV testing faced by this age-group. Innovative strategies to identify adolescents living with HIV that are context- and age-appropriate, sustainable and acceptable to both adolescents and their caregivers are required. Adolescents fare disproportionately poorly across the HIV care continuum following diagnosis and therefore testing strategies must be accompanied by strategies to engage adolescents who test HIV-positive with care services. Evidence for such strategies is sparse. The talk will review existing evidence base for innovative interventions to identify adolescents living with HIV, including both health facility and community-based strategies, and describe how these can overcome barriers to HIV testing and engagement with HIV care in this age-group. It will highlight research priorities to address the burden of undiagnosed HIV in this age-group in lower and middle-income countries.

### 177 Thinking About the Future: Transition for Adolescents With HIV

**Allison L. Agwu**, *Johns Hopkins Univ, Baltimore, MD, USA*

The HIV epidemic among adolescents and youth includes a heterogeneous mixture of survivors of perinatally-acquired HIV infection and those acquiring infection during the second decade of life. Youth living with HIV (YLHIV) have poorer rates of diagnosis, engagement and retention in care, and initiation of combination antiretroviral therapy (cART); and higher rates of cART discontinuation than older adults. All perinatally HIV-infected and approximately 20% (in the U.S.) of non-perinatally HIV-infected youth initially engage in care in pediatric/youth HIV clinics. Studies have demonstrated better ART initiation, retention, and virologic suppression for YLHIV followed in pediatric/youth compared to adult HIV care. However, given the limitations in the structure of these pediatric/youth clinics, YLHIV must transition to adult care, between the ages of 15 and 25 years, depending on the region where they reside. There are a multitude of logistic, structural, and psychosocial barriers to transitioning YLHIV from pediatric/youth to adult care. Therefore, YLHIV are at increased risk of loss to care and associated negative outcomes (e.g., nonadherence, viremia, immunologic deterioration, morbidity, and HIV transmission) during and after this transition period. Guidelines recommend strategic comprehensive, youth-friendly, integrated transition approaches to effectively transition the care of these YLHIV from pediatric/youth to adult HIV care. However, there is limited data on transition strategies, best practices, and clinical outcomes. The aging up of YLHIV creates a critical need to define successful transition, assess different transition strategies, including their implementation, cost, feasibility and sustainability, and importantly, determine the clinical outcomes of the strategies employed. This information is needed to inform guidelines and best practices to optimize retention and clinical outcomes, during and after transition, for YLHIV.

### 178 NK Cell-Mediated Recognition of HIV

**Marcus Altfeld**, *Heinrich Pette Inst, Hamburg, Germany*

NK cells represent the main cytotoxic effector cell population of the innate immune system, and play an important role in the control of viral infections. The mechanisms by which NK cells can recognize HIV-1-infected cells remain incompletely understood. On this presentation, new data will be presented on the receptor/ligand interactions that allow NK cells to recognize and kill HIV-1-infected cells, and on the mechanisms by which HIV-1 can evade NK cell-mediated recognition.

### 179 Signatures of Protective NK Cell Responses

**Catherine A. Blish**, *Stanford Univ, Stanford, CA, USA*

Natural killer (NK) cells play critical roles in immune defense and reproduction, but remain the most poorly understood major lymphocyte population. NK cells contribute to control of chronic HIV infection, yet their role in protection from infection is less clear. NK cell activation is controlled by a variety of combinatorially expressed activating and inhibitory receptors, making NK cell diversity and function closely linked. We recently defined that the human NK cell repertoire is remarkably diverse, with an estimated 6000 to 30,000 phenotypic populations within an individual and >100,000 phenotypes in a small population. Furthermore, our studies revealed immune experience diversifies and specializes the NK cell repertoire. Finally, high NK diversity is associated with increased risk of HIV-1 acquisition in a Kenyan cohort. Overall, these studies suggest that NK diversity may decrease the flexibility of the antiviral response, and that human NK diversity is a previously undefined metric of immune history and function that may be clinically useful in predicting the outcomes of viral exposure.

### 180 Supercharging NK Cells for Cure

**Jonathan Karn**, *Case Western Reserve Univ, Cleveland, OH, USA*

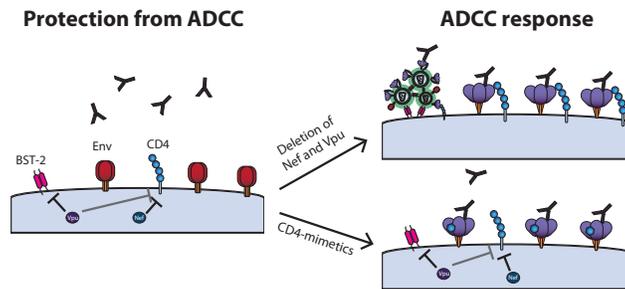
We are developing a novel HIV eradication strategy that combines proviral reactivation, *ex vivo* stimulation, expansion of natural killer (NK) cells, and enhancement of NK cell killing specificity and cytotoxicity via antibody-dependent cell-mediated cytotoxicity (ADCC). Our main hypothesis is that MHC Class I downregulation induced by the expression of Nef

creates an opportunity for activated NK cells to target and kill latently infected primary T cells. We further hypothesized that broadly neutralizing antibodies (bnAb) recognizing the HIV envelope would be capable of mediating ADCC to target a wide range of HIV proviruses. Novel assays for NK cell effector function have been developed using both primary cell models of HIV latency and patient cells. For the primary cell models loss of HIV GFP expression and delivery of granzyme B are measured by flow cytometry. For patient cells loss of inducible cell-associated HIV mRNA and proviral DNA is measured using highly sensitive next generation sequencing readouts. When NK cells were cocultured with cells expressing IL-21, we observed dramatic NK cell expansion and enhancement of cytotoxicity against reactivated latently infected primary T cells. Using this protocol we are able to expand large numbers of highly cytotoxic NK cells that maintain their specificity for killing of infected cells and their ability to mediate ADCC. Importantly, this protocol is able to efficiently expand and activate NK cells from HIV+ donors. We have identified a combination of the histone deacetylase inhibitor (HDACi) Panobinostat with the PKC agonist Bryostatins or the combination of IL-15 with the HDACi SAHA as regimens that can optimally reactivate HIV-1 while inducing minimal changes in cell surface expression profiles. Coculture of NK cells with infected primary T cells led to preferential killing of reactivated cells. We have identified at least one anti-Env antibody that can strongly mediate ADCC and enhance selectivity of killing reactivated HIV-1-infected CD4+ T cells while sparing uninfected cells. This antibody demonstrates ADCC activity at both low antibody and low NK cell to target cell ratios. We have also identified three more anti-Env antibodies that can enhance ADCC. Enhancing NK cell activity may represent an important new approach to virus eradication. We are optimizing protocols that can be rapidly translated into the clinic that involve proviral and NK cell reactivation and enhancement of killing by ADCC.

**181 Exposing Env: A New Strategy to Target HIV-1–Infected Cells by ADCC**

**Andrés Finzi**, CRCHUM, Montreal, QC, Canada

Prevention of HIV-1 transmission and progression likely requires approaches that can specifically eliminate HIV-1-infected cells. There is increasing evidence supporting a role of Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) in controlling HIV-1 transmission and disease progression. However, Env epitopes targeted by antibodies effective at mediating ADCC are poorly exposed on the unliganded HIV-1 envelope glycoprotein (Env) trimer. Indeed, HIV-1 has evolved a sophisticated mechanism to avoid exposure of ADCC-mediating Env epitopes by downregulating CD4 and by limiting the amount of Env at the cell surface. We observed that interaction of Env with the CD4 receptor was required for efficient exposure of ADCC-mediating Env epitopes. In that context, HIV-1-infected cells presenting HIV-1 Env in the CD4-bound conformation were found to be preferentially targeted by ADCC-mediating antibodies present in HIV+ sera. We therefore tested the capacity of rationally-designed CD4-mimetic compounds (CD4mc) to promote the CD4-bound conformation of Env and thereby sensitize HIV-1-infected cells to ADCC. We observed that certain CD4mc were able to induce the CD4-bound conformation of Env, thus enhancing the susceptibility of HIV-1-infected cells to ADCC. Of note, the ADCC-mediating activity in HIV+ sera appears to be mainly directed against the gp120 inner domain. Upon testing a panel of anti-Env antibodies we found that the anti-cluster A class mediated the most robust ADCC response. These antibodies bound Env with a unique angle of approach resulting in optimal Fc exposure. Thus, allowing for efficient engagement by Fcγ receptors present on effector cells. Altogether, our data suggest that forcing Env to expose highly-conserved epitopes, recognized by antibodies present in the sera of HIV-1-infected individuals, might represent a new approach for HIV eradication strategies.



# POSTER ABSTRACTS

## 182 Identification of MDM2/HDM2 as a Positive Regulator of HIV-1 in Human Macrophages

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**Background:** Macrophages play an important role in the establishment and propagation of HIV-1 infection. Upon exposure to HIV-1, only a small proportion of macrophages are infected whereas most remain uninfected. To shed light on this issue, transcriptomic and proteomic analyses were performed to compare the infected and bystander populations and determine the molecular basis of HIV-1 permissiveness in macrophages. Precise mapping of the interactions between HIV-1 and host signaling pathways identified several new potential regulators of HIV-1 replication in macrophages. One of these was the human ortholog of murine double minute 2 (*mdm2*), HDM2, an E3 ubiquitin ligase regulating the turn-over of various proteins, including p53.

**Methods:** We developed an experimental model based on the identification and isolation of infected monocyte-derived macrophages (MDMs) within a heterogeneous population. MDMs are exposed to a competent R5 HIV-1 reporter virus expressing all viral genes before immuno-magnetic sorting. Transcriptomic and proteomic analyses led to the selection of 50 genes that were all tested for their functional role on HIV-1 replication by siRNA screen. MDMs were reverse transfected with 2 different siRNA sequences per target and infected with GFP- or luciferase-encoding HIV-1.

**Results:** Although silencing of some specific targets resulted in an increase in viral replication, only the knockdown of MDM2/HDM2 induced a 2-fold decrease of HIV-1 expression. This reduction in viral replication was confirmed by flow cytometry and p24 ELISA immunoassay. Viability assays further demonstrated that no apparent toxicity was associated with MDM2/HDM2 silencing at 72h. Efficiency of siRNA-mediated silencing was assessed by qRT-PCR and western blot analysis and revealed that no change in the mRNA level of MDM2/HDM2 could be observed at 72h post-transfection. Earlier time points were thus tested and showed an efficient silencing of MDM2/HDM2 only at 24h post-transfection.

**Conclusions:** Our results indicate that the resistance to HIV-1 associated with MDM2/HDM2 silencing is maintained in MDMs even if MDM2/HDM2 mRNA level is restored, thus suggesting that this protein might be indirectly involved in HIV-1 infection. Identification of viral cofactors regulated by MDM2/HDM2 will bring a new understanding of signaling events controlling HIV-1 replication in macrophages.

## 183 HIV-1 Induces p21-Mediated Cellular Senescence in Human Primary Macrophages

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**Background:** Cellular senescence (CS) represents a state of permanent cell cycle arrest in response to a variety of stressors in which cells irreversibly stop dividing but remain metabolically active. This state is maintained by either or both the p53/p21 and p16INK4a/pRb tumor suppressive pathways that cross-regulate each other. HIV-1 infection is a known external inducer of cell cycle arrest and cellular senescence and although is associated with clinical symptoms of accelerated aging, it has been difficult to detect an accelerated aging effect at a molecular level.

**Methods:** Monocytes purified from healthy blood donors were differentiated to macrophages (MDM) with M-CSF and infected with a full-replicative R5 HIV-1 strain BaL. Senescent cells were identified by staining of SA-β-gal and visualization under optical microscope. mRNA relative levels of the interest genes were measured by qPCR and protein expression was assessed by Immunoblot. Cell cycle profile and proliferation status was investigated by flow cytometry. siRNA interference against the Rb gene was performed in isolated monocytes and the differentiated MDM were infected with VSV-pseudotyped NL4-3 GFP-expressing virus and a R5 HIV-1 strain BaL. Total viral DNA formation was quantified by qPCR and HIV replication measured by flow cytometry.

**Results:** Monocyte differentiation into macrophages with M-CSF led to cell proliferation and susceptibility to HIV-1 infection that, in turn, induced cell cycle arrest at G2-M. Established HIV-1 infection activated STAT1 phosphorylation, transcription of interferon-stimulated genes and production of β-galactosidase, a marker of senescent cells. In addition, there was an increased expression of p21 and subsequent inactivation of cyclin-CDK2 activity leading to a hypo-phosphorylated active retinoblastoma protein (pRb) and deactivation of E2F1-dependent transcription. Additionally, HIV infection led to downregulation of the ribonucleotide reductase subunit R2 (RNR2) and reactivation of the HIV-1 restriction factor SAMHD1. pRb knockdown in primary MDM does not block HIV-1 viral DNA formation and HIV-1 replication

**Conclusions:** HIV-1 induces a state of cellular senescence in primary macrophages. In addition, characterization of the cell-signaling pathway involved in the establishment of the HIV-1 induced senescence phenotype showed that it is driven by p21-dependent CDK inactivation that leads to pRb hypo-phosphorylation and SAMHD1 reactivation.

## 184 HIV-1 Attached to Monocytes, but Not Lymphocytes, Transmits Infection to Human Tissue

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**Background:** Semen of HIV-1 infected men contains free virus as well as infected cells. It remains a matter of debate whether cell-associated or cell-free virus in the semen is predominantly transmitted in the course of HIV-1 sexual transmission. Seminal infected cells produce virus that upon release will essentially become free. However HIV can be also adsorbed on seminal cells, such as seminal monocytes and lymphocytes. Here, we focused on this cell-adsorbed virus and investigated whether it could contribute to HIV sexual transmission from an infected man to his sexual partner.

**Methods:** We developed a protocol to adsorb HIV on the cell surface and investigated whether this adsorbed virus can induce productive infection of human tissue *ex vivo*. Briefly, elutriated blood human lymphocytes or monocytes were gamma-irradiated (25 Gy) and then exposed to free R5 HIV-1<sub>BaL</sub> for 2h at 4°C to prevent virus internalization. To evaluate adsorbed HIV-1, cells were treated with trypsin, washed and lysed for p24 quantification.

**Results:** We demonstrated that, under our protocol, ≥ 95% of virions were located on the cell surface (cell-associated p24: 0.92 ng/ml before and 0.043 ng/ml after trypsin treatment; n=3). We compared the ability of cell-adsorbed virus (on lymphocytes or monocytes) and cell-free virus to infect human tissues *ex vivo*. Towards this goal, we prepared free viral suspension with p24 concentration equal to that of cell-adsorbed virus and inoculated matched tissues with both viral preparations. HIV-1 adsorbed on Lymphocytes did not transfer infection to tissue *ex vivo* even after 15 days of culture (n=5). Using TZM-bl cells, we showed that lymphocyte-associated viruses were not transferred to target cells either. In contrast, HIV-1-adsorbed on monocytes were able to induce productive infection in tissue *ex vivo* similarly to that of free virus (p=0.41), as assessed by flow cytometry and by measuring p24 in cultural media (n=8). When 25% of human semen was added to monocyte-adsorbed HIV, we observed that viral replication in tissue was increased by 4.

**Conclusions:** Our results suggest that HIV-1 adsorbed on the surface of monocytes, but not on lymphocytes, can initiate a productive infection in human tissues with the same efficiency as free HIV, and that semen facilitates free HIV-1 infection probably by increasing virus transfer to target cells.

**185 Essential Role for Vpr in Productive Infection of Dendritic Cells by HIV-1**

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**Background:** Viral protein R (Vpr) is a small accessory protein that is packaged into the budding HIV-1 particle through its association with the p6 region of Gag, and is thus present at a high quantity in target cells upon infection. While numerous functions have been attributed to Vpr, very few of these have been studied in the context of HIV-1 infection of dendritic cells (DCs). In this work, we show that Vpr expression is essential at early steps of viral replication in DCs and that infections with  $\Delta$ Vpr viruses or viruses that encode Vpr mutants unable to interact with the DNA damage repair complex SLX4 (SLX4com), display a significant replication defect within a single round of infection.

**Methods:** To determine the mechanism of Vpr-dependent enhancement of virus replication in DCs, we constructed a panel of replication competent and single cycle (luciferase expressing) viruses with point mutations in Vpr at F34I, W54R, Q65R and H71R, or frame shift mutation ( $\Delta$ Vpr). Effects of selective Vpr inactivation on integration, viral protein expression, and virus spread in infected DC cultures was determined by *Alu*-PCR, Western blot analysis, luciferase expression, and ELISA.

**Results:** We observed reduced virus spread in DCs infected with viruses lacking Vpr expression ( $\Delta$ Vpr), or those encoding mutant Q65R- or H71R-Vpr alleles that disrupt the previously characterized association between Vpr and the Cul4a/DCAF/DBB1 ubiquitin ligase complex as well as SLX4com. Attenuated replication of HIV-1/ $\Delta$ Vpr in DCs was not due to a defect in provirus integration or in viral glycoprotein production. Finally, a 8-10 fold decrease in luciferase expression was observed in infections of DCs with single cycle viruses encoding  $\Delta$ Vpr, Vpr-Q65R or Vpr-H71R compared to WT virus infection suggesting that Vpr interaction with the DNA damage response pathway is critical for inducing optimal levels of transcriptional activity from the viral LTR.

**Conclusions:** Together, this data suggests that Vpr expression modulates the level of infection in a single round of replication in DCs. Contrary to previous reports in other cell types, this is not due to effects of Vpr on integration or viral glycoprotein production. Rather, Vpr affects the level of viral LTR transcriptional output through a mechanism that is mediated by the recruitment of the SLX4com and associated ubiquitin ligase machinery. These interactions are necessary for robust viral transcription during infection of DCs by HIV-1.

**186 Cell Membrane Binding Determines Ability of bNAb to Prevent HIV Cell-to-Cell Transfer**

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**Background:** Mature monocyte-derived dendritic cell (MDDC)-mediated HIV-1 transmission to CD4<sup>+</sup> T cells (trans-infection) occurs across tight cellular junctions called virological synapses (VS). We have previously reported that anti-gp120 bNAbs inefficiently block HIV-1 transmission across VS due to steric hindrance, whereas MPER recognizing bNAbs that bind to MDDC plasma membrane efficiently inhibit this transfer. In this study, we determined if binding of newer and more potent bNAbs to plasma membrane of virus-transmitting MDDCs as well as CD4<sup>+</sup> T cells is necessary for efficient neutralization of cell-to-cell transmission of HIV-1.

**Methods:** HIV-1 primary isolates were incubated with LPS-matured MDDCs or used to infect activated primary CD4<sup>+</sup> T cells. Neutralization of virus transfer from these HIV-1 laden cells was examined by measuring luciferase activity after incubating with TZM-bl reporter cell line in the absence or presence of increasing concentrations of anti-gp120 (2G12, VRC01, PGT121 and PGT126) or MPER (4E10 and 10E8) bNAbs and was compared with that of cell free virus using the Wilcoxon rank sum test. Immunofluorescence microscopy and ELISA was used to examine bNAb binding to MDDCs or CD4<sup>+</sup> T cells.

**Results:** Compared to the ability to block infection of TZM-bl by cell free virus, 10E8 showed similar ability to inhibit MDDC-mediated trans-infection ( $p > 0.05$ ), but it was significantly less potent in blocking CD4<sup>+</sup> T cell transfer ( $p = 0.04$ ). PGT121 neutralized cell free virus, MDDC-mediated trans-infection, and CD4<sup>+</sup> T cell mediated cell-to-cell virus transfer with similar efficiency. VRC01 was less efficient in blocking both MDDC and CD4<sup>+</sup> T virus transmission as compared to cell-free virus. Binding assay revealed that both 2G12 and VRC01 displayed negligible binding to MDDC or CD4<sup>+</sup> T cell plasma membrane. Intriguingly, PGT121 robustly bound plasma membranes of both MDDCs ( $p = 0.0013$ ) and CD4<sup>+</sup> T cells ( $p = 0.0135$ ), while 10E8 bound only MDDC surface ( $p = 0.002$ ) but not CD4<sup>+</sup> T cell surface ( $p > 0.05$ ).

**Conclusions:** Ability to block cell-to-cell transmission is strongly correlated with the ability of bNAbs to bind cell surface of virus transmitting cell even in the absence of virus antigen, suggesting that binding to host cell membrane is an important parameter of determining the neutralization potential of anti-HIV-1 bNAbs. Since cell-to-cell transmission is one of the major HIV-1 transmission pathways at mucosa, it would be interesting to test these membrane-binding bNAbs as topical antivirals.

**187 Enhanced Replication of Transmitted/Founder HIV-1 in Gut CD4 T Cells**

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**Background:** Sexual transmission of HIV-1 results in an extreme genetic bottleneck. Reconstruction of transmitted/founder (TF) and matched Chronic Control (CC) viruses revealed that TF strains had higher infectivity and replication capacity. However, these studies were performed in mitogen-activated PBMCs, which do not capture the T cell subset composition in mucosal compartments. Further, it remains unknown if TF HIV-1 are more efficient at killing gut CD4 T cells. We used the Lamina Propria Aggregate Culture (LPAC) model to analyze R5-tropic HIV-1 replication and CD4 T cell depletion with gut mucosal bacteria increased in abundance during HIV-1 infection (HAMB). Here, we compared the relative replication and depletion capacity of TF and CC strains in the LPAC model.

**Methods:** HIV-1 subtype B TF and CC virus stocks from 3 patients (CH40, CH470, CH58) were prepared from 293T-transfected infectious molecular clones. Lamina propria mononuclear cells (LPMCs) isolated from human jejunums ( $n = 10$  donors) were spinoculated with either mock, TF or CC HIV-1 (200ng p24/10<sup>6</sup> LPMCs) for 2h at 1500xg and cultured for 6 days +/- *Prevotella stercorea*, a prominent HAMB species. Infectious titers were analyzed by TZM-bl assay and cellular infection by intracellular p24 staining. CD4 T cell depletion was calculated from total cell counts relative to mock. Wilcoxon t-test was used for statistical analyses.

**Results:** TF infectious titers at 6 dpi were significantly higher than CC strains (CH40  $p = 0.004$ ; CH470  $p = 0.002$ ; CH58  $p = 0.002$ ). The %p24+ CD4 T cells was also higher for CH40 TF vs CC strains (TF 34.8% vs CC 28.0%;  $p = 0.027$ ). Co-incubation with *P. stercorea* led to a 3.8x increase in infectious titer ( $p = 0.0003$ ), a 10.4x increase in %p24+ cells ( $p < 0.0001$ ) and marked T cell depletion ( $p < 0.0001$ ). With *P. stercorea*, TF strains produced more infectious virus than CC strains ( $p < 0.0001$ ). However, there were no consistent differences in CD4 T cell depletion between TF vs CC strains. Gut CD4 T cell depletion did not differ between TF and CC viruses alone. With *P. stercorea*, depletion was increased for CH58 TF vs CC ( $p = 0.037$ ), but not for CH470 ( $p = 0.08$ ) and CH40 ( $p = 0.16$ ).

**Conclusions:** HIV-1 TF strains are more infectious than CC in gut T cells with or without a gut microbe linked to HIV-1 gut dysbiosis. TF and CC strains did not exhibit consistent differences in gut CD4 T cell killing. Our findings suggest that mucosal transmission and/or initial expansion selects for HIV-1 strains with enhanced replication in gut mucosal CD4 T cells.

**188 Human Th17 Cells Are Highly Permissive to Productive HIV Infection**

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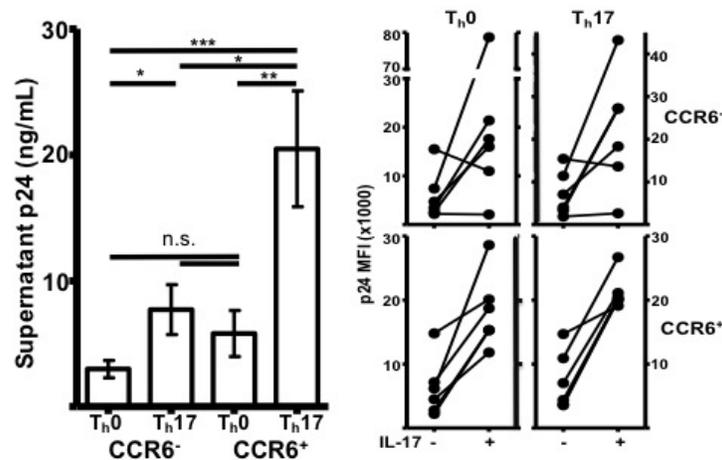
**Background:** Human Immunodeficiency Virus (HIV) preferentially depletes IL-17-producing "T helper 17" (T<sub>H</sub>17) cells during the acute phase of infection. The loss of T<sub>H</sub>17 cells impairs the integrity of the gut mucosal barrier, promoting chronic immune activation - a key determinant of disease progression. The susceptibility of T<sub>H</sub>17 cells has been attributed to high CD4, CCR5, and CXCR4 expression, but we hypothesized that intracellular factors also contribute to their preferential depletion during acute HIV infection.

**Methods:** Primary human CD4<sup>+</sup> T cells from HIV-negative blood donors were sorted according to their expression of CCR6 and activated in T<sub>H</sub>17- or T<sub>H</sub>0-polarizing conditions. Polarized cells were infected with one of four HIV isolates: HIV<sub>BAL</sub>, HIV<sub>IIIIB</sub>, HIV<sub>AD17</sub> (a transmitted-founder isolate) and HIV<sub>AMLV</sub> (a pseudotype HIV vector). Infected samples and

uninfected controls were analyzed by flow cytometry for their expression of immunological markers, cytokines and the HIV capsid protein, p24. Supernatant p24 was also measured by ELISA. Additionally, we used RNA microarrays and confirmatory immunoblots to compare gene expression patterns of cells from different polarizing conditions.

**Results:**  $T_H17$ -polarizing cytokines (IL-1b, IL-6, IL-23 and TGF- $\beta$ ) markedly increased HIV infection, relative to IL-2 treated, donor-matched controls (mean %p24<sup>+</sup> increased 2.75-fold, t-test  $p < 0.001$ ,  $n = 10$ ). Supernatant p24 from CCR6-sorted, HIV<sub>BAL</sub> infected cells was highest among donor-matched,  $T_H17$ -enriched populations ( $p < 0.001$ ,  $n = 5$ , left figure panel). HIV infection was significantly higher among  $T_H17$  cells compared with IL-17<sup>-</sup> or IFN $\gamma$ <sup>-</sup> cells, even upon infecting with HIV<sub>AMLV</sub>, a replication-defective HIV vector with a pseudotype envelope (%IL-17<sup>+</sup> cells were overrepresented among p24<sup>+</sup> cells by 2.9-fold,  $p < 0.001$ ,  $n = 6$ ). Further, HIV<sub>AMLV</sub>-infected  $T_H17$  cells produced more viral capsid protein per cell. In CCR6<sup>+</sup>-sorted cells, we observed a 3.0-fold increase in p24 geometric mean fluorescence intensity among IL-17<sup>+</sup> cells, compared with IL-17<sup>-</sup> cells ( $p < 0.01$ , right figure panel). Our data also reveal that  $T_H17$ -polarized cells have diminished expression of Ribonuclease A superfamily proteins.

**Conclusions:** Here we show that  $T_H17$  cells exhibit heightened permissiveness to productive HIV infection. Our findings link  $T_H17$  polarization to increased HIV replication, suggesting that  $T_H17$  cells might be major sources of viral production during acute HIV infection.



### 189 Spread of HIV-1 Is Delayed in T-Cells in the Absence of Integrin LFA-1

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**Background:** LFA-1 (Lymphocyte function-associated antigen 1) is found on all T-cells and is involved in recruitment to the site of infection. It binds to ICAM-1 on antigen-presenting cells and functions as an adhesion molecule. LFA-1/ICAM-1 interaction has been shown to be important for T cell-T cell interactions, leading to T cell proliferation and differentiation. LFA-1 is formed by the integrin alpha L chain ITGAL and beta 2 chain ITGB2. We wanted to study whether LFA-1 is important for HIV-1 replication in T-cells.

**Methods:** Jurkat T-cells were engineered using the CRISPR/CAS9 technology targeting ITGAL to generate LFA-1 knock-out cells. This LFA-1 KO cells showed no ITGAL expression but normal levels of ICAM-1 on the cell surface. Single-round HIV-1 reporter vectors and multiple round (virus spreading) experiments using HIV-1 NL4-3 were applied to compare wild type with LFA-1 KO cells.

**Results:** HEK293T express only very low levels of ICAM-1. Ectopic expression of ICAM-1 in HEK293T cells caused enhanced ICAM-1 incorporation in HIV-1 particles. HIV-1 luciferase reporter viruses - normalized for reverse transcriptase activity- gained infectivity (three to five-fold) by overexpression of ICAM-1 compared to particles made without ICAM-1 co-expression. Wild type and LFA-1 KO cells revealed no differential permissivity to HIV-1 reporter viruses, independent whether authentic Envelope or heterologous VSV glycoprotein was used for pseudotyping. This finding correlated with equal particle binding to wild-type and KO cells. However, replication competent HIV-1 showed a three-to-four day delayed replication kinetic in the LFA-1 KO cells in comparison to regular Jurkat cells.

**Conclusions:** The cellular factors that modulate HIV-1 replication in T-cells are only partially described. The ICAM-1 incorporation into HIV-1 affects the viral infectivity. Our data implicate that in T-cells the spreading replication is enhanced by LFA-1 expression. This data in addition show that LFA-1 is not an essential dependency factor for HIV-1, but might be relevant to describe disease variability in patients.

### 190 Inhibition of TCR Signaling Is Impaired in Nef Clones Derived From Elite Controllers

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**Background:** Nef is multifunctional HIV-1 accessory protein that is critical for pathogenesis and infectivity. Nef inhibits T cell receptor (TCR) signaling in infected CD4 T cells, presumably to reduce activation-induced cell death and increase progeny virion production, which may contribute to disease outcome. Elite controllers (EC) are a rare group of individuals who spontaneously maintain undetectable viral load in the absence of treatment. We previously showed that Nef clones derived from EC were impaired for numerous functions compared to clones from chronic progressors (CP). Here, we examined the ability of EC Nef clones to inhibit TCR signaling.

**Methods:** 45 EC and 46 CP Nef isolates were cloned into an expression plasmid. Jurkat cells were co-transfected with a plasmid expressing Nef and a NFAT driven-luciferase reporter plasmid. After 18 hours, cells were stimulated with anti-CD3 antibody to activate calcineurin via a calcium flux and luminescence was measured at 6 hours. The ability of Nef clones to inhibit TCR signaling was normalized to WT Nef (SF2 strain), such that function greater or less than WT is represented as >100% or <100%, respectively. Chimeric Nef constructs between 3 notably defective EC-derived isolates and SF2 Nef, and Nef point mutations, were generated by overlap extension PCR.

**Results:** Nef clones derived from EC displayed significantly lower ability to inhibit TCR signaling (median 87 [interquartile range (IQR) 75-93] %) compared to CP-derived clones (median 95 [IQR 89-98] %) ( $p < 0.001$ ). Codon-function analysis revealed a significant association between Nef R21K and reduced function (median 75% versus 92%) ( $p < 0.001$ ). Point mutations in SF2 Nef confirmed this result, with R21K, R21L and R21T displaying function 15%, 15% and 11% lower than WT SF2, respectively. Chimeric constructs mapped the contributing mutations of 3 notably defective EC clones to the first 32 amino acids of Nef. Rare polymorphisms were observed in these EC clones at highly conserved residues, R21L and P25T. A single amino acid reversion (T25P) in one EC clone restored function from 27% to 70%.

**Conclusions:** EC Nef clones displayed an attenuated ability to inhibit TCR signaling. These results highlight the potential impact of natural sequence variation on this function, especially within the N-terminal arm of Nef. Differences in Nef's ability to modulate T cell activation status could contribute to improved clinical outcome in some EC.

**191 HIV-1 Nef Dimerization Is Important for AP2 Recruitment and CD4 Downregulation**

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**Background:** The HIV-1 Nef accessory factor enhances viral infectivity, immune evasion, and AIDS progression. Nef hijacks host cell trafficking pathways to downregulate membrane-bound receptors, including CD4 via the adaptor protein 2 (AP2) complex. CD4 downregulation is linked to enhanced viral infectivity and immune escape, identifying this important Nef function as a rational target for new antiretroviral drug discovery.

**Methods:** We developed a cell-based bimolecular fluorescence complementation (BiFC) assay to visualize the interaction of Nef with the AP2 complex and CD4. Interacting protein pairs were fused to complementary, non-fluorescent fragments of YFP and co-expressed in 293T cells. Nef interactions with CD4 or AP2 result in complementation of YFP and a bright fluorescent signal by confocal microscopy.

**Results:** Nef interaction with both CD4 and the AP2  $\alpha$  and  $\sigma 2$  subunits was readily visualized in live cells by BiFC assay. Co-expression of the AP2  $\alpha$  subunit enhanced the Nef:AP2  $\sigma 2$  BiFC signal and vice versa, suggesting that the AP2  $\alpha/\sigma 2$  hemi-complex interacts cooperatively with Nef. Mutagenesis of Nef residues R134, E174, and D175, which stabilize the Nef docking surface for AP2 in a recent co-crystal structure (PDB: 4NEE), substantially reduced AP2 interaction without affecting CD4 binding. Remarkably, a dimerization-defective mutant of Nef failed to interact with either CD4 or AP2 in the BiFC assay, indicating that the quaternary structure of Nef is required for CD4 and AP2 recruitment as well as CD4 downregulation. Small molecule inhibitors of Nef dimerization also reduced Nef interactions with AP2 and CD4 by BiFC, providing further support for this idea and identifying the Nef dimer interface as a target for inhibitor action.

**Conclusions:** Here we describe a fluorescence complementation assay to visualize interactions between Nef, CD4 and the AP2 complex in live cells. Using this assay, we observed that the AP2  $\alpha$  and  $\sigma 2$  subunits interact cooperatively with Nef, and that the interaction is dependent upon an internal Nef salt bridge present in the recent crystal structure of the Nef:AP2 complex. Complex assembly also requires an intact Nef dimerization interface, consistent with previous observations that dimerization-defective Nef mutants fail to downregulate CD4. Small molecules that inhibit Nef dimerization also suppress AP2 and CD4 recruitment, which may provide a mechanistic explanation for their effects on HIV infectivity.

**192 Quantification of HIV-1 Splicing Using a PrimerID-Tagged Deep Sequencing Assay**

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**Background:** HIV-1 RNA is spliced to produce more than 40 different transcripts. Most previous research on HIV-1 splicing used model single exon systems and PCR gel quantification. Our straightforward assay quantifies HIV-1 RNA splicing patterns in the context of viral infection using Illumina paired-end deep sequencing and PrimerID. This system can be used to assess changes in splicing profiles by mutations that affect splice regulatory elements and to assess the differential effect of APOBEC3G-mediated mutagenesis on the 4 kb partially spliced mRNA population compared to the 1.8 kb mRNA population.

**Methods:** PrimerID is a random sequence tag in the cDNA primer, which is incorporated into a cDNA and all PCR products subsequently made from that tagged cDNA. Each cDNA gets a unique PrimerID and after sequencing, the PCR products can be sorted by their PrimerIDs. Each occurrence of a PrimerID is counted exactly once, and skewing in the PCR steps is filtered out. Additionally, a consensus sequence can be made from multiple reads with the same PrimerID, making it possible to filter out PCR and sequencing errors. PrimerID-tagged reverse primers specific for either the 1.8kb or 4kb splice classes and a common forward primer were used to tag the 40+ HIV-1 spliced mRNAs. Following amplification and Illumina paired-end deep sequencing, mRNAs were quantified according to splice type and collapsed into consensus sequences. Mutations in a stem near A1 and in the ESE(GAR) sequence located between A5 and D4 were correlated with splicing outcomes.

**Results:** Mutations to a highly conserved stem-loop containing splice acceptor A1 caused a 10-fold reduction in A1 usage, resulting in reduced vif transcript levels. The ESE(GAR) splice control element between A5 and D4 was analyzed for APOBEC3G mutations. Reduced vif transcripts led to increased C=>T mutations, which were unevenly skewed towards the 1.8kb splice class. T=>C mutations, though less frequent, skewed towards the 4kb splice class. As this panel of mutations in the ESE(GAR) region illustrates, C=>T and T=>C transitions differentially affect upstream (A5) splicing and/or downstream (D4-A7) splicing.

**Conclusions:** Combining PrimerID with paired-end deep sequencing allows mutations in a splice control element to be screened and correlated to real-time changes in HIV-1 splicing. We identified mutations near A1 and between A5 and D4 that affect utilization of the proximal splice sites.

Consensus Sequence of WT ESE(GAR) Splice Regulatory Element	D4-A7 Spliced to Unspliced Ratios	Fraction of A5 Usage in 1.8kb Size Class	Fraction of A5 Usage in 4kb Size Class	1.8kb:4kb A5 Usage Ratio
TTTGATAGAGAAGCTTGATGAGCTGACTGTTCTGATGAGCTCTTCGTCGCTGTCCGCT	0.992720104	0.638856523	0.863414678	0.739918534
<b>Consensus Sequences with Mutations Shown in Red</b>				
CTTGATAGAGAAGCTTGATGAGCTGACTGTTCTGATGAGCTCTTCGTCGCTGTCCGCT	1.281435144	0.691358025	0.855855856	0.807797271
TCTGATAGAGAAGCTTGATGAGCTGACTGTTCTGATGAGCTCTTCGTCGCTGTCCGCT	0.708577847	0.565217391	0.850877193	0.664276109
TTTGATAGAGAAGCTTGATGAGCTGACTGTTCTGATGAGCTCTTCGTCGCTGTCCGCT	0.796722193	0.632653061	0.888888889	0.711734694
TTTGATAGAGAAGCTCGATGAGCTGACTGTTCTGATGAGCTCTTCGTCGCTGTCCGCT	0.812713076	0.571428571	0.859504132	0.664835165
TTTGATAGAGAAGCTTGATGAGCTGACTGCTCTGATGAGCTCTTCGTCGCTGTCCGCT	0.708763436	0.671641791	0.855421687	0.785158713
TTTGATAGAGAAGCTTGATGAGCTGACTGTTCTGATGAGCTCTTCGTCGCTGTCCGCT	2.132335199	0.617647059	0.835714286	0.739064857
TTTGATAGAGAAGCTTGATGAGCTGACTGTTCTGATGAGCTCTTCGTCGCTGTCCGCT	4.745046288	0.25984252	0.531914894	0.488503937
TTTGATAGAGAAGCTTGATGAGCTGACTGTTCTGATGAGCTCTTCGTCGCTGTCCGCT	2.747073652	0.493670886	0.821782178	0.600732042
TTTGATAGAGAAGCTTGATGAGCTGACTGTTCTGATGAGCTCTTCGTCGCTGTCCGCT	3.24701875	0.493197279	0.748427673	0.658977877
TTTGATAGAGAAGCTTGATGAGCTGACTGTTCTGATGAGCTCTTCGTCGCTGTCCGCT	3.433786694	0.501628664	0.834394904	0.601188552
TTTGATAGAGAAGCTTGATGAGTTGACTGTTCTGATGAGCTCTTCGTCGCTGTCCGCT	3.206683113	0.59047619	0.765217391	0.771645022
TTTGATAGAGAAGTTGATGAGCTGACTGTTCTGATGAGCTCTTCGTCGCTGTCCGCT	1.313824664	0.708333333	0.923076923	0.767361111

**193 Effects of Differential Cell Signaling in HIV Transcription**

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**Background:** After cessation of antiviral treatments, HIV viral levels rebound due to the existence of latent reservoirs. The elucidation of signals that bias a provirus towards a latent or robust infection is necessary for more thorough treatment of HIV/AIDS patients. We propose a model system by which signals from CD3 and CD28, receptors necessary for T cell activation, can be manipulated at the time of infection of CD4+ T cells. This allows us to alter the balance between productive infection and the establishment of latent reservoirs.

**Methods:** Lentiviral vectors were used to transduce Jurkat and primary T cells with chimeric antigen receptors, which act as surrogates for the T cell receptor upon engagement with the corresponding ligand. These receptors include intracellular CD28 and CD3-zeta signaling domains fused to one of several anti-Her2 scFvs displaying different quantifiable affinities for Her2. Flow cytometry was used to isolate transduced cells. Expression of HIV was measured via luciferase or GFP. Integration of HIV was measured via nested Alu RT-PCR.

**Results:** Receptors were successfully transduced into CD4+ T cells. Preliminary data indicates that CAR activation leads to a more than 3 fold increase in HIV expression. Receptor affinity for Her2 does not correlate linearly with the level of HIV expression, indicating that signaling thresholds must be met for robust proviral transcription.

**Conclusions:** Adjusting the intensity of cellular signals at the time of infection allows us to control the robustness of HIV transcription and the course of the viral infection.

#### 194 High-Frequency Illegitimate Strand-Transfers Result in Defective HIV Genomes

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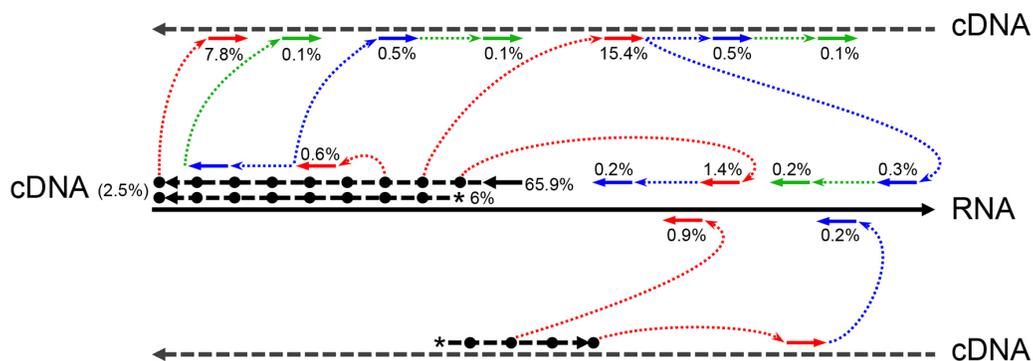
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**Background:** Two strand-transfers of nascent DNA fragments during reverse transcription are required to convert viral RNA genomes into a double-stranded proviral DNA genome for completion of each genome replication cycle. However, whether strand-transfers occur at illegitimate sites and how this may affect viral genome replication are not well understood.

**Methods:** Both HIV and HAV RNA templates were prepared in an in vitro T7 transcription system. The reverse transcription were carried out with reverse transcriptases from three retroviruses: HIV-1, HIV-2 and murine leukemia virus (MLV). The nascent cDNA fragments were directly cloned without PCR amplification. The sequences from individual reverse transcript clones were compared to the HIV or HAV template sequence to determine if new sequences contained mismatched sequences caused by illegitimate strand-transfers.

**Results:** A total of 1067 nascent reverse transcripts were sequenced. Most of them (72%) matched to the template sequences, although they randomly stopped across the RNA templates (~400bp). However, over one-quarter of reverse transcripts (28%) contained mismatched 3' sequences due to illegitimate strand-transfers. The majority (81%) of the illegitimate strand-transfers were disassociated from RNA templates and realigned onto opposite complementary DNA strands. Up to three strand-transfers were detected in a single sequences although the majority of them (93%) contained one strand-transfer. Since the majority of illegitimate strand-transfer fragments were generated from templates at two opposite orientations, they resulted in defective viral genomes and could not be detected by previous methods. Further analysis showed that mutations at pause/disassociation sites in new reverse transcripts resulted significantly higher strand-transfer rates. We also found significantly higher illegitimate strand-transfer rates for HIV-2 RT (38.2%) and MLV RT (44.6%) than for HIV-1 RT (5.1%).

**Conclusions:** Illegitimate strand-transfers frequently occur during reverse transcription and result in defective HIV genomes. These high frequency illegitimate strand-transfers may play an important role in retrovirus genome replication.



#### 195 Gag-Protease-Mediated Replication Capacity Differs According to HIV-1 Subtype

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**Background:** The HIV-1 epidemic is heterogeneous with various subtypes prevalent. Subtype-specific differences in disease progression rate have been reported; however the mechanisms underlying this heterogeneity are still unknown. We investigated whether there were subtype-specific replication capacity differences in recombinant viruses possessing patient-derived *gag-protease* genes from the Majengo sex worker cohort based in Nairobi, Kenya.

**Methods:** Antiretroviral therapy-naïve patients were recruited (n=103). Patient-derived *gag-protease* NL4-3 chimeric viruses were generated and their replication capacities assayed in an HIV-1-inducible green fluorescent protein reporter T cell line. Replication capacities of NL4-3 recombinant viruses bearing subtypes A, C, D and inter-subtype recombinants *gag-protease* were compared. An exploratory codon-by-codon analysis was performed using Kruskal-Wallis test to identify amino acid residues associated with differences in replication capacity for the subtype A recombinant viruses.

**Results:** There were 57 (56%) subtype A, 16 (15.5%) C, 13 (12.6%) D and 17 (16%) inter-subtype recombinant viruses studied. There were no significant differences in CD4 T cell counts or viral loads according to subtypes (ANOVA; p=0.53 and p=0.91 respectively). There were significant differences in viral replication capacities between the different subtypes (p=0.0001, ANOVA), with subtypes A and C displaying lower replication capacity compared to subtype D (p=0.0006 and p=0.0004 respectively) and inter-subtype recombinants (p=0.0006 and p=0.0006 respectively). We identified six amino acids that were significantly (p<0.05 and q<0.2) associated with reduced replication capacity in subtype A (L75I, I107L, S125S, S126S, N315N and S499S). The polymorphism 107L and consensus amino acid 315N were significantly more frequent in subtype A (Chi square test; p=10<sup>-8</sup> and p=0.01 respectively) and were associated with reduced replication capacity.

**Conclusions:** These data show a hierarchy of Gag-protease driven replicative fitness where subtypes A/C are less fit than D, which is also less fit than inter-subtype recombinants. The data is consistent with reported differences in subtype-specific differences in rate of disease progression. We identified amino acids that may contribute to these differences. Further studies to better understand the mechanisms underlying these differences are warranted. Furthermore, these data may have implications for the uneven spread and expansion of HIV-1 subtypes in the global epidemic.

## 196 Capsid Plays a Distinct Role in HIV Infection of TCR or TLR2-Activated CD4+ T Cells

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**Background:** HIV-1 capsid (CA) proteins play a crucial role in multiple stages of HIV infection including nuclear import. However, the function of the CA is not fully understood in primary resting CD4+ T cells. We have previously shown that TLR2 activation enhances HIV infection by promoting nuclear import in resting CD4+ T cells. In this study, we examined the role of the HIV CA protein and their analogs in TLR2-mediated enhancement of HIV infection in resting CD4+ T cells.

**Methods:** VSV-G pseudotyped luciferase reporter virus with wild-type (WT) CA or CA mutants were prepared by transfecting HEK293T cells. PBMCs were isolated from buffy coat of healthy donors by Histopaque gradient centrifugation. Primary resting CD4+ T cells were purified by negative selection from PBMCs, and exposed to pseudotyped virus for 2 h. Cells were stimulated with TLR2 ligand (Pam<sub>2</sub>CSK<sub>1</sub>) or immobilized anti-CD3 Ab in the presence of IL2. HIV infection was determined by measuring luciferase activity 3 days after infection. Cell pellets were collected 12 and 48 h after infection. Total DNA was isolated and analyzed for HIV late reverse-transcribed (RT) and 2LTR products by quantitative real-time PCR.

**Results:** E45A, E71A, and ΔcPPT mutations did not affect HIV infection of primary CD4+ T cells in response to TLR2 or TCR activation. Conversely, P38A, K70A, and L136D mutants were not infectious in TCR- or TLR2-activated CD4+ T cells. T54A/N57A, Q63A/Q67A, E128A/R132A, R143A, and Q219A mutants were infectious in CD4+ T cells in response to TCR activation, but not TLR2. The levels of late RT products were reduced in TLR2-activated CD4+ T cells infected by N74D mutant, but not other mutants. The levels of 2LTR circles, markers for HIV nuclear import, were also reduced in N74D mutant-infected cells. 2LTR circles were diminished in TLR2-activated CD4+ T cells with infection by P38A, T54A/N57A, Q63A/Q67A, K70A, E128A/R132A, L136D, and R143A mutants, suggesting a crucial role for these residues in nuclear import. Infection by Q219A led to greater levels of 2LTR circles compared to WT in TLR2-activated cells, suggesting that Q219 may be important for viral integration.

**Conclusions:** Mutations in the CA have differential impacts on HIV infection of TLR2- and TCR-activated CD4+ T cells. CA mutations altered 2LTR production suggesting its role in HIV nuclear import in primary CD4+ T cells in response to TLR2 activation. Our study provides an insight for developing antiretroviral agents targeting HIV CA proteins.

## 197 Consequences of p24 Gag Mutations for Capsid Stability and Viral Fitness

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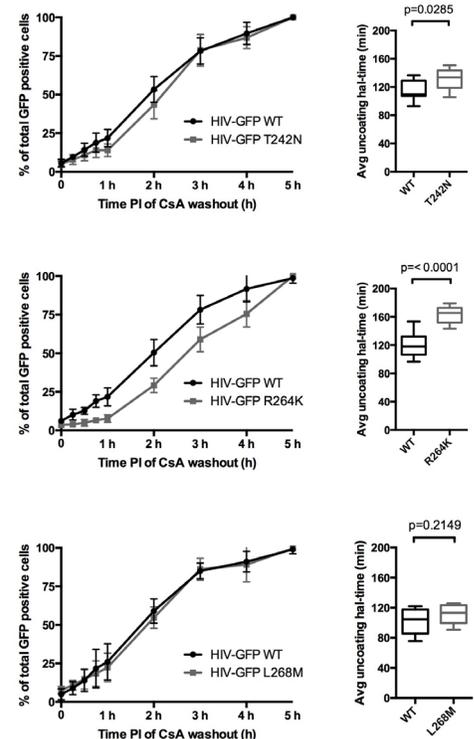
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**Background:** HIV-1-infected individuals encoding for specific HLA alleles, such as B27 and B57, exhibit better control of viremia. This has been associated with strong and highly potent HIV-1-specific CTL responses restricted by these alleles, forcing the virus to evade immune pressure through the selection of CTL-escape mutations with reduced viral fitness. While immunodominant HIV-1-specific CTLs in HLA-B 57+ subjects are directed against the TW10 epitope within p24 Gag, CTLs in HLA-B27+ subjects are directed against the KK10 p24 Gag epitope, resulting in CTL escape mutations (B57+: T242N, B27+ R264K and L268M) in these epitopes. The goal of our studies was to determine the consequences of these mutations within HIV capsid for viral uncoating and fitness.

**Methods:** An HIV-1 NL4-3 deltaEnv strain expressing GFP (HIV-GFP) in the place of Nef was modified using site-directed mutagenesis to encode for the p24 Gag mutations T242N, R264K and L268M, respectively. The capsid stability was determined using a Cyclosporin A (CsA) washout assay. Owl-monkey-kindey (OMK) cells, in which Trim-Cyclophilin A (CypA) inhibits HIV-infection by stopping the uncoating of the intracellular capsid, were infected with HIV-GFP variants in the presence of CsA. CsA blocks Trim-CypA in OMK cells. Washing-out CsA in a time-dependent manner allowed quantification of HIV Gag uncoating kinetics. Viral fitness was assessed in parallel by infecting primary PBMCs with the described HIV-GFP variants.

**Results:** The HIV p24 Gag mutations T242N, L268M and R264K resulted in significant reduced viral fitness. Compared to wildtype NL4-3, the T242N, L268M and R264K mutations showed an decrease of 35%, 17% and 85% of infected PBMCs, respectively ( $p=0.048$ ,  $p=0.014$  and  $p=0.028$ ). Significant differences were observed in uncoating kinetics between WT NL4-3 and T242N mutants (mean: 115 min SEM +/- 6min vs. 132 min +/- 5 min,  $p=0.03$ ) as well as between WT NL4-3 and R264K mutants (120 min: +/- 6 min vs. 164 min +/- 4 min,  $p<0.0001$ ). In contrast, no difference was observed between WT NL4-3 and L268M mutants. (Fig.1)

**Conclusions:** HIV-NL4-3 strains containing the HLA-B57- and HLA-B27-associated p24 Gag capsid mutations T242N and R264K uncoat significantly slower compared to the wildtype virus, indicating enhanced capsid rigidity and altered interactions with CypA. No significant differences in uncoating kinetics were observed for the L268M mutation, in line with previous data indicating that this mutation restores the viral fitness of R264K viruses.



## 198 The HIV Env Signal Peptide Impacts the Glycosylation and Antigenicity of gp120

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**Background:** Transmission of HIV across mucosal tissues involves a complex yet inefficient series of events that are not yet fully understood. The HIV-1 envelope protein (Env) of early-transmitting viruses has been found to present distinct transmission signatures. One such signature involves a reduced number of potential N-linked glycosylation sites, underscoring the importance of post-translational modifications in transmission fitness. Recently, an additional transmission signature has been identified in the signal peptide of Env. This signature involves the over-representation of basic residues at a specific position in the signal peptide. We investigated the potential impact of this signal peptide signature on gp120 glycosylation and antigenicity.

**Methods:** Two recombinant Envs were constructed, one derived from a chronic isolate that lacks the signal peptide signature and a second from a transmitting isolate that includes the signal peptide signature. Chimeric Envs were also constructed in which the two signal peptides were swapped. All four gp120s were probed with glycan-, structure- and interaction-specific probes in a surface plasmon resonance binding assay.

**Results:** The signature found in the signal peptide of Env influences qualitative aspects of Env glycosylation that in turn affect the structure and antigenicity of Env in a major way. The signal peptide impacts Env's affinity for DC-SIGN, a lectin receptor expressed on dendritic cells that is believed to be involved in mucosal transmission. Additionally, affinity for the monoclonal antibody 17b, which recognizes the CD4-induced conformation of Env is also altered.

**Conclusions:** We conclude that the HIV Env signal peptide is an important determinant of Env processing, folding and antigenicity. These observations may aid in the development of Env based HIV vaccines.

**199 Transcriptional Regulation of the APOBEC3 Repertoire Through the CBF $\beta$ /RUNX Axis****Brett D. Anderson**<sup>1</sup>; Reuben S. Harris<sup>2</sup><sup>1</sup>Univ of Minnesota, Minneapolis, MN, USA; <sup>2</sup>Howard Hughes Med Inst, Minneapolis, MN, USA

**Background:** Several members of the APOBEC3 family of DNA cytosine deaminases have the capacity to potently restrict HIV-1 replication by inducing extensive G-to-A mutations in the viral cDNA during reverse transcription. HIV-1 overcomes this antiviral defense by encoding the small protein Vif, which recruits a cellular ubiquitin ligase to target the APOBEC3 proteins for degradation through a polyubiquitination mechanism. Recently, we identified the cellular transcription factor CBF $\beta$  as an obligate Vif binding partner and essential component of this ubiquitin ligase. CBF $\beta$  normally forms a heterodimer with members of the RUNX family of DNA binding transcription factors to regulate expression of numerous genes involved in hematopoietic development and immune function. A recent structural study on the Vif-CBF $\beta$  complex revealed that Vif engages a surface on CBF $\beta$  that overlaps significantly with the known RUNX binding surface. Therefore, we hypothesized that the viral hijacking of CBF $\beta$  to promote APOBEC3 degradation may concurrently promote viral replication by directly impeding the assembly of functional CBF $\beta$ /RUNX heterodimers, ultimately resulting in altered expression of cellular genes.

**Methods:** A combination of shRNA knockdown and CRIPSR/Cas9 gene disruption approaches were employed to deplete CD4+ T cells of endogenous CBF $\beta$ . CBF $\beta$ -dependent changes in cellular gene expression were measured by immunoblotting and RT-qPCR, and HIV-1 replication kinetics in CBF $\beta$ -depleted cell lines were assayed in single-cycle and spreading infection experiments.

**Results:** Surprisingly, we found that CBF $\beta$  functions in complex with the RUNX proteins as positive regulators of the APOBEC3 genes themselves in CD4+ T cells, and that genetic knockdown and knockout of CBF $\beta$  is sufficient to render T cells permissive to Vif-deficient HIV-1 replication due to a loss of antiviral APOBEC3 gene expression.

**Conclusions:** Based on these results, we propose a two-pronged model whereby HIV-1 Vif counteracts the APOBEC3 antiviral defense by directly promoting APOBEC3 polyubiquitination while simultaneously suppressing APOBEC3 gene transcription by sequestering CBF $\beta$  from RUNX-associated transcription complexes.

**200 Is Endogenous APOBEC3H an HIV-1 Transmission Barrier?****Jiayi Wang**<sup>1</sup>; Allison M. Landl<sup>1</sup>; Brian J. Hoium<sup>1</sup>; Eric W. Refsland<sup>1</sup>; Elizabeth M. Luengas<sup>1</sup>; Romel D. Mackelprang<sup>2</sup>; William L. Brown<sup>1</sup>; Michael Emerman<sup>3</sup>; Jairam R. Lingappa<sup>2</sup>; Reuben S. Harris<sup>4</sup><sup>1</sup>Univ of Minnesota, Minneapolis, MN, USA; <sup>2</sup>Univ of Washington, Seattle, WA, USA; <sup>3</sup>Fred Hutchinson Cancer Rsr Cntr, Seattle, WA, USA; <sup>4</sup>Howard Hughes Med Inst, Minneapolis, MN, USA

**Background:** Several members of the APOBEC3 family of DNA cytosine deaminases can potently inhibit HIV-1 replication by catalyzing extensive cytosine deamination in viral cDNA during reverse transcription. HIV-1 counteracts restriction with the virally encoded Vif protein, which adapts the APOBEC3 proteins to a cellular ubiquitin ligase to target them for proteasomal degradation. In humans, A3H is the most polymorphic member of the family and includes seven haplotypes with three encoding for stable proteins and the rest unstable. Stable A3H proteins contribute to HIV-1 restriction and can only be counteracted by hyper-functional but not hypo-functional Vif variants (dictated by amino acids at key positions). We hypothesize stable A3H enzymes provide a natural barrier to HIV-1 acquisition from patients with unstable A3H.

**Methods:** A3H haplotypes of 597 serodiscordant couples with both transmission and non-transmission events were determined from blood spot genomic DNA using novel quantitative PCR assays. The vif genotype for each patient was determined by sequencing RT-PCR products from patient serum samples. Consensus vif open reading frames were cloned into expression vectors and assayed against stable A3H in single-cycle infectivity experiments.

**Results:** HIV-1 restriction by stable A3H can only be counteracted effectively by hyper-functional Vif with F39, H48, and EKGE60-63. In the serodiscordant couples cohort, the transmission rate from index patients with unstable A3H to partners with stable A3H is 34%, which is the same as the overall transmission rate of all analyzed couples. Vif genotyping of the index patients within the unstable-to-stable subgroup show that the majority (65%) of the Vif variants are predicted to be hyper-functional and resistant to stable A3H.

**Conclusions:** Endogenous stable A3H proteins restrict infection by HIV-1 with hypo-functional Vif variants in primary T lymphocytes. However, on population level, stable A3H is not a protective factor in HIV-1 acquisition from individuals with unstable A3H. Nonetheless, natural resistance to infection is likely to be due to many distinct mechanisms, stable A3H enzymes may provide a transmission barrier against HIV-1 isolates harboring hypo-functional Vif alleles.

**201 Characterization of Novel Human APOBEC3A Variants****Matthew Hernandez**; Lara Manganaro; Marcel Ooms; Lubbertus C. Mulder; Veronica Iannucci; Marsha Dillon-White; Sandra N. Terry; Ekta Sharma; Viviana A. Simon  
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**Background:** The APOBEC3 cytidine deaminases (A3A to A3H) are part of the host's intrinsic defense against retroviruses. The human APOBEC3 locus has rapidly expanded through multiple gene duplication events: mice only encode a single APOBEC3 gene while primates have seven different APOBEC3 proteins. Such genomic regions are often hot spots for structural genomic variation. A common somatic structural variant deletes the 3'UTR of A3A and the coding region of A3B. The impact of this copy number variation on A3A transcripts is currently not well understood. We speculate that the loss of the A3B coding region results in novel A3A splice variants and/or A3A-A3B chimeras. Because A3A has been implicated in the restriction of HIV as well as HTLV-1, we hypothesize that novel A3A variants could have a significant impact on viral replication and pathogenicity.

**Methods:** A3B copy number variation was determined by real-time qPCR from peripheral blood lymphocytes of 156 HIV negative blood donors. A3A and A3A-A3B chimera transcripts were amplified, cloned and sequenced of a subset of donors lacking A3B (N=3) or carrying one copy (N=5), two copies (N=2), or three A3B copies (N=1). Wild-type A3A and novel A3A variants were cloned into a mammalian expression vector. The catalytic activity of A3A was assessed by co-transfecting each A3A variant with a plasmid expressing a blue fluorescent protein (BFP) followed by flow cytometry analyses. The cellular localization of the different splice variants was determined by confocal microscopy.

**Results:** We cloned and sequenced 112 A3A transcripts from 11 donors that differ in A3B copy number. We identified novel A3A splice variants and detected A3A-A3B chimeric transcripts from cells lacking one or both A3B copies. These A3A variants resulted in longer and shorter variants and one A3A variant acquired 50 unique residues compared to the 199 amino acid long A3A reference. The catalytic deaminase activity of the A3A variants was significantly affected by certain A3A variants when compared to the reference A3A. Confocal microscopy revealed that the reference A3A and most A3A variants have a pan-cellular localization, whereas a shorter A3A variant exclusively shows a perinuclear localization.

**Conclusions:** We found that the A3B deletion polymorphism in the APOBEC3 locus results in novel A3A and A3A-A3B chimeric transcript variants. These A3A variants have profound effects on catalytic activity and cellular localization and we are currently characterizing their antiviral effects.

**202 CBF $\beta$  Protects HIV-1 Vif From MDM2-Mediated Degradation****Yusuke Matsui**; Keisuke Shindo; Kayoko Nagata; Noriyoshi Yoshinaga; Kotaro Shirakawa; Masayuki Kobayashi; Akifumi Takaori-Kondo*Kyoto Univ, Kyoto, Japan*

**Background:** HIV-1 overcomes the host restrictive APOBEC3 (A3) proteins by organizing an E3 ubiquitin ligase complex together with Vif and a host transcription factor CBF $\beta$ . CBF $\beta$  is essential for Vif function by increasing steady-state level of Vif protein and enabling the ubiquitin ligase recruitment, however, the mechanisms by which CBF $\beta$  up-regulates Vif protein remains unclear. Since we have previously reported that murine double minute 2 homolog (MDM2) is an E3 ligase for Vif protein, we hypothesized that CBF $\beta$  might protect MDM2-mediated degradation of Vif.

**Methods:** To test whether CBF $\beta$  interferes with the interaction between Vif and MDM2, co-immunoprecipitation of MDM2 with Vif derivatives that do not bind CBF $\beta$  was performed. To ask whether MDM2 is relevant to Vif metabolism, MDM2-null cells were used for knockdown by siRNA against CBF $\beta$  and for cycloheximide chase experiments of one of the Vif derivatives. To identify Vif residue that interacts with MDM2, Vif-degradation assays, co-immunoprecipitation and single-cycle infection of HIV were performed with Vif amino acid substitution derivatives.

**Results:** Vif derivatives that do not bind to CBF $\beta$  preferentially bind to MDM2, and over-expression of CBF $\beta$  disrupted the interaction between MDM2 and Vif. Knockdown of CBF $\beta$  reduced steady-state level of Vif in MDM2-proficient cells, but not in MDM2-null cells. Kinetic studies revealed that Vif E88A/W89A, which is deficient in CBF $\beta$  binding, degraded faster than wild-type Vif in MDM2-proficient cells, but not in MDM2-null cells, suggesting that Vif stabilization by CBF $\beta$  is mainly caused by impairing MDM2-mediated degradation. We identified Vif R93E as a Vif variant that does not bind to MDM2, and the virus with this substitution was more resistant to A3G than parental virus. Arginine residue at position 93 is located on the surface of the crystal structure of Vif complex, and not fully covered by, but very close to CBF $\beta$ , supporting our hypothesis. Combinatory substitution of Vif residues required for binding CBF $\beta$  and MDM2 showed comparable steady-state levels to that of wild-type Vif, but partial counteraction to A3G, suggesting other essential roles for CBF $\beta$  than Vif augmentation.

**Conclusions:** Vif augmentation by CBF $\beta$  is mainly due to protection from MDM2-mediated degradation. R93 of Vif is involved in MDM2 binding, and R93E-harboring virus is more resistant to A3G. Stabilization of Vif is not the only function of CBF $\beta$  for supporting Vif-mediated counteraction to A3 proteins.

### 203 MARCH8 Restricts HIV-1 Infection by Reducing Envelope Incorporation Into Virions

**Kenzo Tokunaga**<sup>1</sup>; Takuya Tada<sup>1</sup>; Yanzhao Zhang<sup>1</sup>; Takayoshi Koyama<sup>1</sup>; Minoru Tobiume<sup>1</sup>; Yasuko Tsunetsugu-Yokota<sup>1</sup>; Shoji Yamaoka<sup>2</sup>; Hideaki Fujita<sup>3</sup>

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**Background:** Membrane-associated RING-CH (MARCH) 8 is a member of a recently discovered MARCH family of RING-finger E3 ubiquitin ligases, 11 members of which have been identified. MARCH8 downregulates multiple host transmembrane proteins, such as MHC-II, CD86, IL-1 receptor accessory protein, TRAIL receptor 1, and transferrin receptor. However, its physiological roles remain largely unknown. Here, we identify MARCH8 as a novel antiviral factor.

**Methods:** An envelope (Env)-deficient HIV-1 luciferase reporter construct was cotransfected with wild-type and mutant MARCH8 expression plasmids, together with a plasmid expressing vesicular stomatitis G (VSV-G) or HIV-1 Env, into 293T cells. The resulting viruses were used for infectivity/entry assays and ultracentrifugation analyses, and producer cells were subjected to flow cytometry, immunofluorescence or immunoblotting analyses. Interaction between MARCH8 and HIV-1 Env was analyzed by co-immunoprecipitation. MARCH8 knockdown/knockout experiments in macrophages were performed using lentiviral shRNA and CRISPR/Cas9 systems.

**Results:** The ectopic expression of MARCH8 in virus-producer cells, but not in target cells, impaired viral infectivity. MARCH8 substantially reduced viral entry by blocking the incorporation of HIV-1 Env into virus particles. This was due to MARCH8-mediated Env downregulation from the cell surface likely through an interaction. The inhibitory effect of MARCH8 on VSV-G was similar but even more marked, implying a broad-spectrum inhibition of enveloped viruses. Notably, the endogenous expression of MARCH8 was especially high in monocyte-derived macrophages and dendritic cells. HIV-1 infectivity and multiple replication were strongly enhanced by MARCH8 depletion in macrophages.

**Conclusions:** Our findings indicate that MARCH8 is highly expressed in terminally differentiated myeloid cells and is a powerful antiviral protein that targets viral envelope glycoproteins by reducing their virion incorporation.

### 204 Sirtuins Modulate HIV Integration and Replication: New Cellular Anti-HIV Targets

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**Background:** Highly active antiretroviral therapy (HAART) has increased the survival of HIV-infected individuals, although it cannot eradicate the virus.

At the molecular level, HIV life cycle critically relies not only on the action of viral proteins, but also on host genes. In particular, integration of the proviral DNA into the host genome is due to the interaction between proviral DNA complex, integrase, and broken-DNA fixing proteins, provided by the host cell, in a process called "post-integration repair". We investigated the potential impact of those enzymes that catalyse the removal of acetyl groups from chromatin proteins on HIV integration. In particular, we studied the sirtuin class of proteins, since this family of NAD<sup>+</sup>-dependent deacetylases is recruited to the sites of DNA damage to establish functional interactions with "repairing factors", such as Ku70 or the ATM/Nsb1 complex.

**Methods:** To quantitatively assess the integration of viral DNA into the host genome, we challenged HeLa cells with an HIV-based, replication-defective, lentivirus that carries the GFP reporter gene. By infecting HeLa cells in a range of 0.1-0.2 MOI, we were able to measure "integrational" events by flow cytometry, counting and sorting cells characterized by a brighter green fluorescence, enabled by the integration of the GFP gene into the host genome. *Alu-gag* integrated DNA was measured to confirm the results. Sirtuin inhibitors were identified and tested from a library of commercially available and newly synthesized compounds.

**Results:** Two molecules, B2 and compound-2, were capable to inhibit HIV integration as efficiently as the integrase inhibitor raltegravir with a good concentration/toxicity profile. Importantly, flow cytometry data were validated by the detection and quantification of the *Alu-gag* sequences, via nested PCR.

To further show the involvement of sirtuins in the molecular steps leading to viral integration, treatments with resveratrol, which activates the whole family of de-acetylases by increasing intracellular NAD<sup>+</sup> levels, enhanced HIV integration.

**Conclusions:** Taken together, our observations provide the first evidence that sirtuins are involved in the HIV integration process, hence they may be considered as a new potential class of targets for HIV therapy. Importantly, this paves the way to the development of drugs able to inhibit HIV life cycle on the cellular side in order to avoid resistance to therapies.

### 205 SAMHD1 Phosphorylation Affects dNTPase Activity and HIV-1 Replication Capacity

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**Background:** SAMHD1 is a triphosphohydrolase that restricts HIV-1 by limiting the intracellular dNTP pool required for reverse transcription. Loss of SAMHD1 antiviral activity in cycling cells has been associated to CDK-dependent phosphorylation at residue T592, without affecting its triphosphohydrolase activity. In addition, an endonuclease function of SAMHD1 has been postulated as the mode of anti-HIV-1 activity. A thorough evaluation of SAMHD1 function depending on its phosphorylation state is required.

**Methods:** HIV-1 replication capacity was assessed in CD4<sup>+</sup> MT4 and TZM cell lines. Knock-out (KO) of SAMHD1 was achieved through CRISPR-Cas9 genome-editing of SAMHD1 TZM cells. SAMHD1 phosphorylation sites were predicted *in silico* and phosphorylation defective mutants were constructed by site-directed mutagenesis. Functional evaluation of SAMHD1 mutants was performed by transient expression in SAMHD1 KO cells. HIV-1 replication capacity in the presence of AZT or control drugs was evaluated by b-Gal staining at 72h post-infection. HIV-2 Vpx expression was achieved by cell transduction with viral like particles containing Vpx. SAMHD1 expression and phosphorylation was analyzed by Western blot.

**Results:** SAMHD1-induced degradation with HIV-2 Vpx affects the dNTP pool and HIV-1 replication capacity, measured through its sensitivity to AZT. Similarly, SAMHD1 KO cells showed increased replicative capacity in the presence of AZT. Re-expression of wild type SAMHD1 reduced virus replication in the presence of AZT but sensitivity to a non-nucleoside inhibitor (nevirapine) or the integrase inhibitor raltegravir was not affected. Five phosphorylation sites were: 4 located at the N-terminus (S18, T21, T25, S33) and T592 at the C-terminus. Phosphorylation defective mutants were constructed and expressed in KO cells. Single point mutations at the N-terminal region of SAMHD1 did not significantly affect the overall phosphorylation. However, the combination of three mutations (S18A, T21A, T25A) or mutation T592A significantly prevented SAMHD1 phosphorylation and decreased HIV-1 replication in the presence of AZT, indicating that a change in dNTP levels affected HIV-1 replication.

**Conclusions:** Phosphorylation of the N-terminal region as well as residue T592 affects SAMHD1 function affects virus restriction capacity. Thus, inactivation of SAMHD1 by phosphorylation affects HIV-1 replication through modifications in the dNTP pool, suggesting that the SAMHD1 triphosphohydrolase activity is responsible for HIV-1 restriction.

**206 Novel Mutant HIV-1 Strains With Greater Resistance to Cynomolgus Macaque TRIMCyp**

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**Background:** An antiretroviral factor, TRIM5a, produced by Old World monkeys strongly suppresses HIV-1 replication. A fusion protein comprising cynomolgus macaque (CM) TRIM5 and cyclophilin A (CM TRIMCyp) also potently suppresses HIV-1 replication. However, CM TRIMCyp fails to suppress a mutant HIV-1 that encodes a mutant capsid protein containing a SIVmac239-derived loop between  $\alpha$ -helices 4 and 5 (L4/5) though it costs replicative capability. There are seven amino acid differences between HIV-1 L4/5 and SIVmac239 L4/5. Here, we investigated the minimum numbers of amino acid substitutions that would allow HIV-1 to evade CM TRIMCyp-mediated suppression.

**Methods:** We used PCR-based random mutagenesis to construct a library of HIV-1 variants that contained mutations in L4/5. We then applied a functional screen to isolate resistant viruses; specifically, we recovered replication-competent viruses from CD4+ MT4 cells that expressed high levels of CM TRIMCyp.

**Results:** We generated novel mutant HIV-1 strains that were resistant to the CM TRIMCyp. CM TRIMCyp-resistant viruses were obtained after three rounds of selection in MT4 cells that expressed CM TRIMCyp. Viruses isolated under CM TRIMCyp-selection pressure contained four amino acid substitutions in L4/5 of a capsid protein that is H87R, A88G, P90D, and P93A. These four amino acid substitutions were then confirmed to confer CM TRIMCyp resistance to HIV-1 with efficient replicative capability. We also obtained novel CM TRIM5a-resistant HIV-1 strains that were generated via a similar selection method, which required six rounds of selection and rescue cycles. Sequence analysis of CA region of provirus in MT4 cells expressing CM TRIM5a revealed V86A and G116E mutations. V86AG116E mutation conferred partial resistance to CM TRIM5a without substantial fitness cost.

**Conclusions:** Four amino acid substitutions in L4/5 of HIV-1 capsid protein are enough to confer resistance against CM TRIMCyp, while another two amino acid substitutions are required for partial resistance against CM TRIM5a. These results suggested that random mutagenesis of live viruses followed by the selection with restriction factors would be useful in understanding detailed molecular interaction between virus and host.

**207 The G1/S Specific Cyclin D2 Acts As a Viral Restriction Factor in Primary Macrophages**

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**Background:** Macrophages are a heterogenic cell population with properties strongly influenced by stimuli present during their differentiation from monocytes. Distinct differentiation stimuli lead to differential cell activation and proliferation, differential expression of proteins controlling cell cycle progression and differential capacity to support HIV-1 replication. Cell cycle control plays also a fundamental role in SAMHD1-mediated virus restriction, as it is regulated through phosphorylation by cyclin-dependent kinases (CDK). Here, we describe an HIV-1 novel restriction pathway determined by the differential expression of the G1/S-specific cyclin D2.

**Methods:** Primary monocytes were transfected with siRNA and differentiated into monocyte-derived macrophages (MDM). Monocyte differentiation consisted in 4-day incubation with M-CSF or GM-CSF. Susceptibility to HIV-1 infection was examined by flow cytometry after infection of a VSV-pseudotyped NL4-3 GFP-expressing virus. Total viral DNA formation was quantified by qPCR in MDM infected with full-replicative R5 HIV-1 strain BaL. Co-immunoprecipitation (CoIP) was performed in lysates from 293T cells overexpressing cyclin D2-HA or Flag-p21 fusion proteins and were incubated with anti-FLAG or anti-HA antibodies covalently attached to agarose. Protein expression and phosphorylation and CoIP were analyzed by immunoblotting.

**Results:** Differential gene expression profile of cell cycle proteins was observed in GM-CSF vs. M-CSF MDM, being the most relevant the upregulation in GM-CSF MDM of all D-type cyclins (D1, 2-fold,  $p=0.0006$ ; D2, 40-fold,  $p=0.0045$ ; and D3, 3-fold  $p=0.0002$ , respectively) and the CDK inhibitor p21 (4-fold,  $p=0.038$ ). Efficient and specific siRNA-mediated downregulation of cyclin D2 led to a significant increase of HIV-1 replication and total viral DNA formation only in GM-CSF MDM (3-fold increase;  $p=0.0037$ ), but not in M-CSF MDM. CyclinD2 knockdown was linked to an increased phosphorylation of SAMHD1, without additional differences in the expression profile of cell cycle proteins. Characterization of the restriction mechanism by CoIP and additional siRNA experiments led to the identification of a protein complex formed by cyclin D2 and p21 as the responsible of a novel viral restriction mechanism.

**Conclusions:** The G1/S specific cyclin D2 acts as a restriction factor for HIV-1 in primary macrophages. These results further demonstrate the importance of cell cycle control in viral replication.

**208 Characterizing Dynamics of Proteo-Transcriptomic Response to HIV-1 Infection**

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**Background:** During the 24-hour-long replication cycle, HIV-1 enters the host cell, integrates its genome, and utilizes the host cellular machinery in order to produce new virions. T cells respond to infection by transcriptional and proteomic changes. Here, we have conducted genome-wide measurements on transcriptomic, proteomic, and phosphoproteomic levels, in uninfected and infected CD4+ T cells, in order to define the dynamic, integrated proteo-transcriptomic response of the cell to infection.

**Methods:** SupT1 cells were either mock-treated or infected with a VSV-G pseudotyped HIV-based vector (NL4-3ΔEnv/GFP) at a high multiplicity of infection to reach universal infection. Cells were collected at 6h intervals during 24h. Both mock and HIV-infected samples were subjected to transcriptome analysis with mRNA-Seq, and proteome and phosphoproteome analysis using SILAC. An integrative data analysis based on hierarchical clustering was used to identify infection-specific proteome and transcriptome temporal patterns.

**Results:** We evaluated time-series transcript and protein levels in both HIV-infected and mock samples for 3285 genes and detected phosphorylation events in 524 of them. The 3285 genes were further stratified based on their combined proteo-transcriptomic HIV/mock temporal progression. We isolated gene clusters enriched in canonical pathways, including nuclear import and cell cycle, as well as HIV co-factors, supporting different stages of HIV-1 replication.

We observed specific post-transcriptional and post-translational regulation after infection. A total of 307 transcriptionally invariable genes in HIV/mock, enriched in HIV-1 life cycle and host interaction factors, expressed differential behavior at proteome or phosphoproteome level. Of 279 differentially expressed proteins in HIV/mock, 16.13% ( $n=45$ ) showed differential expression also at the transcriptome level. A clear separation between genes being progressively up or down-regulated over 24h was observed, as well as a consistent 6-hour delay in the protein response compared to the transcriptome. Among 93 differentially phosphorylated genes in HIV/mock, 21.5% ( $n=20$ ) showed differential transcriptome or proteome expression.

**Conclusions:** Integrated time-series proteo-transcriptomic analyses of HIV-1 and mock-infected T-cells define host response to HIV-1 infection. This study exposes novel regulation mechanisms of virus-host interaction through an integrated temporal investigation of different omics measurements.

**209LB Kinesin-1 and Nup358 Cooperatively Mediate the Nuclear Import of HIV During Infection**

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**Background:** Fusion of viral and target cell membrane releases the viral conical capsid in to the cytoplasm of target cells which houses the viral RNA and accessory proteins for viral reverse transcription and integration in to the host cell nuclei. The viral capsid undergoes a gradual dissociation of the viral capsid core, a process called uncoating allowing subsequent nuclear import of the viral genome. Nup358, a component of the nuclear pore complex has been shown to be important for HIV-1 nuclear import and infectivity.

Nup358 has already been shown in previous studies to be an adaptor protein for trafficking by the microtubule motor KIF5B. We therefore asked if KIF5B exerts its effect on viral uncoating through Nup358. In this study.

**Methods:** We utilized fluorescent microscopy, including colocalization and Proximity Ligation Assay, to measure the association of the viral core with Nup358 in primary macrophages. We also utilized viral CA mutants which are known to enter the nucleus independently of NUP358 (P90A, N74D) to determine the role of KIF5B in the nuclear import of the viral genome, using quantitative PCR.

**Results:** We show here that depletion of the kinesin-1 motor causes a change in viral capsid and Nup358 localization. Also we show here Nup358 has a dispersed cytoplasmic staining in infected macrophages, a natural target cell type for HIV-1, and these Nup358 signal colocalize with viral capsid in the cytoplasm. This Nup358 relocalization and capsid colocalization was absent in N74D and P90A capsid mutants. Finally we show that kinesin-1 and Nup358 are important determinants for the nuclear import of wildtype virus and not for N74D and P90A.

**Conclusions:** Taken together, our data reveal that the cooperative activities of KIF5B and Nup358 are required for the normal uncoating and nuclear import of the HIV-1 genome during infection. In addition to identifying a key player in HIV-1 infection, these studies may reveal new opportunities to prevent the nuclear import of the viral genome and thereby trigger the antiviral response induced by the accumulation of viral DNA in the cytoplasm.

## 210 Phylogenetic Analysis of HIV Full Genomes in London, UK: Initial Results From ICONIC

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**Background:** The ICONIC (Infection response through virus genomics) project applies whole genome sequencing (WGS) to guide clinical management, infection control and public health approaches of viral diseases including HIV. It has developed an automated high-throughput pipeline that rapidly generates HIV clinical genomes (from gag to nef) from large amounts of short read data.

**Methods:** The pipeline was applied to 420 HIV samples from antiretroviral-naïve patients, collected at University College London Hospital and Barts Health NHS Trust (London) and sequenced using an Illumina MiSeq at the Wellcome Trust Sanger Institute (Cambridge). The consensus genomes were subtyped using COMET and Rega, and unique recombinants were studied with jPHMM and SimPlot. Maximum-likelihood phylogenetic trees were constructed (RAxML) to identify transmission networks using the Cluster Picker. Drug-resistance mutations (DRMs) and co-receptor usage were analysed using the Stanford HIVdb and Geno2pheno tools, respectively.

**Results:** The pipeline generated genomes for 375/420 samples (89%), with a median length (7.4Kb) that covered 86% of the clinical genome. The most frequent HIV strains were subtypes B (n=153, 41%) and C (n=80, 21%) and CRF02\_AG (n=33, 9%). In total, we found 14 different CRFs (n=68, 18%) and multiple URFs (n=32, 8%) that involved recombination between 11 different subtypes/CRFs. The most frequent URFs were A1/D, B/C, B/CRF01 and B/CRF02 (3 cases each). Half (54%) of the URFs lacked breakpoints in PR/RT, rendering them undetectable if only that was sequenced. Major DRMs were found in 29 (8.3%) PR/RT and 2 (0.8%) integrase sequences. Accessory DRMs were found in 36 (15%) integrase sequences. Usage of X4 co-receptor, which confers resistance to entry inhibitors, was detected in 21 (7%) samples. We detected 21 sequence clusters: 19 pairs (mostly subtypes B and C) and 2 triplets (both CRF02\_AG).

**Conclusions:** The initial analysis of genome sequences detected substantial hidden variability in the London HIV epidemic. Analysing full genome sequences, as opposed to only PR/RT, detected DRMs in all target genes (including integrase and env), and identified previously undetected recombinants. It provides a more reliable description of CRFs (that would be otherwise misclassified) and transmission clusters. Further analyses will study intra-sample minority viral populations. ICONIC-HIV will generate thousands of additional genomes that will help to determine whether WGS should be implemented routinely in clinical care.

## 211 Comparing Three HIV-1 Subtyping Tools and a Novel Phylogenetic-Based Method

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**Background:** HIV-1 evolves rapidly, increasing its genetic diversity and complexity, with group M containing >80 subtypes and circulating recombinant forms (CRFs). Subtype determination is important epidemiologically and can impact treatment and vaccine development. Multiple automated subtyping tools are available; however, differences in their subtype/CRF assignments in a predominantly subtype B setting or with large surveillance data have not been fully evaluated.

**Methods:** We included polymerase sequences ≥500-bp in length reported to the U.S. National HIV Surveillance System for HIV-1-infected persons (one sequence/person). We assigned HIV-1 subtype or CRF using COMET (Context-based Modeling for Expeditious Typing), REGA V3, and SCUEAL (Subtype Classification Using Evolutionary Algorithms). For sequences not classified as subtype B by all three methods (including those classified as non-B), we performed phylogenetic analysis using a fast, novel method (phylopartitioning) that combined FastTree approximate maximum likelihood inference using 2,864 curated reference sequences with cluster analysis to identify subtype using Phylopart. We compared results of these subtyping approaches.

**Results:** Of 71,659 sequences, subtype B classification varied by method (COMET:94.8%; REGA:91.6%; SCUEAL:89.6%, p<0.0001). In all, 95.7% were determined to be subtype B by at least one method, and 85.6% were classified as subtype B by all three methods. Of 67,973 sequences assigned as subtype B by COMET, 99.3% were assigned to subtype B by REGA, SCUEAL, or both. Of 6,624 sequences assigned to subtype B by COMET that were not subtype B by all three tools, 3,798 (57.3%) were B by REGA but not SCUEAL, 2,319 (35.0%) were B by SCUEAL but not REGA, and 475 (7.2%) were not B by either REGA or SCUEAL. Of these 6,624, 6,580 (99.3%) were subtype B by phylopartitioning. For non-B subtypes/CRFs, agreement between the three methods also varied, with almost 90% of COMET and REGA assignments, but only 65.4% of SCUEAL assignments, matching results from phylopartitioning. REGA and SCUEAL identified a higher percentage of all sequences as unique recombinants than COMET (REGA: 4.4%; SCUEAL: 6.7%; COMET: 1.2%).

**Conclusions:** In a setting dominated by subtype B, overall results varied by subtyping method. REGA and SCUEAL reported a high number of unique recombinants. COMET and phylopartitioning, on the other hand, identified a larger number of subtype B sequences.

## 212 Identification of Rare HIV-1 Group N and HTLV-3 Strains in Rural South Cameroon

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**Background:** South Cameroon is a hot spot for newly emerging strains of HIV and HTLV, making this region a critical location for monitoring circulating variants that have the potential to spread globally. In this study we aim to monitor the prevalence and diversity of HIV and HTLV, as well as detect emerging viral strains by conducting surveillance of HIV/HTLV in South Cameroon, which can inform diagnostics and research.

**Methods:** Study participants, recruited in 7 towns in South Cameroon, were screened for HIV infection using the national algorithm from 2010-2015. All collected specimens from 2010 were selected for further testing; however, in 2011-2015 the selection was weighted to include 30-40% HIV positive specimens. All selected specimens were tested with ARCHITECT HIV Ag/Ab Combo assay (Abbott Diagnostics), and a subset with remaining volume was tested with ARCHITECT rHTLV I/II assay. HIV serotype was determined by a peptide multiplex immunoassay, which classified infections as either HIV-1 group M, N, O or HIV-2. Molecular characterization was performed on a subset of reactive HIV and HTLV specimens.

**Results:** In 2010, the prevalence of HIV in South Cameroon was 8.5% amongst study participants. The serotype immunoassay identified the majority of HIV infections in 2010-2015 as group M (99%); group O (n=22) and group N infections (n=2) were also identified and confirmed by sequence classification of *env* gp41 or *pol*-integrase regions. Based on *env*

gp41, gag, and/or pol-integrase sequences from 415 HIV-1 group M specimens, CRF02\_AG accounted for 62.6% of HIV infections; 6 subtypes, 11 CRFs and 21 URFs were found. From 2010-2015, the prevalence of HTLV was 1% (95% confidence interval: 0.70-1.33) in the study population, with no significant effect of HIV co-infection. Sequence classification of the *tat* and/or *gag* region identified HTLV-1 (n=32), HTLV-2 (n=5), and HTLV-3 (n=1) infections.

**Conclusions:** Our findings confirm that South Cameroon has a high burden of HIV infections and indicate a high level of HIV-1 and HTLV strain diversity in the region. Only 4 previous HTLV-3 infections have been reported, making our identification and sequencing of a new HTLV-3 infection of great significance. Likewise, the 2 HIV-1 group N infections we report are 9% of the 22 total group N infections reported and add to the limited sequence information available. Continued HIV/HTLV surveillance in South Cameroon will be essential to ensure diagnostic tests and HIV research keep pace with these rapidly evolving viruses.

### 213 Complete Genomes Reveal Complex HIV-1 Diversity in the Democratic Republic of Congo

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**Background:** Surveillance of human immunodeficiency virus-1 (HIV-1) strain diversity is fundamental to collective efforts towards the prevention, diagnosis, and treatment of HIV-1 infections globally. However, limited surveillance has been conducted in the Democratic Republic of Congo (DRC), where a high level of strain diversity and intersubtype recombination has been reported. Since partial sequences may underrepresent recombination and diversity, complete genome sequencing is essential to improving HIV surveillance in the DRC. In this study we have sequenced the complete genomes of rare variant HIV-1 specimens from the DRC to meet neglected surveillance needs and to examine HIV-1 diversity.

**Methods:** HIV-1 specimens were collected at two rural hospitals in the DRC between 2001-2003. Out of 172 HIV-1 specimens classified by phylogenetic analysis of the envelope immunodominant region sequence, 18 rare and unclassified subtypes were selected for next generation sequencing (NGS) using an established HIV-specific primer approach. The viral load for 9 of these specimens was less than 5 log<sub>10</sub> copies/ml, and NGS library preparation conditions were further optimized for these specimens. Genomes were assembled by aligning reads to references and *de novo* assembly using CLC Bio software. Strain classification was determined by phylogenetic and recombination analysis.

**Results:** For low viral load specimens, the highest genome coverage was obtained by concentrating the total nucleic acid extract before reverse transcription at 42°C using a SMART cDNA kit (Clontech). Genome sequences with >99.5% genome coverage, an average read depth of >10, and a sequence length of >9500 nucleotides were obtained for 14 HIV-1 specimens. The remaining four genomes had coverage of >60%. Phylogenetic and recombination analysis of the 14 complete genomes identified pure subtypes D (n=1), H (n=3), and CRF25 (n=1). The remaining genomes were simple recombinants of a single subtype with unclassified or CRF sequence (n=6), or complex recombinants of 4 or more subtypes, including A, G, H, K, and unclassified (n=3). Two of these complex recombinants shared 97% sequence identity.

**Conclusions:** The sequence complexity of the reported genomes demonstrates a high level of diversity in HIV-1 strains circulating in the DRC. These complete genomes are a valuable contribution towards HIV-1 surveillance, and the complex recombinants will be useful for modeling the history of HIV-1 in the DRC.

### 214 HIV-2 Group A in France Displayed 2 Clades With Distinct Geographical Origins

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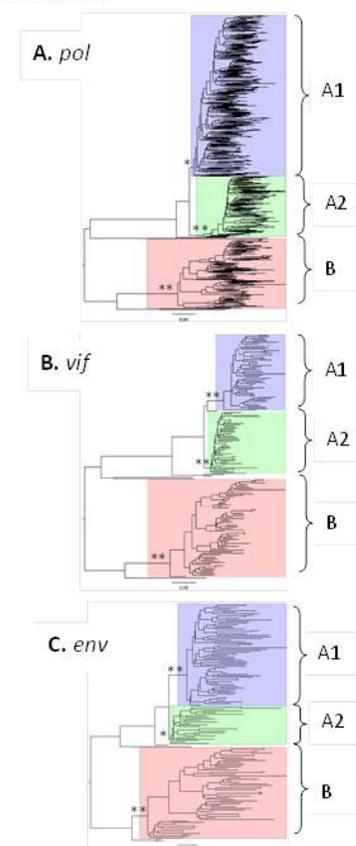
**Background:** Damond *et al.* 2000 showed that HIV-2 group A can be divided in two distinct genotypes. We analysed all the HIV-2 *pol*, *env* and *vif* sequences available in France to better characterise genetic variations between these two genotypes, analyse their relative prevalence in France and to explore a potential link with patient's country of birth.

**Methods:** Maximum likelihood phylogenetic trees were reconstructed from 446 HIV-2 partial *pol* (PR and RT; 1350 nt), 155 *vif* (655 nt) and 154 partial *env* (525 nt) sequences sampled from 386 patients followed up in France, using FastTree 2.1 under the GTR evolutionary model. Publicly available sequences sampled outside of France were included for *pol* and *vif* fragments (207 and 22 sequences, respectively). Recombination analyses were conducted with the RDP4 software. Patients' country of birth was retrieved for the patients included in the French ANRS C05 HIV-2 cohort (n=272).

**Results:** The group A formed two distinct and strongly supported clusters, herein called A1 and A2, in all trees (cf. Figure 1), suggestive of a past founder effect. Overall, 72% and 28% of the group A sequences belonged to cluster A1 and A2 respectively, with 20% of the latter being sampled in France. Among the 193 HIV-2 A sequences obtained from public databases 19 (10%) belong to clade A2. Inter-cluster median genetic distances were 0.12 [IQR=0.11-0.14], 0.12 [0.11-0.13] and 0.15 [0.12-0.18] substitutions/site for *pol*, *vif* and *env*, respectively. For the 163 viruses with more than one genetic region available, 17 (10%) presented inconsistent clade assignments across trees, suggesting potential recombination events. A1 viruses were most prevalent amongst patients born in coastal Western African countries (i.e. Senegal, Gambia, Guinea Bissau and Guinea) with 40 A1- and 8 A2-infected patients. Inversely, A2 strains were predominantly found among patients originating from inland Western countries such as Mali and Burkina Faso with 8 A1- and 29 A2-infected patients, suggesting distinct origins of the two clades. Sequences issued from patients born in Ivory-coast displayed a balanced prevalence of these clades with 13 A1- and 16 A2-infected patients).

**Conclusions:** This study provides an enhanced understanding of the geographical and genetic diversity of HIV-2 group A. It highlights the co-circulation of two distinct clades in France that likely appeared from an ancient divergent event, followed by a founder effect explaining the distinct geographical patterns in Western Africa.

**Figure 1.** Maximum likelihood phylogenetic trees were reconstructed from HIV-2 partial *pol* (A), *vif* (B) and *env* (C) genes. \* and \*\* indicates a branch support value >65% and >90%, respectively.



## 215 Infer and Characterize a Transmission Network in an Opioid-Driven HIV-1 Outbreak

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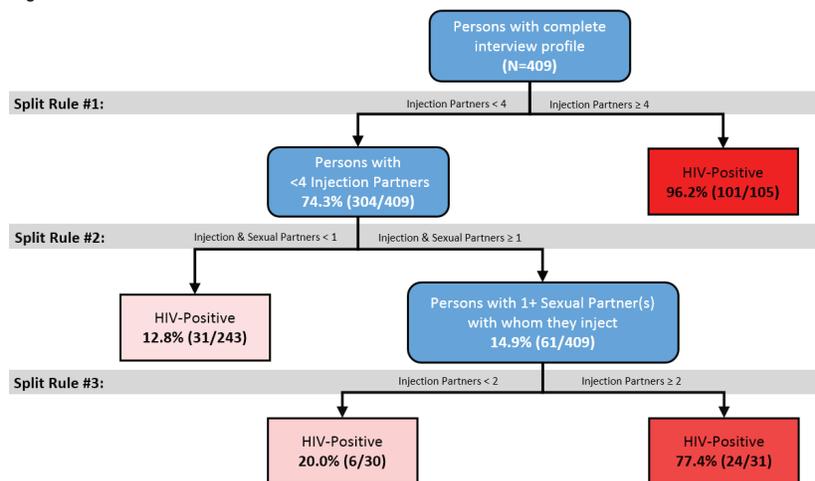
**Background:** In January 2015, investigation of a sudden upsurge in new HIV-1 infections in a rural county in Indiana linked to injection drug use (IDU) identified a large outbreak (n=181). Here we describe the integration of epidemiologic and laboratory data to infer and characterize the transmission network to inform future prevention efforts.

**Methods:** Serum specimens were used to determine recency of infection and to obtain HIV-1 polymerase (*pol*) sequences for genetic analysis. Putative undirected transmission links were drawn between sequences with genetic distance <1.5%. Standardized interviews were conducted with HIV-positive patients to collect high-risk behavior and contact data. High-risk contacts (sexual, IDU, or both) were considered as continuous risk factors, regardless of reporting direction. The reported contact network and inferred transmission network were compared. A decision tree was generated and logistic regression performed concerning contact type and occurrence with respect to infection status.

**Results:** HIV-1 *pol* sequences were obtained from 157 persons epidemiologically linked to the outbreak. Phylogenetic analysis inferred a monophyletic *pol* clade with limited diversity. Of 123 specimens available for avidity testing, 113 (91.9%) were recent ( $\leq 8$  months prior to collection). Network analysis showed that each type of high-risk contact was correlated with HIV infection ( $p < 0.01$ ). However, 82.3% of potential transmission events corresponded to a reported IDU contact, as opposed to 11.0% for reported sexual contacts. The decision tree revealed that the likelihood of infection for persons with  $\geq 4$  recent injection partners was 96.2% (101/105). 77.4% (24/31) of persons with multiple sexual partners with whom they also share injection equipment, and who also had 2 or 3 additional recent injection partners, were infected. In contrast, 20% (6/30) of persons with the same number (2 or 3) of recent injection partners and who shared syringes with only one sexual partner were infected.

**Conclusions:** A single HIV-1 strain was detected in this outbreak of HIV among persons who inject drugs, suggesting recent and rapid transmission. Comparison of reported contact and transmission networks reveal that IDU drove transmission and led to explosive growth of the outbreak. Integration of sequence and epidemiologic data facilitated a better understanding of transmission dynamics in a rural community of persons who inject prescription opioids.

Figure 1



## 216 HIV Phylodynamics in North Carolina: Detecting “Active” Clusters for Intervention

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**Background:** Phylogenetic analyses of HIV sequences can be used to monitor epidemic trends and help target prevention through the identification of transmission clusters. Characterizing such clusters in NC, where >1,500 new HIV diagnoses are reported annually despite widespread prevention, is needed. We evaluated temporal cluster expansion and epidemic growth to detect “active” clusters as potential intervention targets.

**Methods:** We analyzed 15,247 HIV-1 *pol* sequences (each from an individual patient) derived from genotypes sampled in NC from 1997-2014 by the largest commercial laboratory. Putative transmission clusters were clades with high branch support ( $\geq 0.90$ ) and maximum pairwise genetic distance  $< 3.5\%$  on a maximum-likelihood (ML) tree with the GTR model. Clusters were confirmed and transmission dynamics evaluated using BEAST with Bayesian skyline and relaxed molecular clock priors, inferring internal node ages (for each cluster  $\geq 10$  members). Reproductive numbers ( $R_0$ ) for individual large clusters were estimated using birth-death models.

**Results:** Most samples were from men (71%) with median age 40 years (IQR 32-48), and HIV-1B (98%). 7,647 (50%) sequences were identified in 2,318 clusters (median size 3 members). 74 large clusters ( $\geq 10$ ) involved 1,062 patients and were highly supported in BEAST. Compared to non-clustered patients, large clusters were associated with sex (81% vs. 70% men), more recent sampling year (65% vs. 46%  $\geq 2009$ ), younger age (49% vs. 15%  $\leq 30$  years), and sampling from Raleigh metropolitan area (54% vs. 42%) [all  $P < 0.001$ ]. However, 16 (22%) large clusters were  $\geq 50\%$  women and had earlier origin dates (1996 vs. 1999) and longer time spans (18 vs. 15 years) than male dominated clusters. The 8 largest clusters (n=22-36 members) originated between 1997 and 2004 and spanned a mean 12.5 years with 2.2 transmissions/year. The median estimates of  $R_0$  ranged 1.2-4.1. Most (75%) had highly supported internal nodes 2010-2014, indicating cluster expansion. Most of these clusters were homogenous by sampling region, and all but one were >90% men.

**Conclusions:** Phylodynamics revealed transmission cluster expansion in this densely sampled region and allowed estimates of  $R_0$  to help identify active clusters contributing to recent transmission. While large clusters originated >10 years ago, most continue to expand, particularly among young men. Enhanced prevention interventions to active clusters are planned to halt further propagation.

## 217 HIV Transmission Hotspot Detection Combining Sexual and Phylogenetic Network Analysis

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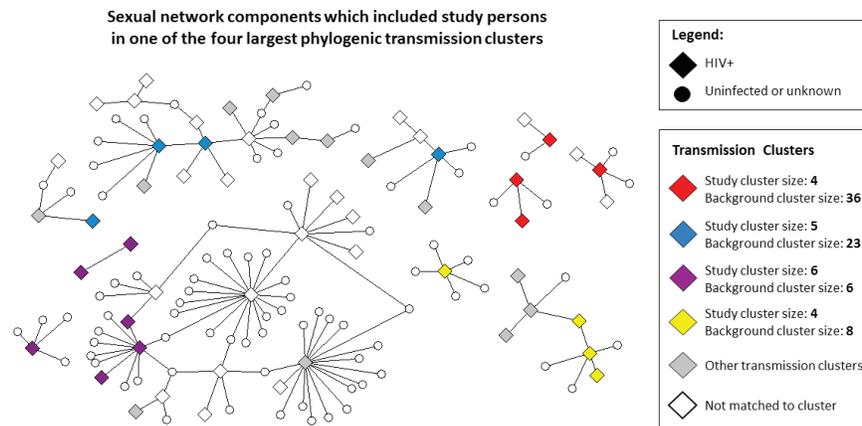
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**Background:** Studying local HIV transmission using molecular approaches may provide new avenues to supplement routine contact tracing. We investigated the overlap between phylogenetic transmission clusters and sexual networks involving new HIV cases in metropolitan Wake County, NC in the context of the statewide epidemic.

**Methods:** Sexual networks were constructed using partner contact tracing data for index cases newly diagnosed with HIV from 2012-2013 (N=280) and their HIV+ and HIV- partners. HIV *pol* sequences (n=15,247; one per individual), from genotypes performed by the largest reference lab in NC from 1997-2014, were matched to HIV+ study cases with the remainder used as background references. Maximum-likelihood phylogenies were constructed. Transmission clusters (TC) were defined as clades with short branch lengths and high support ( $\geq 0.90$ ) and confirmed with Bayesian methods. "Study" TCs were clusters including  $\geq 2$  persons from the traced Wake County network.

**Results:** Of 280 index cases, 80% were interviewed by the health department and reported 854 sex partners and 34 social contacts; 446 (50%) were traceable. The resultant sexual network included 663 unique persons in 137 network components including 411 HIV+ cases. Most persons were Black (64%), MSM (59%), and between ages 25-45 years. 230 cases (53% index and 63% HIV+ partners) had a *pol* sequence. Of these, 171 (74%) were identified in 118 TCs (range 2-36 members; 804 total sequences); 87 (38%) were in 34 study TCs. However, only 48% (42/87) in a study TC were also in the same network component as at least one other study person in their TC. Both partners were in the same study TC in every heterosexual pair where each had a sequence. TC members were more likely to be men (77% vs. 57% women), men reporting male contacts vs. no named partners, and younger (median age 29 vs. 37 years). The four largest TC (range 6-36 members) involved 14 index and 5 partner cases distributed in 11 components (Figure). Three large TCs had growth after 2012 (75% sequences were sampled 2012-2014) indicating significant ongoing transmission.

**Conclusions:** Phylogenetic analysis offers considerable promise to identify transmission in high risk populations that is not detected through contact tracing. Overlapping sexual networks with phylogenetic clusters provides novel methods for understanding HIV transmission dynamics and identifying transmission hotspots where interventions can be intensified, particularly among young MSM.



## 218 Metamorphosis of the Montreal MSM Epidemic: Large Cluster Viruses Fuel Transmission

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**Background:** The 25-50% decline in HIV transmissions with antiretroviral therapy has raised optimism that Treatment-as-Prevention (TasP) may control the global pandemic by 2030. Paradoxically, transmissions among MSM have not declined, raising concerns that early-stage infection, frequently undiagnosed, may offset the benefit of TasP. Phylogenetics ascribe the growth of the Montreal MSM epidemic to large cluster outbreaks, averaging 43 linked transmissions/cluster. This study characterized the distinct genotypic and phenotypic features of large cluster viral variants favoring their selection advantage.

**Methods:** Phylogenetic analysis was performed on RT/protease sequences from all newly infected MSM (n=4319, 2002-2014), assessing clustering dynamics (frequency, size, periodicity). Nucleotide mixed base calls estimated recency of infection, using a 0.44% cut-off for primary HIV infection (PHI). PHI cohort data evaluated the natural history (viral load, quasispecies diversity) in subjects belonging to large clusters vs. solitary transmission groups. Viruses were amplified from both cluster groups to ascertain infectivity, tropism, drug susceptibility and emergent resistance to integrase inhibitors.

**Results:** There has been a significant rise in the relative contribution of X-large clusters representing 29%, 34%, and 46% of new infections over the 2002-2005, 2006-2009 and 2010-2013 periods. Overall, 40 viral lineages led to 1235 transmissions as compared to 1375 solitary "dead-end" transmissions (n=1375). Primary infections (0-0.44% genetic diversity) accounted for 57% and 26% of transmissions in large cluster and solitary transmission groups, respectively. PHI cohort data revealed viruses belonging to large clusters showed prolonged high viremia for 2 years as compared to unique transmissions where viremia declined to set points by 6 months. Amplified viruses from representative large clusters harbored X4/R5 dual tropic viruses (n=5/7) as compared to dominant R5 tropism in the unique transmission group (0/6). Large cluster variants developed drug resistance mutations under dolutegravir and elvitegravir pressure in 6-12 weeks as compared to solitary transmission variants that retained wild-type genotype at 26 weeks.

**Conclusions:** Failure to control early stage transmission is leading to worrisome trends towards the selection of super-viruses showing prolonged viremia, dual tropism, rapid tropism shift, and/or facilitated escape from drug pressure.

## 219 Assessing HIV Transmission Networks in a Thai Cohort With Acute HIV Infection

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**Background:** Bangkok has an ongoing HIV epidemic in MSM but phylogenetic support as to whether this occurs within networks or at random is lacking. Studies from other metropolitan areas suggest local transmission among networks of MSM with certain characteristics, which might allow for targeted interventions. The RV254 study enrolls subjects in the earliest stages of HIV infection. Baseline drug resistance genotyping allows for inventory of subtypes and clustering in this cohort.

**Methods:** Real-time screening of 141,233 samples, using nucleic acid testing and sequential EIA, identified 330 acute HIV-1 infections, of whom 93% were MSM. Demographic and risk-factor data were collected at baseline by interview and questionnaire. Date of infection was estimated from self-reported sexual exposure. Protease and reverse transcriptase gene sequences from 273 (268 Thai) subjects were retrieved from plasma. Maximum likelihood trees and pairwise genetic distances were generated. A distance of  $\leq 1.5\%$  and visual inspections of signature nucleotides were used to identify linked transmission clusters.

**Results:** CRF01\_AE was predominant at 86% (234) (N), followed by 8% (21) subtype B and 6% (15) CRF01/B. A West African CRF02\_AG, a South African C and a B/C strain were also identified. 13/21 B strains clustered with Thai B'. The 5 non-Thai participants had 3 CRF01\_AE, 1 subtype B and 1 CRF01/B. Thirty-three subjects (31 CRF01\_AE and 2 B) formed 14 clusters: 11 of 2, 2 of 3, and 1 of 5 individuals. All clusters represent only Thai MSM and CRF01\_AE, but for one cluster of 2 B. Only 2 clusters of 2 are known to be linked. 30/33 clustering subjects live in the Bangkok area. Subjects who clustered were not significantly different from non-clustering participants in terms of age, Fiebig stage, plasma HIV-RNA level, days since exposure, reported drug use at exposure, and number of sexual partners preceding enrolment. Median (range) time between infections was 590 (0-1211) days for the clusters of 2, 158 (84-277) days for the clusters of 3, and 144 (43-194) for the cluster of 5 subjects.

**Conclusions:** The predominance of CRF01\_AE sequence clusters suggests local MSM transmission networks. The majority of Bangkok MSM with acute HIV infection in our cohort do not cluster, suggesting that numerous sexual transmission networks contribute to the ongoing epidemic of HIV among MSM in the city. The presence of HIV-1 strains from other regions indicates global transmission networks.

**220 Increasing Role of Young MSM to HIV Epidemic Spread and Renewal**

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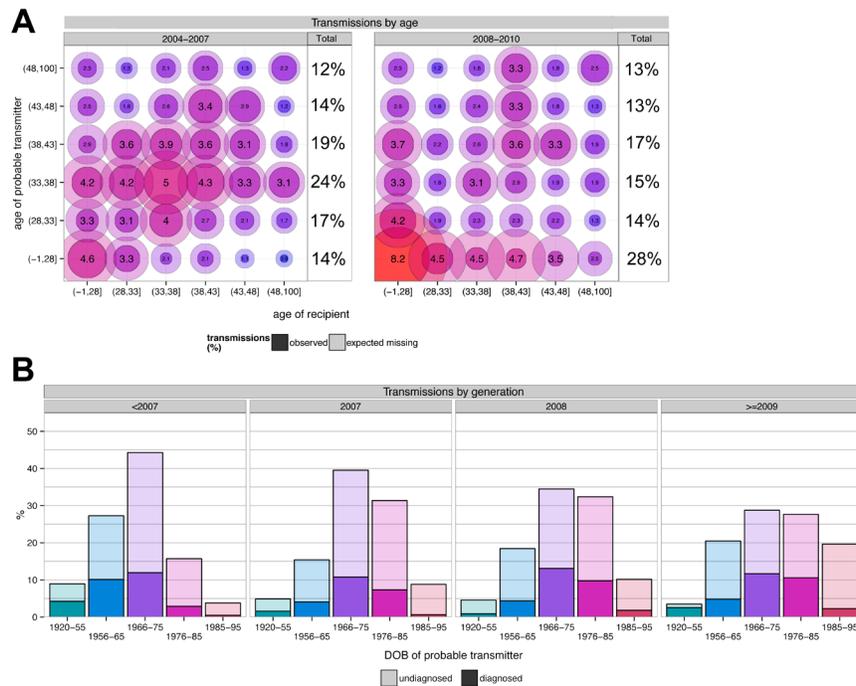
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**Background:** In the Netherlands, the age at diagnosis amongst men having sex with men (MSM) has been continually increasing from 37 years in 1996 to 41 years in 2013. This challenges the perception that young, high-risk MSM are the predominant source of infection in high-income countries. Using in depth records from the Netherlands' ATHENA HIV observational cohort, we previously identified and characterized 617 transmission events to MSM with evidence for recent infection (12 months) at time of diagnosis. Here, we use this cohort to evaluate the sources of the ongoing MSM epidemic in the Netherlands by age, date of birth, and diagnosis status.

**Methods:** 903 probable transmitters with date of diagnosis between 1996 and 2010 were identified through phylogenetic analysis. Demographic and clinical data from the ATHENA cohort were used to characterize these transmission events in detail. Statistical modelling adjusted for sampling and censoring biases. The proportion of transmissions attributable to age/birth groups was calculated by averaging individual-level viral phylogenetic transmission probabilities across recipients. Limited sequence coverage required us to restrict this multivariate analysis to 509 transmission events between 2004 and 2010.

**Results:** Figure 1A shows the estimated proportion of transmissions by age of the recipient and their probable transmitters. Overall, transmissions were not concentrated within age groups. Further, the age structure of the transmission events in the cohort shifted substantially over calendar time. After 2008, men aged < 28 years continued to be infected from older men, and transmitted increasingly amongst peers as well as to older men. After 2008, an estimated 28% of all transmissions originated from men aged < 28 years, compared to 14% between 2004-2008. This led to a rapid shift to transmissions from younger generations (figure 1B). The proportion of transmissions from undiagnosed transmitters remains high across generations (figure 1B).

**Conclusions:** We evaluated 509 phylogenetically reconstructed transmission events amongst MSM in the Netherlands. The increasing age at diagnosis is a consequence of complex, changing transmission dynamics by age. Young men are increasingly linked within the epidemic and infect relatively more older men than previously. Prevention of new infections to and from young MSM is especially difficult, but must be urgently improved.



**221 Differences in Outbreak Size for HIV-1 Non-B Subtypes Amongst MSM in the Netherlands**

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**Background:** The HIV-1 epidemic amongst men having sex with men (MSM) in the Netherlands is mainly driven by subtype B. Non-B subtypes are found mostly amongst heterosexuals immigrants from Sub-Saharan Africa. Therefore considering a phylogenetic tree of non-B polymerase sequences allows identifying the introductions into the MSM

population in the Netherlands. This can be used to estimate the proportion of introductions that actually resulted in onward transmission and assess whether this differs between subtypes.

**Methods:** The ATHENA observational cohort includes anonymized patient data of virtually all patients with HIV in care in the Netherlands. Polymerase sequences were available for 39% (8978) of patients in November 2014. Maximum likelihood trees were built by subtype in MEGA with 500 bootstraps, under a General Time Reversible model and five gamma distributed categories. We considered clusters with bootstrap values  $\geq 70\%$  including  $\geq 2$  MSM (indicating onward transmission), and those including  $\geq 3$  MSM (as indicative of ongoing established transmission clusters). The remaining MSM sequences were regarded as separate introductions, although some sequences formed clusters that were not statistically supported. The mean outbreak size per subtype was calculated as the number of sequences from MSM divided by the number of introductions amongst MSM.

**Results:** In ATHENA, 2131 of patients diagnosed between 1981-2014 with an HIV-1 non-B infection have a sequence available. Of these, 1467 self-reported being infected through heterosexual and 337 through MSM contact. Of the heterosexuals, 71% (1037) originated from Sub Saharan Africa, and 18% (262) from the Netherlands; of the MSM 8% (26) originated from Sub Saharan Africa and 66% (222) from the Netherlands. We built separate trees for subtypes A, CRF01AE, CRF02AG, C, D, F, G. The main results are summarized in table 1. The largest tree of 636 subtype CRF02AG sequences also contained most sequences from MSM. The proportion of successful introductions was highest for subtype F, the tree had the highest proportion of sequences from MSM, and was found to have the largest average outbreak size. Interestingly the median viral load at first hospital visit was significantly higher for MSM infected with subtype F compared to the MSM infected with other non-B subtypes.

**Conclusions:** Non-B subtypes are being introduced into the MSM population in the Netherlands and have gone on to form national sub-epidemics with different mean outbreak sizes.

Subtype	A	CRF01AE	CRF02AG	C	D	F	G
Percentage (number) of sequences from MSM	13 (38)	24 (76)	14 (87)	12 (60)	7 (6)	46 (30)	6 (8)
Number of MSM clusters of size $\geq 2$	6	13	11	5	0	2	2
Percentage (number) of sequences from MSM in clusters of size $\geq 2$	55 (21)	37 (28)	53 (46)	62 (37)	-	83 (25)	50 (4)
Number of introductions counted amongst MSM	23	61	52	28	6	7	6
Percentage of introductions amongst MSM that have transmitted onwards	26	21	21	18	-	29	33
Number of MSM clusters of size $\geq 3$	3	2	4	3	0	2	0
Percentage (number) of sequences from MSM in clusters of size $\geq 3$	42 (16)	9 (7)	37 (32)	55 (33)	-	83 (25)	-
Percentage of introductions amongst MSM that resulted in an established cluster	12	3	7	10	0	27	0
Mean outbreak size per subtype amongst MSM	1.7	1.2	1.7	2.1	1	4.3	1.3
Median viral load at the first visit (copies/ml)	17359	17663	17910	17795	18653	18401	16845

## 222 Spatiotemporal Dynamics of HIV-1 Transmission in France, 1999-2014

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**Background:** Identifying and monitoring HIV transmission networks can be important in understanding the evolutionary patterns and geospatial spread of the epidemic. Here, we reconstructed the broad molecular epidemiology of HIV in France from individuals enrolled at the time of primary HIV-1 infection (PHI) in the national ANRS PRIMO Cohort over 15 years.

**Methods:** Sociodemographic, geographic, clinical, and *pol* sequence data from 1356 cohort participants were collected between 1999 and 2014. Viruses mostly belonged to subtype B (71.5%) and CRF02\_AG (15.3%). Network analysis was performed to infer genetic relationships, i.e. clusters, between HIV sequences. Bayesian coalescent-based methods were used to examine the temporal and spatial dynamics of identified clusters from different regions in France and the geographical patterns of viral spread and growth rate of HIV-1 subtype B and CRF02\_AG subtypes.

**Results:** Participants were mostly Caucasian (85.9%) and men (86.7%) who reported sex with other men (MSM, 71.9%). Overall, 387 individuals (29%) were involved in 78 dyads ( $n=156$ ) and 231 participants fell in the remaining 42 clusters with 3 or more people (median size: 4, range 3-41). Compared to those who did not cluster ( $n=969$ ), those in clusters with  $\geq 3$  people were more frequently men (98.3% vs 83%,  $p<0.01$ ), MSM (88.3% vs 65.6%,  $p<0.01$ ) and infected with CRF02\_AG (27.3% vs 13.4%,  $p<0.01$ ). Participants entering the cohort after 2011 (21.3%) clustered significantly more than participants entering before 2011 (20.6% in 2006-2010 and 10.1% before 2006,  $p<0.01$ ). Phylogeographic analyses found a higher density of CRF02\_AG clustering among participants residing in Paris versus those living outside Paris ( $p<0.01$ ). Evolutionary analyses revealed that HIV-1 CRF02\_AG epidemic arose more recently (time from the most common ancestor [TMRCA]=17.3 years, mean logistic growth rate [LGR]=0.051 year<sup>-1</sup>, mean evolutionary rate=4.91x10<sup>-3</sup>subs/site/year) and spread more rapidly among individuals with PHI in France than subtype B lineages (TMRCA=26.4 years, mean LGR=0.048 year<sup>-1</sup>, mean evolutionary rate=2.89x10<sup>-3</sup>subs/site/year).

**Conclusions:** These analyses support the hypothesis of a recent and rapid rise of CRF02\_AG within the French HIV-1 epidemic among MSM. Since CRF02\_AG is historically associated with various parts of Africa, such results may help us understand the relationships between migration of human populations and HIV-1 diffusion.

**223 Potential Misclassification in Standard HIV Phylogenetic Clusters Association Studies**

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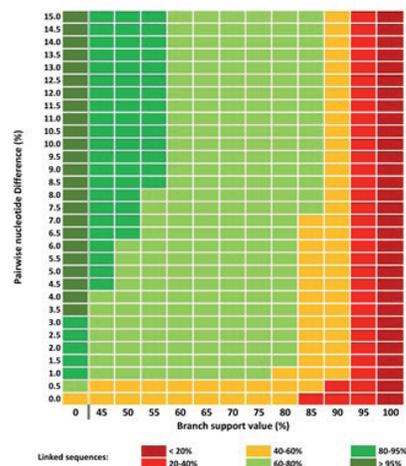
**Background:** The phylogenetic identification of transmission chains from partial HIV *pol* sequence data is a cornerstone of HIV molecular epidemiology. The standard method proceeds by identifying clusters in the viral phylogeny under a combination of strict criteria (e.g. minimal intra-cluster genetic distance, high branch support). Individuals with sequences in the same cluster are then declared phylogenetically linked. However, HIV sequences from infected individuals are typically sampled some time after infection, which could lead to substantial bias under these criteria. To quantify potential bias in the phylogenetic identification of HIV linkage, we mined data from two British HIV observational cohorts for epidemiologically linked sequences, and evaluated what proportion are excluded from cluster association studies under standard protocols.

**Methods:** Routine drug genotyping HIV sequence data from the UK HIV Drug Resistance Database and UK Register cohorts were mined for longitudinally sampled intra-host sequences (n=16,842 pairs). These were considered linked. Maximum likelihood phylogenies were reconstructed with FastTree v.2.1.7 and clustered pairs of sequences identified using a range of criteria. Potential bias was defined as the proportion of linked sequences that are not recognised as such under any given set of viral phylogenetic clustering criteria.

**Results:** Figure 1 quantifies the extent of potential bias under standard protocols in the UK cohorts. With 95% support and 2% genetic distance thresholds, the proportion of linked pairs that are excluded from further analyses was 69%. With 90% bootstrap and 5% genetic distance thresholds, the proportion of linked sequences that are excluded from further analyses remained substantial at 52%. Larger genetic distances are expected between linked sequences that had more time to diverge. Therefore, authentic transmitters that are excluded from viral phylogenetic cluster association studies are more likely to be late presenters, untreated for a long period, or treated and successfully virally suppressed for long periods.

**Conclusions:** Standard viral phylogenetic protocols may exclude a substantial proportion of authentic transmitters from further epidemiological analysis in cluster association studies.

**Figure 1. Proportion of truly linked sequences that are not excluded from HIV phylogenetic association studies.** The potential bias associated with these protocols was defined as 1 minus the proportions in the figure.



**224 Partner Services in Acute and Early HIV-Infected Adults**

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**Background:** We hypothesized that provision of Partner Services (PS) to acute and early HIV infected (AEH) index subjects in San Diego would prove effective in identifying genetically linked HIV-infected sex partners not previously aware of their HIV positive status.

**Methods:** We analyzed data generated by the San Diego Primary Infection Resource Consortium from 1996 to 2014. Persons with AEH infection were offered PS and enrollment to PIRC. Sex partners of AEH index subjects were offered HIV screening and HIV drug resistance testing if infected. Partnerships were categorized as genetically linked if viral protease and partial reverse transcriptase sequences isolated from the partners were at most 1.5% distant under the TN93 model. Number needed to interview (NNTI), i.e. number of AEH index subjects required to interview to find one partner or new HIV diagnosis was calculated. Mixed-effects logistic regression and two-tailed  $\chi^2$  analyses were used to test whether partners who enrolled within 30 days of the index subject enrollment were more likely to be 1) newly HIV diagnosed and 2) genetically linked to the index subject.

**Results:** PS provided to 96 AEH index subjects led to recruitment of 105 sex partners (total of 114 partnerships); 55 (52.4%) sex partners were HIV-infected, of whom 35 (63.6%) were newly HIV-diagnosed. Thirteen (37.1%) newly diagnosed sex partners were themselves AEH infected. Table 1 illustrates PS yield and efficiency for each diagnostic outcome. Demographics and risk behaviors between HIV uninfected and infected sex partners were similar. HIV-concordant sex partners (N=66) enrolled within 30 days of the index partner (47/66 [71%]) were 5.7 times more likely to be newly diagnosed with HIV infection ( $p < 0.01$ ), and 3.25 times more likely ( $p = 0.04$ ) to be genetically linked to each other (41/66 [62%]), compared to those enrolled after 30 days. There was no difference in the rates of genetic linkage to index subjects between chronic and AEH sex partners ( $p = 0.166$ ).

**Conclusions:** PS delivered immediately following an AEH diagnosis is an effective strategy to identify HIV infected and previously unaware persons, including sex partners with AEH. It is likely that PS delivered within the first month of AEH diagnosis benefits from a more accurate recall of recent sexual contacts, with 62% of sex partners representing putative transmission links as confirmed by genetic linkage. PS delivered to chains of sex partners identified with AEH may be a promising strategy for finding HIV unawares.

Mean number of AEH persons interviewed for:	
Identification of 1 partner	5.5 (574/105)
Identification of 1 HIV-infected partner	10.4 (574/55)
Identification of 1 AEH-infected partner	28.7 (574/20)
Identification of 1 new HIV diagnosis	16.4 (574/35)
<b>Of all 105 partners identified (% , N):</b>	
HIV-infected	52.38% (55/105)
AEH-infected	19.05% (20/105)
Newly HIV-diagnosed	33.33% (35/105)
<b>Of all HIV-infected partners identified (% , N):</b>	
AEH-infected	36.36% (20/55)
HIV-diagnosed	63.63% (35/55)

Table 1: Number needed to interview and yield of San Diego Primary Infection Resource Consortium Partner Services

**225 Probabilistic Estimation of HIV-1 Subtype B Transmission Rates From Viral Phylogenies**

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**Background:** Identifying the determinants of an epidemic is of critical importance for devising prevention strategies. In HIV epidemics, incomplete sampling, large numbers of undiagnosed transmitters and stigma-related misclassifications currently limit our understanding of the transmission dynamics of the virus. We developed a probabilistic model to estimate relative rates of HIV transmission from annotated viral phylogenies that can effectively account for missing or incorrect data, and investigated the rates of HIV-1 subtype B sexual transmission in the UK.

**Methods:** 22,481 HIV-1 subtype B *pol* sequences from the UK HIV Drug Resistance Database linked to epidemiological data from national and cohort clinic data were analysed. Maximum Likelihood phylogenies were reconstructed and 2,860 monophyletic transmission clusters were identified. A maximum likelihood probabilistic model was used to estimate rates of HIV sexual transmission between and amongst men who have sex with men (MSM), heterosexual males ( $H_m$ ) and heterosexual females ( $H_f$ ) within the identified clusters. We also estimated the proportion and most likely risk category of unobserved/undiagnosed transmitters.

**Results:** The highest rate of HIV infection was amongst MSM. Transmission rates from  $H_m$  to  $H_f$  was also high, 11 times the rate from  $H_f$  to  $H_m$ . High infection rates from MSM to  $H_m$  were observed which, when combined with the large number of infected MSM, indicates that a substantial proportion of  $H_m$  in the clusters were infected through sex with men despite reporting sex with a woman as their route of infection. The infection rate between  $H_m$  was higher than from MSM to  $H_m$ , identifying transmission amongst self-declared  $H_m$  as a sub-epidemic distinct from that between MSM. Transmission from  $H_m$  to  $H_f$  was 58 times higher than that from MSM to  $H_f$ , highlighting the linking role of  $H_m$  between MSM and women. Despite their importance in the infection dynamics,  $H_m$  were the most likely group to be unobserved.

**Conclusions:** Rates of HIV transmission estimated from self-reported acquisition routes are likely to underestimate homosexually acquired subtype B infections. Heterosexual men who have sex with men are more likely to do so with other self-identified heterosexual men rather than declared MSM.  $H_m$  provide the dominant link between MSM and heterosexuals, while also being the group most likely to be missing from the cohort. This work highlights the power of likelihood based statistical models to analyse pathogen transmission patterns.

**226 HIV-1 Subtypes, 5' LTR-Leader Sequence Variants, and Late HIV Seroconversion**

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**Background:** A minority within the Pumwani sex worker cohort in Kenya remain persistently seronegative despite frequent exposure to HIV-1. A small proportion of this group seroconverted several years later. This study attempts to identify viral factors, specifically 5' leader sequence variations that might contribute to the late seroconversion. The 5' leader sequence contains sites essential for replication and genome packaging, viz, primer binding site (PBS), major splice donor (SD1) and major packaging signal (PS).

**Methods:** Partial proviral 5' long terminal repeat (LTR) region of 20 late seroconverters and 122 early seroconverters were amplified, cloned and sequenced. Sequencher, MEGA, SPSS and HelixTree SNP and Variation Suite version 6.4.3 were employed to classify HIV subtypes and specific sequence variants correlated with the late seroconversion.

**Results:** Phylogenetic analysis of 3667 5'LTR-leader sequence clones showed that HIV-1 subtype A1 and subtype D of Ugandan origin were over-represented in the viral population infecting the late seroconverters ( $P=3.68 \times 10^{-93}$ ). Recursive partitioning analysis of sequence variants of PBS, SD1 and PS showed that specific sequence variants of PBS ( $P_c=2.12 \times 10^{-55}$ ), SD1 ( $P_c=1.31 \times 10^{-137}$ ) and PS ( $1.90 \times 10^{-19}$ ) were identified only in the viral population of either early or late seroconverters. Furthermore, combinations of sequence variants of PBS [PBS-2 ( $P=0.001$ ;  $P_c=0.04$ ) and PBS-3 ( $P=6.24 \times 10^{-211}$ ;  $P_c=1.57 \times 10^{-173}$ )] variants with specific SD1 sequences were only present in late seroconverters. Molecular Phylogenetic analysis PBS sequence variants identified only in late (PBS-1) or early (PBS-4) seroconverters showed that PBS sequence variants identified only in late seroconverters co-clustered with PBS reference sequences utilizing tRNA<sup>Arg</sup> molecules. Whereas, the PBS sequence variants identified only in early seroconverters (PBS-4) co-clustered with PBS wild type references PBS- tRNA<sup>Lys3</sup> and its variants.

**Conclusions:** This study reports, for the first time, specific PBS, SD1 and PS sequence variants within 5' leader sequence are associated with clinical outcome upon HIV-1 exposure and the findings could aid designing effective anti-HIV strategies.

**227 HIV-1 Stop Codon Usage and Its Clinical Impacts in Rural Ugandan Patients**

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**Background:** Retroviruses have high mutation rates resulting in highly variable codon usage. Stop codon usage (TAA, TAG, TGA) is known to affect translation termination efficiency. In this study, we hypothesize that despite its high mutation rate, HIV-1 stop codon usage in clinical samples is conserved, and variability is associated with pre- and post-therapy clinical parameters.

**Methods:** Pre-treatment baseline plasma samples were collected from initially treatment-naïve HIV-infected patients ( $n=454$ ) in Mbarara, Uganda where subtype A1 and D co-circulate (44% vs 37% by *pol*). Near-full-genome Sanger sequences were obtained by nested PCR and ABI 3730. Subtypes were inferred by Los Alamos RIP. Clinical correlates examined included: Pre-therapy viral load and CD4 count, post-therapy time to virologic suppression ( $\leq 400$  copies/mL) and time to virologic rebound (first of two consecutive viral load  $>400$  copies/mL post-suppression). Statistical significance was defined as  $p < 0.003$  after Bonferroni correction. As a comparator, stop codons were extracted from all available sequences in the Los Alamos HIV sequence database.

**Results:** In the 454 Ugandan patients, HIV-1 stop codon usage was highly conserved and each gene had a specific preference: *gag* (90% used TAA), *pol* (85% TAG), *vif* (99% TAG), *vpr* (84% TAA) and *vpu* (81% TAG), *env* (94% TAA), *nef* (96% TGA). Stop codon usage was not associated with subtype except in *gag*: A1 (93% TAA, 202/217) and D (83% TAA, 111/134),  $p < 0.001$  Fisher's exact. Stop codon usage was also not significantly associated with any pre-/post-therapy clinical correlates in all genes examined after Bonferroni correction (all  $p > 0.01$ ). HIV sequences archived in the Los Alamos database that were predominantly subtype B (58%) and C (10%) showed similar pattern of usage conservation: *gag* (99% TAA), *pol* (82% TAG), *vif* (100% TAG), *vpr* (100% TAG), *vpu* (70% TAG), *env* (100% TAA), *nef* (98% TGA).

**Conclusions:** HIV-1 stop codon usage was highly conserved, implying strong selection pressure(s) on stop codon usage and/or on codon positions in genes with overlapping reading frames. However, stop codon variability was not associated with the pre- and post-therapy clinical parameters examined in this study.

## 228 Intrahost Viral Competition and Archiving Dynamics in HIV-1 Superinfection

Antoine Chaillon; Davey M. Smith; Susan J. Little; Gemma Caballero; Douglas D. Richman; **Gabriel Wagner**

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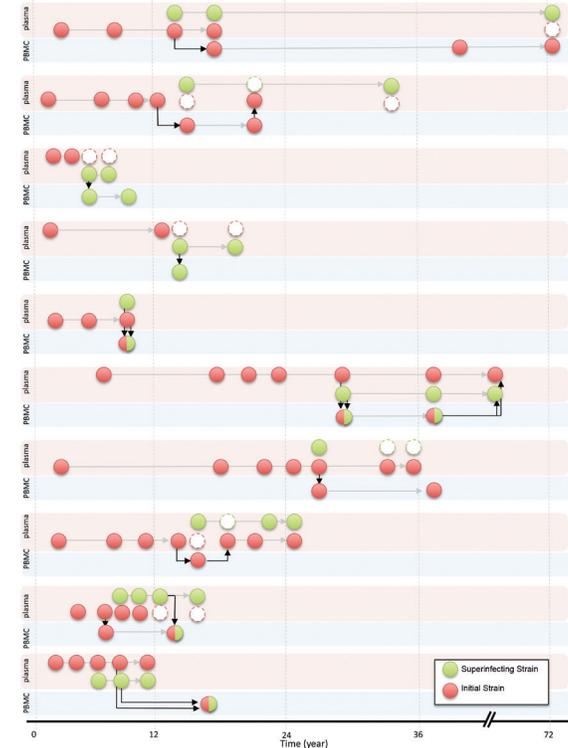
**Background:** The frequent occurrence of HIV-1 superinfection has been increasingly described, but little is known about the competition dynamics of the initially infecting and superinfecting virus strains over time and which variants are archived. We hypothesized that characterizing HIV-1 RNA and DNA populations of superinfected individuals with next generation sequencing (NGS) would determine the relative efficiencies of the initial and superinfecting viruses to be archived into the latent reservoir.

**Methods:** Blood plasma and peripheral blood mononuclear cell (PBMC) samples were longitudinally collected from antiretroviral (ARV) naive participants of the San Diego Primary Infection Resource Consortium with previously documented superinfection ( $n=10$ ). RNA and DNA were extracted, and coding regions within HIV-1 *env*, *gag*, and *pol* were PCR-amplified. Amplified products were then deep sequenced (454 FLX, Roche). Phylogeographic techniques were utilized to infer intrahost viral population migration of sampled viral lineages in the sampled PBMC and plasma.

**Results:** HIV-1 DNA NGS data derived from 19 PBMC samples of 10 individuals (2 median samples per subject; IQR: 2.0-2.8 samples) with HIV-1 intrasubtype B superinfection were analyzed in conjunction with viral sequences generated from HIV-1 RNA populations in the participants' blood plasma (median 6 samples per subject; IQR: 4.3-7.8 samples). At the latest sampled timepoint (median 18.8 months post-estimated date of infection; IQR: 12.9-28.1 months), only 4/10 subjects (40%) had archived the initial and superinfecting strains. Among the remaining 6 individuals: 2 (20%) only had evidence of the superinfecting strain in PBMC DNA, concurrent with replacement of initial virus by superinfecting virus in blood plasma, while the other 4 (40%) only archived the initial strain despite ongoing blood plasma circulation of superinfecting virus in 3 of the 4. Viral gene flow analyses between HIV-1 RNA and DNA populations showed bidirectional migration predominantly from plasma to PBMC ( $p=0.04$ ).

**Conclusions:** Overall, archiving of both initial and superinfecting strains was common (40%) in the cohort, although only a single variant was archived in the remaining individuals with the initial virus predominating. As might be expected with phylodynamic analysis, viral populations flowed freely from plasma to PBMC in these ARV naive participants. It is not clear what mechanisms determine whether superinfecting virus ultimately becomes archived in PBMC.

Overview of the dynamics between initial and superinfecting viral strains in blood plasma and PBMC in 10 participants with HIV-1 superinfection. Time after estimated date of initial infection is shown on the x-axis and 10 superinfected participants are listed on the y-axis. Red and blue shaded areas correspond to blood plasma and PBMC compartments, respectively. PBMC: peripheral blood mononuclear cells.



## 229 Spatiotemporal Dynamics of Drug Resistance Evolution and Persistence of RT-SHIV

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**Background:** To better design therapeutic approaches that prevent the evolution of drug resistance in HIV-1, it is important to understand how drug resistance spreads and establishes within a patient. There is mounting evidence that intrahost viral evolution is a non-homogenous process within the body and therefore must be understood spatially and temporally.

**Methods:** We examined >3300 single-genome sequences from four macaques infected with RT-SHIV, a SIV with an HIV reverse transcriptase (RT). Macaques were given monotherapies to induce the emergence of drug resistance within RT between weeks 12-20 post-infection. Both viral RNA and DNA (vRNA and vDNA) were sampled from four different compartments (lymph node, vagina, gut and PBMC) in addition to plasma vRNA between 13 and 30 weeks post-infection. Compartmentalization was assessed using the variance partitioning statistic  $\Phi_{ST}$  from population genetics.

**Results:** Acquisition of drug resistance is a highly dynamic process with periods of stasis. Although compartmentalization is observed, both natural selection within and migration between compartments are important for establishing drug resistant lineages. We observe differential dynamics between compartments, with the gut being particularly notable. In all four macaques, gut vRNA has the greatest rate of turnover, both compared to gut vDNA and to vRNA in other tissues. In addition, drug resistance increases in frequency more quickly in gut vDNA compared to vDNA other compartments. We find that RT-SHIV does not acquire detectable drug resistance immediately after treatment initiation, but takes more than one week. Upon treatment removal, plasma vRNA does not change significantly over the following 6 weeks, suggesting that drug resistance can have limited fitness cost.

**Conclusions:** Drug resistance evolution depends on the interactions between partially independent compartments, some of which have SHIV populations that show more persistence than others or respond more quickly to pressures imposed by treatment. Characterizing the differences and relationships between the compartments could be important in understanding how multidrug resistance emerges, particularly outside of the blood, and could inform the administration of treatment. That many of these findings are reliant on the sampling scheme of both vDNA and vRNA, across compartments and time demonstrates the importance of varied sampling to obtain a more complete picture of how intrahost evolution proceeds.

## 230 Limited Evidence for a Bias Toward Consensus Residues Upon HIV-1 Transmission

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**Background:** Understanding selective mechanisms governing HIV transmission can help design anti-HIV strategies. Carlson and colleagues recently observed that amino acids (AA) identical to the population consensus were preferentially transmitted among transmission pairs (TP), implying a selection bias for increased fitness at the HIV heterosexual transmission bottleneck (Science 2014).

**Methods:** Phylogenetic trees were reconstructed using TP-derived sequences along with two consensus sequences (independent sequences in the Zambian cohort (Con.ZM); subtype C 2004 consensus (Con.C) and the subtype B sequence HXB2, used as an outgroup to infer the most recent common ancestor (MRCA) of TP sequences.

**Results:** Phylogenetic trees showed that the transmitter's sequence was usually more proximal, i.e. closer to the root than the recipient's sequence (in 42/49, 76/83, and 70/82 TP in *gag*, *pol*, *nef*, respectively). Sequences from recipients were significantly more divergent from the consensus and MRCA than sequences from transmitters (Wilcoxon matched-pairs signed rank test, all  $p$ -values  $< 0.0001$ ). When based on AA sequences, the divergence from either Con.C, Con.ZM, or the MRCA were larger in the sequences from the recipients than in those from the transmitters. The difference was significant in Pol ( $p < 0.0001$ ) and Nef ( $p \leq 0.036$ ), but not in Gag ( $p \leq 0.874$ ). Similar to the distances constrained on the trees, measures based on pairwise distances showed that sequences from the recipients were more distant from the consensus sequences or the MRCA both at the nucleotide (all  $p$ -values  $< 0.0001$ ) and AA level ( $0.044 \leq p \leq 0.136$  for Gag;  $p < 0.0001$  for Pol and Nef).

Next, we identified sites that varied between transmitter and recipient and evaluated whether the residue in the recipient was the consensus. About half of the sites in the recipient had the consensus (Table 1), hence not suggesting a bias toward the consensus at transmission.

**Conclusions:** Our phylogenetic analyses showed that sequences from the recipients were further away from the consensus than sequences from the transmitters, conforming to HIV's diversification since the beginning of the epidemic. Our results suggest that the evidence for a selection bias for increased fitness upon transmission may not be generalizable when HIV sequences as a whole, rather than independent AA, are considered. Given that setpoints viral loads have remained stable over the epidemic, we conclude that a bias toward the consensus at transmission has a limited impact.

**Table 1.** Among sites that differed between the transmitter and recipient, the number of amino acid sites in the recipient that matched ("To") the consensus (either the subtype C consensus (Con.C) or the consensus derived from independent sequences from the Zambian cohort (Con.ZM)) or not ("Away") are reported for each transmission pair, and summarized for each protein.

	n subjects	To Con.ZM	Away	Total	Fraction To Con.ZM
Gag	49	514	547	1061	0.484
Pol	83	550	628	1178	0.467
Nef	82	474	427	901	0.526
Total		1538	1602	3140	0.490

	n subjects	To Con.C	Away	Total	Fraction To Con.C
Gag	49	520	541	1061	0.490
Pol	83	549	629	1178	0.466
Nef	82	431	470	901	0.478
Total		1500	1640	3140	0.478

### 231 Mode of Sexual Transmission Can Exert an Impact on Shaping the HIV-1 Founder Virus

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**Background:** While the global spread of HIV-1 has been fueled by sexual transmission the genetic determinants underlying the transmission bottleneck remains poorly understood. We sought to investigate whether the mode of sexual transmission has an influence on the multiplicity of infection and further examined whether other intrinsic differences exist between heterosexual (HSX) and men who have sex with men (MSM) founder viruses.

**Methods:** Samples from 74 acute HIV-1 infected subjects were subjected to whole genome deep sequencing. Logistic regression coupled with cross validation was used to assess the performance of a newly adapted genetic-distance based measurement to relate quantitative (*env* diversity) viral predictors to qualitative (single/multiple infection) outcomes. To distinguish the pattern of selection between MSM and HSX founder viruses a comparative codon-based phylogenetic framework was implemented and the transmission indices calculated. Lastly, a phylogenetic corrected approach was used to search for signature sites associated with MSM or HSX transmission.

**Results:** Implementation of our genetic distance based approach to deep sequence data revealed that 83% of MSM infections in our cohort are established by a single founder virus. Combination of these data with previously published studies extended this analysis to a total of 354 subjects and further supported that there are no significant differences between the mode of transmission and the rate of multivariant infections. However comparison of envelope sequences revealed that HSX founder viruses exhibited a greater number of codon sites under positive selection, as well as higher transmission indices. Moreover, specific genetic signatures within MSM and HSX founder viruses were identified, with single polymorphisms within gp41 enriched among HSX viruses while MSM viruses appear to select for variants surrounding the CD4 binding site.

**Conclusions:** These results illustrate how consequential the mode of sexual transmission is in shaping the genetic composition of the HIV-1 founder virus. While our findings suggest that the number of viral variants establishing infection is not influenced by the mode of transmission there are marked differences during the transmission bottleneck that influences the selection and genetic make-up of the founder genome. These data provide new insights into the HIV-1 bottleneck process that may in turn facilitate the design of a more effective HIV-1 vaccine or other therapeutic and prevention strategies.

### 232 Characterizing the Multiplicity of HIV Founder Variants During Sexual Transmission

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**Background:** Infections with multiple founder viral strains have been associated with faster HIV disease progression. Most previous studies have estimated the number of founder variants based on the analysis of viral populations sequenced from acutely HIV-infected individuals. We hypothesized that including sequences collected from source partners would improve resolution and characterization of transmission events.

**Methods:** Blood plasma samples were collected from both the source and recipient in 31 epidemiologically and phylogenetically linked transmission pairs from the San Diego Primary Infection Resource Consortium (SD PIRC). All were men who have sex with men (MSM), sampled on average 64 days (range: 11-170) after the recipient's estimated date of infection. Deep sequencing (454 FLX, Roche) of HIV-1 *env* (C2-V3) was performed for all samples. A combination of highlighter plots, tree topologies and sequence diversity were used to determine the number of founder viruses with and without the inclusion of source partner data. Each phylogenetically distinct clade with limited diversity ( $< 2.5\%$ ) in the recipient viral population was deemed to arise from a single founder variant.

**Results:** Using sequence data from the recipient partner, we were able to estimate the number of founders in 26/31 (84%) cases. Seven of these had high diversity ( $> 2.5\%$ ) at baseline, consistent with the existence of multiple founder variants. Undetermined results were produced for 5 cases (16%) due to inconsistency among the applied methods to recipient data. By incorporating sequence data generated from the source partner, we were able to derive the number of founder variants for all 31 transmission pairs. Overall, 16/31 cases (51.6%) involved transmission of multiple founders and we found a moderate agreement between the two approaches with and without source data (73% congruent,  $k = 0.46$ , 95%IC=0.12-0.8). To further evaluate the transmission bottleneck, we focused on the 15 single founder transmissions, and identified four recipients (27%) who had

founder variants that were inferred to arise from minority viral populations in the source, identified as a distinct clade most closely related to the inferred founder variant. These variant lineages were representative of 1.0 to 5.4% of the sampled source population.

**Conclusions:** Incorporating sequence data from the source increased the ability to determine the multiplicity of founder variants, reduced misclassification, and allowed us to infer the transmission of minority variants.

**233 Gut Integrity, CD4 T Follicular Helper Cells, and IgA+ B Cells in GALT Following ART**

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**Background:** Disruption of the gastrointestinal tract (GIT) epithelial and immune barriers is believed to contribute to microbial translocation, systemic inflammation and progression of HIV-1 infection. ART may lead to improved reconstitution of gastrointestinal-associated lymphoid tissue (GALT), but its impact on the integrity of the GIT epithelial barrier is unclear. We hypothesized that incomplete reconstitution of CD4 T cells in the GALT following ART commenced in chronic HIV-1 infection, compared with commencing in primary HIV-1 infection, may be associated with reduced numbers of CD4+ T follicular helper cells (Tfh). This in turn may result in lower levels of locally resident B cells switched to IgA production, loss of first line defense against luminal microbial products, and reduced GIT epithelial integrity.

**Methods:** Fifty-two subjects were recruited into three groups: healthy adult controls (HC) undergoing routine endoscopy and colonoscopy (n=23); HIV+ subjects commencing ART in either primary HIV-1 infection (PHI; n=8); or in chronic infection; CHI; n=21). Confocal endomicroscopy was used to assess gut integrity in vivo in 5 subjects from each group. Gut biopsies were assessed by histology, and flow cytometry of single cell suspensions from biopsies from left colon (LC) and terminal ileum (TI) were used to accurately count T and B cell subsets, as well as EpCAM+ epithelial cells.

**Results:** Confocal endomicroscopy found no difference in fluorescein cell leakage or tight junction enhancement between the 3 groups. The TI CD4+ T cell counts in gut biopsies were 2 fold lower in all HIV+ versus HC (106,000 v 228,000 cells; p=0.01) and there were similarly less CD4+ T cells relative to epithelial cells in both TI (1/55 v 1/22; p=0.02) and LC (1/115 v 1/62; p=0.05). However, we found no significant differences in Tfh or IgA+ B cell counts at either site between all HIV+ and HC. Further analysis showed no difference in CD4, Tfh or IgA+ B cells between subjects who started ART in PHI compared to CHI.

**Conclusions:** In contrast to the current paradigm, confocal endomicroscopy did not find disrupted GIT integrity in treated HIV-1 infection. We found lower absolute and relative CD4 counts in the TI in HIV+ subjects on ART, but they were not associated with significantly reduced Tfh cell counts or IgA+ B cells, compared with healthy controls, or whether the ART was commenced in PHI or CHI.

	CD4 count (x 10 <sup>3</sup> )	CD4:EpCAM ratio	CD4 Tfh (x 10 <sup>3</sup> )	IgA+ B cells (x 10 <sup>3</sup> )
HC LC	65 <sup>a</sup> (46-148)	1/62 (1/35-1/112)	3.0 (0.91-11.6)	19.7 (11.8-40.0)
HIV+ LC	76 (26-106)	1/115 (1/49-1/238)*	2.4 (0.74-9.3)	32.8 (12.3-48.0)
HC TI	228 (97-443)	1/22 (1/13-1/49)	8.7 (0.68-28.6)	24.9 (10.3-150)
HIV+ TI	106 (32-201)**	1/55 (1/31-1/104)***	2.5 (0.76-12.0)	17.2 (2.3-86.0)

<sup>a</sup> Median (IQR); \* p=0.05 versus HC; \*\* p=0.012 versus HC; \*\*\* p=0.02 versus HC

**234 Microbial Translocation and Inflammation Responses to Lubiprostone in HIV Infection**

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**Background:** Microbial translocation (MT) may play a role in delayed CD4 recovery in HIV infection after initiating antiretroviral therapy (ART). Lubiprostone (LPT), a bicyclic fatty acid with no drug-drug interactions that specifically activates CIC-2, a selective chloride channel constituent of the apical membrane of the human intestine, is indicated for treatment of chronic idiopathic constipation. Luminal action is exerted within 4 weeks of therapy. In animal models LPT stimulates recovery of mucosal barrier function via restoration of tight junction protein complexes through intracellular trafficking of occludins. An intervention that aims to decrease the level of MT using modulators that act at the mucosal tight junction barrier has not been studied in HIV.

**Methods:** In the 2-arm, open-label LAMBCHOP (Lubiprostone Activity among the MicroBiota of the Colon in HIV in Opposing Permeability) study (NCT01839734), HIV-1 infected adults aged ≥18 years from The Ruth M. Rothstein CORE Center with CD4 <350 cells/ul after ≥72 weeks of continuous ART and ≥48 weeks of virologic suppression up to baseline (N=20) were randomized 4:1 to 4 weeks of once-daily LPT (24 µg) vs no drug. Baseline demographics were recorded, serum and plasma specimens were collected, and CD4, HIV RNA, MT biomarkers (intestinal fatty acid binding protein [iFABP], zonulin, sCD14, sCD163) and inflammation markers (IL-6, hsCRP) were measured at baseline and 4 weeks. P-values with α<0.05 by Exact Wilcoxon test were calculated for comparisons of median change from baseline to week 4.

**Results:** Baseline demographics were similar between arms. Median age was 49 years (49.5 in the LPT arm vs 49 in controls; p=0.77), male gender was 81.3% in the LPT arm and 50% in controls (p=0.29), and Black race was 37.5% in the LPT arm and 100% in controls (p=0.13). Change in CD4, MT, and inflammation markers are listed in Table 1. The most commonly reported side effect was soft stools, and there were no LPT discontinuations or HIV RNA virologic rebound events.

**Conclusions:** In this pilot trial, there was no change in MT and inflammation markers in HIV-infected persons with delayed CD4 recovery on suppressive ART following 4 weeks of LPT, a duration of therapy that produces appropriate responses in CIC-2 activation for its indicated use in humans. LPT was well-tolerated with once-daily dosing. Further investigation with LPT is ongoing to characterize possible alterations of gut microbiota in stools samples to evaluate potential mechanisms of MT in HIV infection.

Table 1: Characteristic	LPT Arm, N=16	Control Arm B, N=4	p-value
<b>iFABP (pg/mL)</b>	<b>Median (Q1, Q3)</b>	<b>Median (Q1, Q3)</b>	
Baseline	145.76 (118.14, 169.35)	125.36 (82.43, 267.76)	
Week 4	151.50 (75.86, 206.63)	111.73 (98.44, 139.84)	
Δ baseline to 4 weeks	-9.34 (-74.32, 71.74)	14.47 (-155.83, 43.93)	0.751
<b>Zonulin (ng/mL)</b>			
Baseline	1.00 (0.95, 1.11)	3.24 (1.46, 7.14)	
Week 4	1.06 (0.83, 1.88)	0.98 (0.77, 2.04)	
Δ baseline to 4 weeks	0.05 (-0.08, 0.15)	-1.80 (-6.16, 0.37)	0.211
<b>IL-6 (pg/mL)</b>			
Baseline	1.46 (1.11, 1.66)	1.72 (1.44, 6.51)	
Week 4	1.67 (1.19, 2.48)	2.65 (0.75, 6.64)	
Δ baseline to 4 weeks	-0.12 (-0.49, 0.38)	-0.68 (-1.48, 0.93)	0.211
<b>sCD163 (pg/mL)</b>			
Baseline	73.09 (54.57, 92.88)	51.75 (28.68, 73.13)	
Week 4	69.96 (50.61, 94.16)	56.74 (29.21, 90.24)	
Δ baseline to 4 weeks	-2.56 (-10.39, 6.81)	4.99 (0.54, 17.12)	0.290
<b>sCD14 (pg/mL)</b>			
Baseline	10,807.06 (9198.00, 12183.11)	12,370.30 (9548.08, 13117.20)	
Week 4	11,177.92 (10025.86, 12108.48)	11,345.27 (8565.97, 13704.10)	
Δ baseline to 4 weeks	247.20 (-1499.26, 2414.21)	-930.48 (-1448.47, 1053.26)	0.617
<b>hsCRP (pg/mL)</b>			
Baseline	25.99 (13.97, 60.57)	21.48 (4.73, 48.47)	
Week 4	27.72 (14.76, 47.94)	22.97 (11.53, 54.39)	
Δ baseline to 4 weeks	0.51 (-4.98, 6.17)	6.80 (-1.47, 14.19)	0.437
<b>CD4 (cells/ul)</b>			
Baseline	333 (275, 384.5)	329.5 (229.5, 379.5)	
Week 4	339 (269.5, 405.5)	230 (196, 336)	
Δ baseline to 4 weeks	5.5 (-27.05, 24.5)	-17.5 (-107, 30)	0.682

### 235 A20 May Contribute to Recovery of Intestinal Epithelial Function in Treated HIV

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**Background:** Untreated HIV infection is characterized by mucosal Th17 depletion, gut epithelial barrier dysfunction, and microbial translocation, which are reversed to variable degrees by suppressive antiretroviral therapy (ART). Little is known about the specific restorative mediators of intestinal epithelial cell (IEC) function during ART.

**Methods:** Rectosigmoid biopsies were obtained from 9 risk-matched uninfected controls, 6 treatment-naïve, and 20 ART-suppressed HIV-infected participants. IECs were isolated by EDTA treatment and gut lymphocytes were obtained by subsequent enzymatic digestion. Markers of epithelial function were assessed by qPCR on RNA extracted from isolated IECs. Full transcriptomes of IECs were assessed by RNAseq and compared across subgroups. Cytokine production of CD4 T cells was determined by *ex vivo* stimulation and intracellular flow cytometry.

**Results:** Consistent with prior work, IL-17 producing CD4 T cells were significantly decreased in untreated HIV-infected participants and restored to normal levels in ART-suppressed participants. Expression of mucins and tight junction protein genes by IECs was significantly higher in participants on ART than in untreated participants. Trefoil factor 3, a key epithelial repair factor, was also elevated in IECs from ART-suppressed participants relative to both uninfected and untreated groups. RNAseq analysis in isolated IECs identified A20-encoding *TNFAIP3* as one of the most highly upregulated genes in ART-suppressed participants compared to both uninfected and untreated participants. A20 is a ubiquitin-editing enzyme that is induced by IL-17 and NFκB signaling and which acts in a feedback manner to suppress the transcription of inflammatory mediators as well as to support maintenance of tight junctions between epithelial cells. Other genes involved in the regulation of the NFκB and A20 pathways (*IL1R2*, *NFKBIA*) were also among the most differentially expressed genes between groups, further supporting the prediction that NFκB activity is downregulated in ART-suppressed participants.

**Conclusions:** Restoration of mucosal Th17 cells during suppressive ART appears to coincide with a restoration of intestinal epithelial cell barrier function and an active repair response, which may be in part mediated by upregulation of A20. While the causal pathways have yet to be defined, A20 expression in IECs could represent a novel interventional target for treated HIV-infected individuals with persistent intestinal epithelial dysfunction.

**Table 1: Significantly Differently Expressed Genes from RNAseq Analysis on Intestinal Epithelial Cells**

Symbol	Fold-Change (log2)	FDR-Corrected p-value	Direction Of Change in ART	Relative To
IL1R2	-1.015	0.0019	↓	Uninfected
CSNK2A1	-0.996	0.0168	↓	Uninfected
RPL11	0.394	0.0230	↑	Uninfected
TNFAIP3	1.268	0.0230	↑	Uninfected
IFNGR1	-0.681	0.0341	↓	Viremic
POF1B	-0.584	0.0341	↓	Viremic
NFKBIA	0.887	0.0355	↑	Viremic
FCGBP	0.914	0.0064	↑	Viremic
TRIB1	0.965	0.0446	↑	Viremic
C19orf21	1.011	0.0341	↑	Viremic
DEDD2	1.290	0.0381	↑	Viremic
SFN	1.315	0.0065	↑	Viremic
TNFAIP3	1.345	0.0161	↑	Viremic
HBB	1.664	0.0068	↑	Viremic

### 236 Compartment-Specific Alterations of the Intestinal ILC Pool in HIV(+) Patients

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**Background:** Innate lymphoid cells (ILC) have been shown to display critical effector and regulatory functions in innate immunity and tissue remodeling. Recent reports demonstrate ILCs to promote anatomical containment of gut commensal bacteria and to play an important role in intestinal inflammation. However, most of these studies have been performed in mouse models and, thus, the distribution of ILCs in the human gastrointestinal (GI) tract remained unclear.

**Methods:** In HIV(-) individuals biopsies from macroscopically normal tissue were taken in the oesophagus (n=8), the stomach (n=11), the duodenum (n=11), the terminal ileum (n=13), and the left-sided colon (n=13) during routine endoscopy. Moreover, oral biopsies (n=6) as well as peripheral blood (n=14) was studied. In addition, biopsies from the oesophagus (n=7), the stomach (n=12), the duodenum (n=12), the terminal ileum (n=8), and the colon (n=8), as well as PBMC (n=14) from HIV-infected patients under effective cART were analyzed. ILCs were phenotypically characterized by flowcytometry and tested for expression of IFN-γ, IL-13, and IL-22 following stimulation with PMA and ionomycin.

**Results:** Frequency of total ILC (as % of Lin(-) cells) stepwise increased from the oral cavity to the distal GI-tract, with highest frequencies found in the colon. Frequency of CD94(-) ILC1 was significantly higher in upper GI-tract (mouth, stomach, duodenum) than in ileum and colon. Similar observations were made regarding the distribution of CD103(+)ILC1. The opposite was true regarding ILC3, which represented a major ILC population in the ileum and the colon but only a minor fraction in the oesophagus and stomach, respectively. Substantial proportions of ILC2 were only found in the stomach and the oral cavity. Expression of IL-13(ILC2), and IL-22(ILC3) did not differ significantly between the analysed GI-compartments, whereas frequency of IFN-γ(+) cells was lowest in the colon. Frequency of total ILCs in HIV(+) patients was similar to that observed in healthy controls. However, HIV infection was associated with compartment-specific alterations of the ILC pool as frequency of ILC2 and ILC3 was increased in the duodenum of HIV(+) patients whereas in the colon we observed reduced numbers of ILC3 but increased numbers of NCR(-) ILC1.

**Conclusions:** Our data indicate a compartment-specific distribution of ILCs in the human GI-tract, which is markedly dysregulated in HIV infection.

### 237 IL-33/ST2 Axis as an Inflammatory and Gut Damage Marker in Primary HIV Infection

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**Background:** The alarmin IL-33 and its receptor ST2 mainly expressed by innate lymphoid type 2 cells are involved in microbial invasion, cytokine induction and promotion of cytotoxic CD8 T cells. The soluble ST2 (sST2) binds IL-33 as a decoy receptor to negate its inflammatory/healing effects. sST2 has been validated as a prognostic marker in cardiac insufficiency, IBD, GVHD and been evaluated in HIV. We assessed relationship of IL-33/ST2 axis with gut mucosal damage markers in patients undergoing primary HIV infection (PHI).

**Methods:** A total of 41 patients diagnosed during PHI were followed: 24 remained untreated while 17 initiated early ART. We also assessed chronically infected patients treated (n=15) or not (n=14), elite controllers (EC, n=10) and uninfected controls (n=15). IL-33 and sST2 plasma levels were compared with markers of gut epithelial damage (I-FABP), microbial translocation (LPS, sCD14) and TNF- $\alpha$ , IL-7 and IDO immunosuppressive activity (Kynurenine/Tryptophan). CD4 and CD8 T-cell activation (HLADR/CD38) and exhaustion (PD-1) and Tregs were assessed by FACS.

**Results:** sST2 levels were elevated during primary (18.6 ng/mL, p<0.001) and chronic (15.6 ng/mL, p=0.034) HIV infection as compared to EC and controls (10.5 and 11.2 ng/mL). IL-33 levels were close to limit of detection in all groups and controls. sST2 levels positively correlated with plasma IDO activity (r=0.1981, p=0.021), TNF- $\alpha$  (r=0.3051, p=0.002) and IL-7 (r=0.2269, p=0.006), CD8 count (r=0.2721, p=0.001) and inversely correlated with CD4/CD8 ratio (r=-0.2195, p=0.015). However, no association of sST2 was observed with CD4 count, Treg and viral load (p>0.05). sST2 was associated with expression of activation and exhaustion markers on CD4 & CD8 T cells (p<0.05) and correlated with I-FABP (r=0.2099, p=0.039), LPS (r=0.1865, p=0.042) and sCD14 (r=0.2188, p=0.037). Prospective analysis following PHI showed that early ART had no impact on sST2 and gut damage markers contrasting with normalization of IDO, inflammatory cytokines and activation/exhaustion markers.

**Conclusions:** sST2 was elevated in primary and chronic HIV infection correlating with elevation of CD8 T cells and their expression of activation/exhaustion markers. Similar to other gut damage markers, sST2 never improved following early ART, contrasting with reversal of immune activation/exhaustion markers, IDO and inflammatory cytokines. By linking immune function, and tissue damage, IL-33/ST2 axis may induce gut injury and represents an immunotherapeutic target.

**238 Probiotics Modulate Th1/Th17 and IFN Response in HIV Patients on Suppressive cART**

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**Background:** The gut-associated lymphoid tissue (GALT) is an important site of HIV replication and immune dysfunction; previous works have demonstrated the failure of antiretroviral therapy (ART) in controlling the immune activation and modifying the balance of immune cells profiling in intestinal mucosa; we evaluated the immunomodulatory effect of six-month of probiotics supplementation in ART experienced subjects.

**Methods:** Ten ART treated HIV-infected subjects underwent endoscopic procedures and blood collection prior to initiation of probiotics supplementation (T0) and after 6 months (T6) (Trial #NCT02276326).

Normal mucosa biopsies were obtained from five different intestinal sites; lamina propria lymphocytes (LPLs) were isolated. Cell phenotype (CD3, CD4, CD45Ro, CD27) and activation markers (CD38 and HLA-DR) were detected on freshly samples. Th1 and Th17 cell phenotype were detected by IL-17A and IFN $\gamma$  intra-citoplasmatic staining, respectively, after overnight PMA and ionomycin activation. IFN $\alpha$  subtypes (n=12), IFN $\beta$ , IFN $\gamma$ , IFN $\gamma$  mRNAs were quantified by RT-PCR. Data were analyzed by Wilcoxon signed-ranked test (paired data) and Spearman's correlation using SPSS v22.

**Results:** Subjects assumed 1.8x10<sup>12</sup> live bacteria daily for 6 months; no adverse event was observed during the follow-up. All subjects had undetectable plasmatic viral load at T0 and T6. The median CD4 cell number at T0 was 674 cells/mm<sup>3</sup> and didn't change at T6. Intestinal and blood Th17 and Th1 frequencies at T6 were increased in CD4 population and in EM (Effector Memory) and CM (Central Memory) subpopulations (Tab 1). A reduction of CD38 and HLA-DR was observed at T6 in CD4 and in all EM and CM subpopulations.

The most statistically significant increase in Th17 frequencies in gut was observed in CM population and this level at T6 was inversely correlated to the percentages of CD4 cells expressing HLA-DR in EM subpopulation (r=-0.79 p=0.006). A decrease in IFN $\gamma$  expression and an increase in IFN $\alpha$  subtypes (10,14,17 and 21) expression were recorded at T6 in gut whereas no such differences were observed for the other IFN genes analyzed in gut and peripheral blood compartments.

**Conclusions:** Probiotics supplementation for 6 months in HIV+ individuals on effective ART induced a marked recovery in the levels of both Th17 and Th1 cells in GALT and in peripheral blood and a significant reduction of cellular markers of activation. Furthermore, the use of probiotics was associated with a specific modulation of IFN-mediated immunity in the gut.

	PBMCs			LPLs		
	T0	T6	p	T0	T6	p
<b>Th17</b>	0.4	1.6	0.059	0.9	2.7	0.059
<b>EM Th17</b>	0.3	1.6	<b>0.037</b>	0.0	2.0	<b>0.011</b>
<b>CM Th17</b>	0.5	2.1	<b>0.028</b>	0.2	3.3	<b>0.007</b>
<b>Th1</b>	0.2	15.2	0.14	0.1	19.4	<b>0.005</b>
<b>EM Th1</b>	0.6	31.9	0.11	2.9	13.6	<b>0.047</b>
<b>CM Th1</b>	0.3	17.9	0.23	7.7	26.3	0.059
<b>CD4+CD38+</b>	8,205	2,81	<b>0.005</b>	8.5	2.8	<b>0.005</b>
<b>CD4+DR+</b>	4,32	2,09	<b>0.005</b>	6.5	4.2	<b>0.005</b>
<b>EM_CD4+CD38+</b>	3,5	1,87	<b>0.005</b>	8.3	1.9	<b>0.005</b>
<b>EM_CD4+DR+</b>	10,15	8,12	<b>0.005</b>	5.9	4.8	0.575
<b>CM_CD4+CD38+</b>	8,86	4,885	<b>0.007</b>	9.7	2.9	<b>0.005</b>
<b>CM_CD4+DR+</b>	4,32	2,505	0.074	6.9	4.3	0.059

\*PBMC= peripheral blood mononuclear cell; LPL= lamina propria lymphocytes; EM=Effector memory; CM= Central memory.

**239 Ceniciviroc Decreases sCD14 and LBP Levels Without Affecting Gut Permeability**

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**Background:** Ceniciviroc (CVC), a potent oral, once-daily CCR2/CCR5 antagonist, blocks HIV entry through CCR5 blockade and inhibits monocyte trafficking through CCR2.

Monocyte recruitment promotes atherosclerosis, hepatic fibrosis and other end-organ diseases in HIV infection. In a completed randomized, double-blind study of 48 weeks of CVC or efavirenz (EFV) with tenofovir/emtricitabine (TDF/FTC) (Study 652-2-202; NCT01338883), levels of sCD14, a marker of monocyte activation, significantly decreased with CVC treatment independent of HIV-1 RNA changes but increased in the EFV arm. We aimed to determine whether decreases in this monocyte activation marker with CVC were attributable to decreased intestinal permeability and microbial translocation.

**Methods:** 135 men and 8 women were enrolled, age 35±11 years (46 Black, 35 Hispanic, 53 White, 9 Other). Intestinal fatty acid binding protein (I-FABP), zonulin, flagellin and lipopolysaccharide binding protein (LBP) were measured by ELISA on stored plasma. As sCD14 levels decreased in participants who received TDF/FTC with CVC either 100mg or 200mg (N=115), these arms were pooled and compared to the arm who received TDF/FTC with EFV 600mg (N=28). Median values were compared between groups by Mann-Whitney test and longitudinal within-group comparisons by Wilcoxon matched-pairs signed rank test. Pearson correlation coefficients were calculated.

**Results:** Plasma I-FABP and flagellin levels increased in both CVC and EFV groups whereas LBP levels decreased in both groups during 48 weeks of ART (see table). Zonulin levels did not change significantly. Lower LBP levels were associated with lower CRP levels in both groups at most time points and with lower GGT at weeks 24 and 48 in CVC group. In the CVC group, lower sCD14 levels were associated with lower LBP levels at weeks 0 and 12 and lower I-FABP levels at weeks 12 and 24. Lower sCD14 levels were associated with lower LDL cholesterol levels at all time points.

**Conclusions:** The decrease in sCD14 levels reflecting a reduction in monocyte activation with CVC was not accompanied by decreased intestinal permeability (I-FABP, zonulin) or bacterial products (flagellin). The decrease in LBP with CVC could reflect decreased LPS in the liver, but the consistent correlation with decreases in CRP suggests decreased activation of the acute phase response. Thus, CVC decreases sCD14 levels independently of changes in microbial translocation, viremia or gut integrity, suggesting CVC may directly inhibit monocyte activation.

Group	CVC					EFV				
	Wk 0	Wk 12	Wk 24	Wk 32	Wk 48	Wk 0	Wk 12	Wk 24	Wk 32	Wk 48
I-FABP (pg/mL)	2256	3604*	3830*	2978*	3251*	1742	3691*	5134*	4006*	4887*
Zonulin (ng/mL)	8.39				7.99	9.30				9.10
Flagellin (ng/mL)	1.55	1.65*	1.63	1.55*	1.96*	1.58	1.63	1.67*	1.64	1.82
LBP (ug/mL)	16.3	15.2	12.0*	13.2*	11.9*	19.3	15.0	9.4*	8.0*	8.9*

\* P<0.05 compared to Wk 0

## 240 In Vivo Evidence for a Role of Pyroptosis in HIV Pathogenesis

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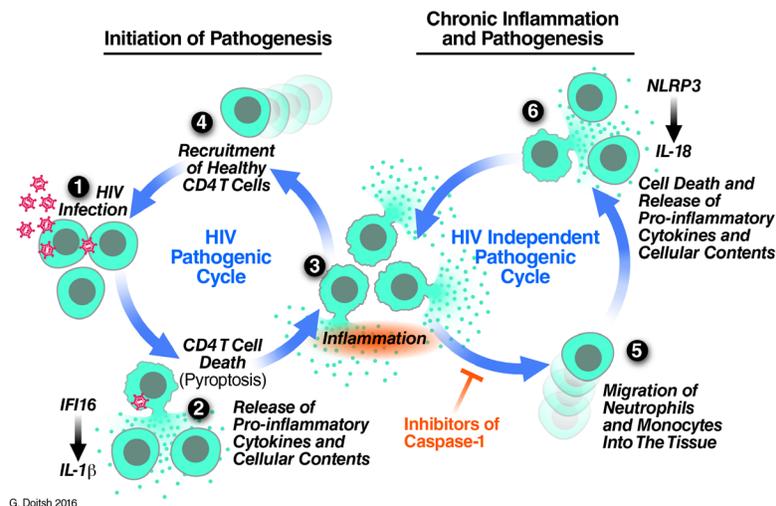
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**Background:** 30% of HIV-infected subjects fail to increase their CD4 T-cell lymphocyte counts during combination antiretroviral therapy (cART) and remain both inflamed and immunodeficient. These individuals are termed “immunological non-responders.” Systemic inflammation persists in these HIV-infected patients despite the absence of detectable viremia. The reason for this persistent inflammation is unknown. We have found that CD4 T cells in lymphoid tissues are naturally primed to mount inflammatory responses—these cells constitutively express high levels of cytoplasmic pro-IL-1 $\beta$ , ASC (caspase-1 adaptor protein), and NLRP3 inflammasome. The release of intracellular ATP by pyroptotic CD4 T cells may provide a second inflammatory stimulus to induce activation of caspase-1 by the NLRP3 inflammasome in surrounding uninfected CD4 T cells that are already primed. Thus, pyroptosis initiated by HIV may result in an avalanche of new rounds of pyroptosis in primed CD4 T cells by the repeated release of intracellular ATP in a *virus-independent manner*. Such an “auto-inflammation” scenario could generate persistent rounds of pyroptosis, chronic inflammation, and loss of CD4 T cells even when viral replication is reduced by antiretroviral therapy

**Methods:** Longitudinal analyses of plasma and peripheral lymphoid tissues from subject with three distinct clinical backgrounds: uninfected, infected untreated, infected treated virological suppressed.

**Results:** We now show extensive caspase-1 activity and secretion of IL-18 in large areas of paracortical zones in lymph nodes freshly recovered from infected untreated patients. Interestingly, while caspase-1 activity is decreased in some subjects following treatment with antiretroviral therapy (ART), other patients exhibit persistent caspase-1 activity even when viral replication was undetectable. Moreover, in tissues where active caspase-1 was detected, we observed a significant loss of myeloid cells including resident macrophages and dendritic cells. This loss was consistent with high levels of extracellular bioactive IL-18, suggesting cell death by caspase-1-mediated pyroptosis. These findings highlight a role for pyroptosis beyond CD4 T cell death, and underscore the involvement of myeloid cells in promoting chronic inflammation and secretion of IL-18.

**Conclusions:** These preliminary findings further underscore the role of pyroptosis during R5-tropic viral infection *in vivo*, raising the possibility for a new class of “anti-AIDS” therapeutics.



## 241 Caspase Inhibition Prevents HIV Replication and Cell Death in Human Lymphoid Tissue

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**Background:** The pathway causing CD4 T-cell death in HIV-infected hosts remains poorly understood although apoptosis has been shown as a key mechanism. Using an *ex vivo* human lymphoid aggregate culture, it has been proposed that most of the quiescent CD4 T cells die by caspase-1-mediated pyroptosis, although the mechanisms of cell death in *ex vivo* lymphoid tissue has not been explored.

**Methods:** Human tonsil histocultures were prepared from 4 healthy individuals and infected *ex vivo* with a X4 virus in the presence or absence of the entry inhibitor JM-2987 (1 $\mu$ g/mL), the integrase inhibitor raltegravir (1 $\mu$ g/mL) and the pan-caspase inhibitor Q-VD-OPh (30 $\mu$ M). Drugs were added to the cultures 2 hours before infection. Culture medium was collected and replaced every 3 days during 12 days and the p24 production was measured in the supernatant by ELISA. Tissue cells were isolated and CD4 T cell depletion (CD3+CD8-), p24 cellular content, and caspase-1 and 3/7 activity (FLICA assay) was analyzed by flow cytometry.

**Results:** After 12 days of culture, viral replication was totally inhibited in the presence of the antiretroviral drugs JM-2987 and raltegravir. Surprisingly, the addition of the pan-caspase inhibitor Q-VD-OPh resulted also in an almost complete inhibition (84%) of the viral replication. Furthermore, a massive cell depletion was observed in the absence of antiretroviral drugs ( $18\% \pm 6\%$  of cells remaining relative to uninfected controls) that was totally prevented by the addition of JM-2987 and raltegravir. Loss of cells was also prevented by Q-VD-OPh treatment ( $63\% \pm 4\%$  relative to uninfected control). HIV infection triggered high levels of both caspase-1 and 3/7 activity ( $34.7\% \pm 4$  and  $20.4\% \pm 3$ , respectively) that were inhibited to the uninfected control level by JM-2987 and raltegravir ( $15.6\% \pm 1$  and  $9\% \pm 0.6$ , respectively) and partially inhibited by Q-VD-OPh ( $24.2 \pm 3$  and  $15\% \pm 1$ , respectively). After infection, increased caspase-3 was mainly detected in the CD3+CD8-CD4- population (productively infected cells) while increased caspase-1 was observed in both CD4+ and CD4- T cell subsets.

**Conclusions:** HIV infection of histocultures of human lymphoid tissue triggered caspase-1 and 3/7 activity that was inhibited with antiretroviral drugs as well as with a pan-caspase inhibitor. Caspase inhibitor treatment prevented both viral replication as massive CD4 T cell depletion suggesting that, caspase activation contributes significantly to viral replication and HIV pathogenesis in lymphoid tissue.

## 242 Follicular Regulatory T Cells Are Highly Permissive to HIV-1 Ex Vivo

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**Background:** The majority of HIV replication occurs in follicular helper T cells (TFH) in secondary lymphoid follicles during asymptomatic disease. Follicular regulatory T cells (TFR) are a subset of TFH that control germinal center (GC) responses. Their role in HIV-1 replication is unknown. We hypothesized that TFR are highly susceptible to HIV and evaluated potential causative mechanisms.

**Methods:** Cells were disaggregated from healthy human tonsils or HIV-uninfected human lymph nodes (LN) and spinoculated with R5-tropic GFP reporter virus or mock spinoculated, cultured for 2 days, and analyzed for GFP expression by flow cytometry. Due to downregulation of CD4 by HIV, cells were gated on CD3+CD8- cells and subsets within were defined as TFR (CXCR5+CD25+CD127-), GC TFR (CXCR5+PD1+CD25+CD127-), TFH (CXCR5+CD25-), and GC TFH (CXCR5+PD1+CD25-). In select experiments, tonsil TFR and TFH were sorted prior to spinoculation. CCR5 and Ki67 expression were determined at baseline prior to spinoculation in a subset of subjects. Statistical analyses were performed by Wilcoxon matched-pairs signed rank test using GraphPad Prism 6.

**Results:** %GFP+ is higher in TFR compared to TFH (median, 4.8-fold higher;  $p=0.008$ ) and in GC TFR compared to GC TFH (median, 4.0-fold higher;  $p=0.008$ ;  $n=8$ ). GFP MFI is not significantly different in TFR compared to TFH (medians, TFR=4599, TFH=6952;  $p=0.09$ ,  $n=6$ ), but is lower in GC TFR compared to GC TFH (medians, GC TFR=4114, GC TFH=6427;  $p=0.03$ ,  $n=6$ ). Spinoculated cultures of sorted tonsil TFH and TFR ( $n=4$ ) confirm differences observed in unsorted cultures (median fold difference %GFP+ TFR:TFH = 3.5). In HIV-uninfected LN ( $n=2$ ), %GFP+ is also higher in TFR compared to TFH (average, 4.7-fold higher) and GC TFR compared to GC TFH (average, 3.4-fold higher). In tonsil cells prior to spinoculation, %CCR5+ is higher in TFR (37%) compared to TFH (11%;  $p=0.03$ ,  $n=6$ ) and GC TFR (38%) compared to GC TFH (11%). %Ki67+ ( $n=3$ ) is higher in TFR compared to TFH (medians, TFR=51%, TFH=14%) and in GC TFR compared to GC TFH (medians, GC TFR=63%, GC TFH=14%).

**Conclusions:** TFR are highly permissive to R5-tropic HIV *ex vivo*, which may promote HIV infection and replication within secondary lymphoid follicles *in vivo*. Potential mechanisms underlying heightened permissiveness of TFR include elevated proliferation and CCR5 expression.

## 243 Dead Cells Tell No Tales: The Enigma of Discerning HIV-Infected Cell Death

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**Background:** Multiple mechanisms contribute to CD4 T cell death in HIV. It is unclear which predominate, and whether cell death occurs mostly in uninfected, non-productively infected, or productively infected cells. This traditionally has been studied by measuring co-expression of markers of HIV infection (viral protein or nucleic acid) and cell death. During cell death, host cell proteases and endonucleases are activated. We hypothesized that activation of these enzymes during cell death degrades common markers of infection and therefore precludes reliable detection of infected-cell death.

**Methods:** Jurkat T cells transiently or stably expressing eGFP, and J-Lat 10.6 cells (which contain integrated HIV provirus encoding GFP) were used. Cells were treated with camptothecin (CPT) or DMSO control to induce apoptosis, and monitored for apparent GFP expression and markers of cell death over time by flow cytometry. Beta-actin gene expression was measured by RT-qPCR.

**Results:** Jurkats transiently expressing eGFP treated with CPT had decreased apparent GFP expression over 48 hrs compared to DMSO treated cells, coincident with loss of membrane integrity measured by vital staining. CPT treated cells also had significantly decreased RT-qPCR signal for the housekeeping gene beta-actin ( $P<0.001$ ) compared to control. Jurkat T cells stably expressing eGFP, sorted twice to ensure >95% baseline GFP expression, were treated in a similar manner. These CPT-treated cells also lost apparent GFP expression by flow cytometry coincident with binding Annexin V (37% loss), expression of active Caspase 3 (50% loss) and becoming TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) positive (80% loss), whereas DMSO treated cells maintained >90% GFP expression. 96% of CPT treated, dead Jurkat-eGFP at 48 hrs by vital staining were apparent GFP-negative. Similar effects were seen with J-Lat 10.6 cells reactivated with prostratin, and subsequently treated with CPT compared to DMSO control.

**Conclusions:** Our data suggest that common markers of cellular HIV-infection, including protein and mRNA expression, diminish as cells progress through the death process. Therefore, determining the proportion of dying or dead cells that are HIV "positive" necessarily under-estimates this fraction. Novel markers of infected cell death are needed. This is important for HIV cure, for which novel strategies, i.e. "shock and kill", require specificity to infected cells versus uninfected cells.

## 244 Multidimensional Profiling of HIV-Infected Human CD4 T Memory Stem Cells

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**Background:** Human CD4 T cells constitute the long-lived HIV reservoir, which prevents the virus eradication in HIV infected patients treated with highly active antiretroviral therapy (HAART). Emerging evidence suggest that the HIV reservoir is formed early upon infection within the CD4 T memory cell population.

A new powerful technology which combines Flow Cytometry and Mass Spectrometry (Mass Cytometry, CyTOF) allows immune profiling of human cells at unprecedented resolution (>30 parameters for single cell). We hypothesized that T memory cells supporting infection differ with respect to phenotype, cell cycle and proliferation.

**Methods:** We compared the immuno-phenotype, cell cycle profiles and proliferation capacity of CD4 T cells stimulated with IL2 or IL15 and infected with replication competent NL4.3-HIV. A custom antibody cocktail was used to detect >20 surface and intracellular markers including p24 by CyTOF. We also conducted quantitative phospho-proteomics of CD4 T cells treated with IL2 and IL15 different cytokines in order to define signaling pathways specific for the given CD4 T cells stimulation.

**Results:** The CyTOF analysis revealed that T memory stem cells (TSCM) display high proliferation capacity, which is specifically amplified by IL15. Moreover TSCM efficiently support HIV infection. The susceptibility to infection was further increased by IL15 stimulation with TSCM constituting up to 9% of the infected cell population. Cell cycle analysis showed a skewing of the infected cell populations towards the G2 phase.

The phosphoproteomic analysis of CD4 T cells showed that 247 proteins were differentially phosphorylated in IL15 compared to IL2. The main processes activated by IL15 were RNA metabolism, transcription regulation, chromatin remodeling, signal transduction and cytoskeleton remodeling. Of note, we also identified up-regulation of SAMHD1 phosphorylation upon IL15 stimulation.

**Conclusions:** Our data indicate that TSCM are cycling and support productive infection. Moreover, the TSCM pool is dynamic and expands in response to IL15. This cytokine activates several pathways favorable to infection (transcription and chromatin remodeling) and counteracts the antiviral activity of SAMHD1. A global characterization of the features of infected TSCM is a key factor for the development of new strategies to eliminating the reservoir.

#### 245LB Single Cell Transcriptome Sequencing of Human Lymph Node HIV-Infected CD4 Cells

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**Background:** Identification of live HIV-infected CD4 cells allows the characterization of individual cells both by flow cytometry and by transcriptomic analysis.

**Methods:** Cervical lymph nodes were obtained from 3 viremic HIV-infected volunteers, median CD4 count 671 (range 247-1,288) and median viral load 17,437 (14,075-29,923). Approximately 90 individual CD45RO<sup>+</sup> PD1<sup>hi</sup> CD4<sup>dim</sup> cells were index sorted into individual wells for each patient. In addition, surface expression of HIV envelope on CD4 T cells was identified using fluorescently labelled PGT121. Whole transcriptome libraries were generated from the purified RNA of sorted single cells using established methods and deep sequenced on an Illumina HiSeq (Trombetta, JJ et al. (2014) *Curr Protoc Mol Biol* 4.222.1-422.17).

**Results:** The median frequency of HIV-RNA containing PD1<sup>hi</sup> CD4<sup>dim</sup> cells was 8.0 (4.6-8.0)%. In cells from two of the three lymph nodes the frequency HIV RNA containing cells was higher in cells staining for PGT121, 15 and 18%, compared to 6 and 5%, respectively. There was no difference in HIV RNA containing cells within PGT121<sup>+</sup> and PGT121<sup>-</sup> populations in the lymph node from the third volunteer. The percentage of total reads containing HIV RNA varied between 0.02-6.0% in individual HIV+ cells. In two of the three volunteers the median frequency of HIV reads was higher in PGT121<sup>+</sup> cells than in PGT121<sup>-</sup> cells, 0.15 and 0.6%, compared to 0.04 and 0.03% PGT121<sup>-</sup> cells. In the other sample there was essentially no difference.

**Conclusions:** These data show that it is possible to identify HIV-infected CD4 T cells at a level which makes whole cell transcriptome analysis of HIV RNA+ and similar HIV RNA- cells possible.

#### 246 BST-2, TRIM22, and RAD51 in Host Susceptibility to HIV-1 Infection and Virus Control

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**Background:** To establish infection and replicate efficiently, HIV-1 must overcome host antiviral restriction factors and utilize host replication cofactors. We hypothesized that expression levels of specific HIV-1 restriction factors or replication co-factors may modulate infection risk among HIV-1 exposed persons or potentiate viral control during early infection.

**Methods:** We measured by quantitative real-time PCR levels of 25 previously validated HIV antiviral factors (IFN- $\alpha$ 2b, IFN $\beta$ , MxA, TRIM5 $\alpha$ , TRIM11, TRIM19, TRIM25, TRIM27, TRIM28, TRIM36, PAF1, CTR9, AP2M1, RTF1, DNMT2, MKRN3, HERC5, C3orf63, SETDB1, RPRD2, COX18, RNF19A, BST-2, APOBEC3F and p21) and two replication cofactors (Rab7A and RAD51) in peripheral blood mononuclear cells (PBMCs) of high risk, HIV-1 uninfected participants, and in recently HIV-1 infected participants. Using two well-pedigreed cohorts, the CAPRISA 002 study, (Training cohort) and the antiretroviral-naïve placebo arm of the CAPRISA 004 study, (Validation cohort), we explored in vivo association with risk of HIV-1 acquisition and biomarkers of disease progression. Generalized Estimating Equations (GEE) models were fitted for expression levels of all HIV-1 restriction factors and replication cofactors, plasma viral loads and CD4+ T cell counts. Differences between groups were evaluated by using Student's t-test.

**Results:** In both the training set cohort and the validation cohort (CAPRISA 004 study) non-seroconverters had significantly higher pre-infection (baseline) levels of the antiviral factor BST-2 than seroconverters ( $p < 0.003$ ). Levels of TRIM22, another antiviral factor, correlated negatively with viral load (all  $p < 0.05$ ) whereas levels of the replication cofactor RAD51 correlated positively with viral load in both cohorts (all  $p < 0.05$ ). None of the other factors consistently associated with HIV-1 outcomes.

**Conclusions:** These data suggest that high expression of BST-2, an antiviral factor that inhibits efficient HIV-1 release is associated with reduced susceptibility to HIV-1 infection, and that some HIV-1 restriction factors and replication factors may play a role that impacts the level of viral load during early HIV-1 infection.

#### 247 6-Amino-Acid Insertion/Deletion Polymorphism in TIM1 Confers Protections Against HIV1

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**Background:** TIM-1 (T-cell immunoglobulin and mucin domain 1), a cell surface glycoprotein, facilitates the entry of enveloped virus, such as HIV, into host cells. Because the length of the mucin domain of TIM-1 is a critical factor in modulating viral entry, we assessed whether the TIM-1 18-bp insertion/deletion polymorphism modulates susceptibility to HIV-1 infection in three independent cohorts of HIV-exposed seronegative (HESN) individuals.

**Methods:** The Tim-1 18-bp insertion/deletion polymorphism was genotyped in three case/control cohorts of HIV sexually-exposed HESN and their HIV-1-infected partners with different geographic origin (Italy, Peru and Colombia); data from an additional cohort were retrieved from a previous study conducted in Thailand. CD4+ T lymphocytes purified from 34 healthy controls (HC) grouped according to their TIM-1 genotype were infected *in vitro* with HIV-1<sub>Ba-L</sub> and viral replication was assessed after 5 days by measuring viral p24 levels produced by the infected cells.

**Results:** In all comparisons, homozygosity for the short TIM-1 allele was more common in HESN than in HIV-1 infected subjects. A meta-analysis of the four association analyses, revealing no heterogeneity among samples, yielded a p value of 0.005. These results were sustained by a drastic and significant reduction of viral replication in CD4+ T lymphocytes isolated from HC that were homozygous for the short TIM-1 allele compared to subjects carrying at least one long allele (t-test,  $p = 0.042$ ).

**Conclusions:** The TIM-1 deletion allele protects from infection with a recessive effect. In vitro infection assays on CD4+ T lymphocytes support this conclusion and underscore a complex interaction between enveloped viruses and TIM molecules that need further investigation.

#### 248 MicroRNA Profile in CD8 T Cells From HIV-Infected Individuals and Disease Progression

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**Background:** The relationship between host microRNAs (miRNA), viral control and immune response has not been elucidated in the field of HIV yet. The aim of this study was to assess the differential miRNA expression profile in CD8+ T-cells between HIV-infected individuals who differ in terms of disease progression.

**Methods:** miRNA profile from resting and CD3/CD28-stimulated CD8+ T-cells from uninfected individuals (HIV-, n=11), Elite Controllers (EC, n=15), Viremic Controllers (VC, n=15), Viremic Progressors (VP, n=13) and HIV-infected patients under antiretroviral treatment (ART, n=14) was assessed using Affymetrix miRNA 3.1 strip arrays. After background correction, quantile normalization and median polish summarization, normalized data were fit to a linear model. Multiple test was corrected using 5% false discovery rate (FDR). The analysis comprised: resting samples between groups; stimulated samples between groups; and stimulated versus resting samples within each group. Enrichment analyses of the putative target genes were performed using bioinformatical algorithms (TargetScan, miRanda, miRWalk).

**Results:** A downregulated miRNA pattern was observed when resting samples from all infected groups were compared to HIV- (hsa-miR-4734 in VP vs HIV-, hsa-miR-4505 in EC vs HIV- and hsa-miR-4492 and hsa-miR-4508 in ART vs HIV-). A miRNA downregulation was also observed when stimulated samples from EC, ART and HIV- groups were compared to the VP, being hsa-miR-4492 the most downregulated. This miRNA targets 2346 predicted genes including the Linker for Activation of T-Cells (LAT), a key protein in

the TCR-signalling pathway. Although a preferential miRNA downregulation was also observed when we compared stimulated to the respective resting samples, VP presented a differential miRNA expression pattern. VP showed a downregulation of both hsa-miR-155 and hsa-miR-181a whereas in the other groups, we observed either an upregulation or no differences, respectively. Overall, functional enrichment analysis revealed that the predicted target genes were involved in metabolic regulation, apoptosis, immune response and signal transduction pathways.

**Conclusions:** Resting CD8+ T-cells do not exhibit a differential miRNA expression between HIV-infected individuals but they do differ from non-infected individuals. Moreover, a specific miRNA pattern is present in stimulated CD8+ T-cells from VP which probably reflects a detrimental pattern in terms of CD8+ T cell immune response.

#### 249 P2X7 Purinergic Receptors Are Required for HIV-1 Infection and Inflammation

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**Background:** HIV-1 infection is incurable and causes a chronic inflammation which causes multiple comorbidities, even with virologic suppression. The mechanism of this inflammation is not clearly understood. Purinergic receptors are known to be mediators of inflammatory responses and can contribute to pro-inflammatory cytokine production and lymphocyte cell death. Purinergic receptor signaling is important for HIV-1 infection and we have recently found that inhibition of the P2X subtype purinergic receptors potently blocks HIV-1 productive infection at the level of membrane fusion. This study examines whether virus-induced purinergic signaling is responsible for inflammatory cytokine release during HIV-1 infection.

**Methods:** We use fluorescent constructs of HIV-1 to evaluate productive infection by flow cytometry in CD4 T cell lines and primary cells in the presence or absence of purinergic inhibitors. Infected supernatants can be subjected to multiplex bead capture assays to test for an array of human cytokines. We have tested the effect of HIV-1 infection on peripheral blood mononuclear cells and observed levels of pro-inflammatory cytokine production.

**Results:** We observe that HIV-1 productive infection in CD4 T lymphocytes is potently blocked by P2X7 selective inhibitors. Exposure of peripheral blood mononuclear cells to HIV-1 results in induction of pro-inflammatory cytokines and levels are reduced with P2X7 inhibition. This suggests that P2X7 inhibitors can block both HIV-1 productive infection and associated inflammation.

**Conclusions:** Our findings distinguish purinergic receptors, specifically P2X7, as key signaling mediators of HIV-1 infection and inflammation. We believe that these drugs could be used as adjunctive antiretroviral therapy that could serve to reduce the morbidity and mortality associated with HIV-1 chronic inflammation.

#### 250 Characterization of Plasma Exosome Protein Cargo in HIV Patients on ART

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**Background:** Exosomes are microvesicles originating from many cell types including immune cells. Prior studies suggest exosomes play a role in HIV pathogenesis and some comorbidities. The relationship of exosome cargo in peripheral blood to HIV infection, immune responses and comorbidities has potential applications for biomarker discovery and new treatment strategies. Here, we perform a cross-sectional study to characterize protein cargo of circulating exosomes in HIV patients and its relationship to virological and immunological markers.

**Methods:** Plasma exosomes were isolated from 53 subs (n=30 HIV+ from NNTC, age 38-54, 66% male, 53% black, on ART with suppressed viral load [VL<1500 copies] & n=23 HIV- controls matched for age, gender, race). Exosome quality was assessed by dynamic light scattering, transmission electron microscopy & immunoblotting (WB) for exosome markers (HSP70, CD9, CD63, CD81). Proteomic analysis was by LC/MS/MS. Following bioinformatic analysis of hits, proteins were confirmed by WB

**Results:** Circulating exosomes were increased in HIV+ subjects compared to controls based on WB for exosomal HSP70 & CD9 (p<0.01). HSP70, CD9 & CD63 were also detected in exosomes released by PBMCs treated with hemin (24 hrs). Exosomal HSP70 & CD9 levels correlated with plasma VL & kynurenine:tryptophan ratio (K:T ratio, a marker of immune activation) in HIV+ subjects (p<0.01). CD81+ exosome numbers (by ELISA) & exosome-associated NOTCH4 (by WB), but not HSP70 & CD9 levels, were higher in HIV+ subjects using cocaine compared to non-users, despite similar VL & K:T ratios (p<0.05). LC/MS/MS proteomics suggested plasma exosomes were mainly derived from myeloid cells (CD33, CD11A, CD11B, CSF1R) & revealed proteins related to exosomes (EXOSC10, BST2, SYNE1, VPS), immune activation/inflammation (CD69, CRP, TNFRSF10, CSF1R, chemokines), Wnt signaling (E-Cadh, DDR1, LRP5, NOTCH4), & metabolism (ADIPOQ); many of these were also detected in exosomes released by heme-treated PBMC. Treatment of THP-1 cells with HIV+ patient-derived exosomes induced modest increases in CXCL10 & other IFN-induced genes, indicating pro-inflammatory effects

**Conclusions:** This study demonstrates associations between exosome proteins & disease markers in HIV patients on ART. Circulating exosomes in HIV+ individuals on ART are mainly derived from myeloid cells, carry protein cargo related to immune responses, inflammation, Wnt signaling and may have pro-inflammatory effects during pathogenesis.

#### 251 A Model of CD4 TCM Cell Death in HIV Infection Based on a Gene Expression Signature

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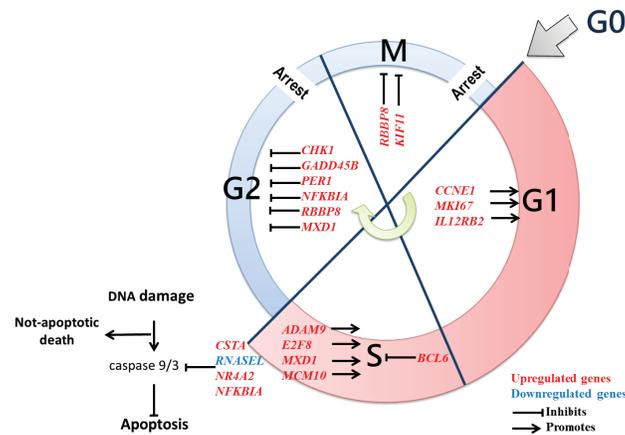
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**Background:** Considering that direct cytopathicity of HIV cannot completely explain HIV pathogenesis, that central memory CD4 T cells ( $T_{CM}$ ) are functionally altered along HIV infection, and given the critical homeostatic role of  $T_{CM}$  in chronic HIV infection, we asked if  $T_{CM}$  from HIV+ patients have an activation-related genetic expression pattern closer to effector memory CD4 T cells ( $T_{EM}$ ), and if differentially expressed genes entail a decrease in their proliferative and survival capacities.

**Methods:** We analyzed mRNA expression patterns (with gene expression microarrays) of 3 CD4 T naive, 3  $T_{CM}$ , and 3  $T_{EM}$  healthy controls' purified cell samples, plus 3 CD4 T naive and 3  $T_{CM}$  cell samples from HIV-1+ patients (purity >90%). Log<sub>2</sub>FC ≥|0.5| and Log (odds)>0 defined differential expression. Gene expression patterns were compared by 2-way hierarchical clustering. Differentially expressed genes discriminating patients' and controls'  $T_{CM}$  cells were subject to functional enrichment analysis (IPA, GSEA, DAVID). 75 genes of  $T_{CM}$  signature genes were validated by qPCR using a qPCR array.

**Results:** Hierarchical clustering grouped each CD4 T cell differentiation subpopulation from patients with its corresponding subpopulation from controls. The expression of differentiation-related genes did not differ between patients and controls, and increased or decreased progressively as follows: T naive à  $T_{CM}$  à  $T_{EM}$ , ruling out  $T_{EM}$ -like differentiation of  $T_{CM}$  in HIV infection. Comparing  $T_{CM}$  cells from patients and controls we found 210 differentially expressed genes. According to functional enrichment analysis this HIV-related  $T_{CM}$  gene expression signature indicated movement to cell cycle phases G1 and S (increased CCNE1, MKI67, IL12RB2, ADAM9, decreased FGF9, etc.), but also arrest in G2/M (increased CHK1, RBBP8, KIF11, etc.). Unexpectedly CSTA, RNASEL, and NR4A2 expression patterns from this signature, among others, predict decreased caspase-mediated apoptosis (Fig. 1). 75 qPCR validated genes independently reproduced the enrichment analysis results. Results also suggested increased IL-1 $\beta$ , IFN- $\gamma$ , TNF, and RANTES activities upstream of the  $T_{CM}$  signature, agreeing with the demonstrated milieu in HIV, and compatible with a non-apoptotic death pathway.

**Conclusions:** Our findings support a model where CD4  $T_{CM}$  cell progressive loss in chronic HIV-1 infection can be driven by increased cell cycle entry followed by mitotic arrest, leading to a non-apoptotic death pathway, possibly contributing to increased turnover.



## 252 IL-7 Responsiveness Is Impaired in CD4 T Cells From HIV Immune Failure Patients

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**Background:** Despite prolonged HIV suppression with anti-retroviral therapy (ART), some patients fail to recover normal CD4 T cell numbers. These patients are referred to here as “immune failure” (IF) patients. Here, we test the hypothesis that CD4+ T cell responses to the homeostatic cytokine, interleukin-7 (IL-7), are impaired in IF patients, potentially contributing to poor CD4 T cell reconstitution.

**Methods:** PBMC were obtained from IF patients (n=13), immune success patients (n=12) and healthy control donors (n=9). IF was defined by at least 2 years of ART and current CD4 count <380, while IS was defined by at least 2 years of ART and current CD4 count >500. Freshly isolated PBMC were examined for expression of CD127 (IL-7R) among CD4+ T cell subsets. Additional PBMC were labeled with CFSE and stimulated with rIL-7 (5 ng/ml). IL-7 induced CD25 expression was measured overnight by flow cytometry. IL-7 induction of P-STAT5 and P-AKT was measured at day 3 by phosflow and IL-7 induction of proliferation (CFSE dye dilution) was assessed after 7 days.

**Results:** We found defects in IL-7 induced proliferation (p=0.019) and CD25 surface expression (p=0.0081) in CD4 T cells from IF patients when compared to IS and HC donors. In contrast, IL-7 induced P-Akt and P-STAT5 were not diminished in CD4 T cells from IF patients. There was a trending relationship between CD127 surface expression and IL-7 induced CD25 expression (r=0.5727, p=0.0708). In contrast, CD127 expression did not correlate with IL-7 induced proliferation (r=0.3, p=0.3713).

**Conclusions:** These results demonstrate that IL-7 responsiveness is impaired in CD4 T cells from immune failure patients and may contribute to incomplete CD4 T cell recovery in HIV infected patients. These results also indicate that deficiencies in CD127 expression may not sufficiently explain impaired IL-7-induced proliferation in T cells from IF patients and the mechanism of impaired IL-7-induced proliferation is likely complex.

## 253 Does Reduced HLA-I Downregulation by HIV-2 Nef Contribute to “Functional Cure”?

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**Background:** The HIV-1 accessory protein nef mediates downregulation of HLA-I molecules to evade recognition of infected cells by cytotoxic T lymphocytes (CTL). Disparate HLA-I molecules are downregulated to differing extents by nef from the laboratory-adapted HIV-1 strain SF2, with HLA-B exhibiting increased resistance, consistent with the observation that HLA-B-restricted responses exert better control on the virus. Primary isolates of HIV-2 nef have however been little studied. The clinical course of HIV-2 infection differs from HIV-1 in that a large proportion of patients are viral controllers, with very similar characteristics to the state of “functional cure” in post-treatment HIV-1 controllers. We aimed to determine whether such viral control was related to the ability of HIV-2 nef to downregulate HLA-A, -B and -C molecules, using nef genes derived from patients with distinct clinical outcomes.

**Methods:** We transiently transfected a series of 16 transgenic cell lines stably expressing chimeric HLA-I alleles, combining cell-surface HLA-A2 with cytoplasmic tails from the major A, B and C families. Cells were transfected with IRES expression vectors containing the nef gene of interest and a fluorescent reporter. The resulting downregulation of surface HLA-A2 was measured using flow cytometry. As controls, cells were also transfected with empty vectors, vectors containing SF2, or one of 8 primary HIV-1 subtype B’ nef alleles.

**Results:** None of the patient-derived HIV-2 nef alleles mediated differential downregulation of HLA-A and -B molecules. Moreover, some patient nef alleles failed to downregulate any HLA-I molecules, but this was not associated with clinical status. Of the HIV-2 nef alleles that did downregulate HLA-I, the extent of downregulation did not differ substantially from HIV-1 nef.

**Conclusions:** We conclude that primary HIV-2 patient nef isolates do not elicit differential HLA-I downregulation *in vitro*, in contrast to that reported in HIV-1. This may explain the lack of association between HLA-B alleles and good clinical outcome in HIV-2 infection. However the extent of HLA-I downregulation in these patients did not correlate with clinical parameters, so is unlikely to explain the stronger HIV-specific CTL response in HIV-2 viral controllers. Differential HLA-I downregulation is also unlikely to account for the reduced immunogenicity of HIV-2 nef: we are now investigating whether abrogated proteasomal processing explains this phenomenon.

## 254 Selective Loss of T-bet and Eomes in HIV-Specific CD4+ T Cells During HIV Infection

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**Background:** T-bet and Eomesodermin (Eomes) are key transcription factors that govern critical antiviral Th1-type CD4+ and CD8+ T cell functions, including cytokine responses, tissue trafficking, memory formation, exhaustion, and cytolytic activity. Loss of T-bet and heightened Eomes expression in CD8+ T cells are associated with T cell exhaustion and loss of cytolytic potential in progressive HIV infection. Given the importance of these transcription factors for effective anti-viral T cell immunity, we here examined whether changes of T-bet and Eomes expression in CD4+ T cells could be linked to progressive disease and CD4+ T cell dysfunction.

**Methods:** Peripheral blood was collected from HIV acute seroconverters (n=12), ART- (n=145), ART+ (n=23), elite controllers (n=12), and 51 healthy controls from sites in Africa, Europe, Asia, and USA. Multi-parametric flow cytometry was used to assess T-bet, Eomes, maturation, activation, exhaustion, Gag-p24, and cytokine/chemokine expression in bulk and antigen-specific CD4+ T cells. Data were acquired by flow cytometry and analyses performed in FlowJo, GraphPad Prism, R and SPICE.

**Results:** We find in HIV-seronegative subjects that CD4+ T cell expression of T-bet and Eomes is associated with effector differentiation, cytolytic potential, and preferential CCR5 expression. However, T-bet+Eomes+ memory CD4+ T cells are preserved in both HIV-1 and HIV-2 infected individuals, despite increased immune activation, exhaustion and senescence compared to total CD4+ T cells. In contrast, HIV-1-specific CD4+ T cells selectively lose T-bet expression following the resolution of acute viremia and do not reacquire T-bet expression into chronic infection or post-ART. Preserved levels of T-bet+Eomes+ CD4+ T cells are also found in individuals with high HIV-1 viremia, and these cells demonstrate low susceptibility to HIV-1 infection *in vitro*. Finally, we show that T-bet+Eomes+ CD4+ T cells are the superior producers of  $\beta$ -chemokines and possess lower CCR5 expression directly *ex vivo* in HIV-1 infected subjects.

**Conclusions:** Memory CD4+ T cells expressing T-bet and Eomes are preserved in HIV infection, probably through the influence of increased  $\beta$ -chemokine production and lower CCR5 expression prohibiting HIV susceptibility. Conversely, lack of T-bet and Eomes expression in HIV-specific CD4+ T cells provides a potential mechanism of increased susceptibility for HIV infection.

## 255 Antioxidants Improve Lung Immunity and T-Cell Proliferation in Immune Non-Responders

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**Background:** HIV-1 immune non-responders are at increased risk for lung infections. Alveolar macrophages express CXCR4 and CCR5 and can be infected by HIV-1. We have previously shown that HIV replication within alveolar macrophages impairs phagocytic function, that HIV-1-infected individuals may have zinc and glutathione deficiency leading to oxidative stress, and that *in vitro* supplementation of zinc and glutathione improves phagocytic function. We hypothesize that dietary zinc and thiol antioxidant S-adenosylmethionine supplementation will enhance alveolar macrophage immune function and reduce macrophage viral burden in immune non-responders.

**Methods:** In a prospective cohort study non-smoking, HIV-1 infected immune non-responders were given zinc 30 mg and S-adenosylmethionine 1600 mg daily. All subjects underwent bronchoalveolar lavage and blood sampling pre-treatment and after 12 months on therapy. Alveolar macrophage phagocytic index [(% positive cells x mean channel fluorescence)/100] was measured by flow cytometry using FITC-labeled *S. aureus*. Proviral DNA was qualitatively measured using a modified version of the Abbott RealTime HIV-1 Assay (Abbott Molecular Inc. Des Plaines, IL). Immunologic parameters, including levels of CD4 and CD8 T cells, their main differentiation subsets, and their levels of proliferation were analyzed by flow cytometry.

**Results:** We enrolled 14 HIV-1 infected subjects (median CD4 count=257/ $\mu$ l), all of whom had blood HIV-1 RNA below level of detection on standard commercial assays. Alveolar macrophage phagocytosis increased significantly with treatment ( $31.4\% \pm 43.9$ ,  $p=0.02$ ). There was no significant change in lavage glutathione levels. HIV-1 proviral DNA was detected in 5/14 patients initially and all were negative after treatment. There were no significant changes in the frequencies of CD4 and CD8 T cells or their main maturation subsets and no significant changes on the frequencies of CD4 and CD8 T cells expressing activation markers (HLA-DR and CD38). However, there was a significant reduction in the frequencies of CD4 ( $p<0.001$ ) and CD8 ( $p=0.04$ ) T-cell proliferation, as assessed by Ki-67 expression.

**Conclusions:** In HIV-1 infected immune non-responders on antiretroviral therapy, dietary supplementation with zinc and S-adenosylmethionine reduced T cell proliferation, improved alveolar macrophage phagocytosis and may improve HIV-1 clearance from the lung, potentially reducing the risk for lung infections.

## 256 The Effect of KIR-Mediated Immunity on HIV Clinical Outcome in South Africa

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**Background:** HLA Class I molecules contribute to the immune control of HIV through antigen presentation to both CTLs and NK cells. CTL-mediated HIV control through antigen presentation by protective HLA alleles (eg HLA-B\*27/57/58:01) is well documented. The role of NK cell response is also well described, with the protective effects of HLA-Bw4 alleles in combination with, killer immunoglobulin-like receptor (KIR) 3DL1 and 3DS. Studies of the role of KIR2D-HLA-C in HIV control, however, remain sparse.

**Methods:** 327 HIV-infected treatment-naïve patients were recruited from South Africa, and the effects of HLA-KIR interactions on clinical outcome were analyzed.

**Results:** Viral setpoint in subjects with KIR2DL3 was significantly higher than in those without it (median 4.6 vs 4.3  $\log_{10}$  copies/ml,  $p=0.02$ ). This difference in viral setpoint between KIR2DL3-positive vs KIR2DL3-negative individuals was significant (median 5.0 vs 3.4  $\log_{10}$  copies/ml,  $p=0.04$ ) only among subjects expressing HLA-C\*16, a member of the HLA-C1 group and a ligand of KIR2DL3, but not among subjects with other HLA alleles in the HLA-C1 group. Subjects expressing alleles from the HLA-C1 group also showed significant viral setpoint differences when they co-expressed KIR2DL3 ( $p=0.01$ ), but not other receptors of HLA-C1 group (KIR2DL2 or KIR2DS2), suggesting some combinations of HLA-C1 alleles and KIR2DL3 may drive changes in viremia despite sharing the same HLA-KIR ligand-receptor structures. Longitudinal analysis of 303 patients showed more frequent ART initiation among subjects with HLA-C\*16+KIR2DL3+ combination than in subjects without it ( $p=0.01$ , Figure). The more frequent ART initiation in HLA-C\*16+KIR2DL3+ individuals remained significant in multivariate analysis (aHR 2.5, 95% CI 1.2-5.1,  $p=0.01$ ) and this was independent of other combinations of HLA-C1 alleles and KIR2DL3. Finally, no benefit of KIR3DL1/S1-HLA-Bw4 combination on clinical outcome was observed in this cohort.

**Conclusions:** In this study, the deleterious effects of KIR2DL3, particularly in combination with its ligand HLA-C\*16, on clinical outcome among HIV-infected South African individuals were identified. These findings highlight the existence of unique anti-HIV innate immune pressures and viral adaptation to this pressure specific to each endemic area.

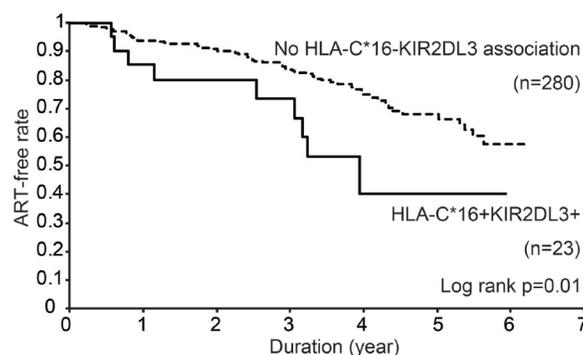


Figure. Kaplan-Meier curves showing the difference in ART-free rate between subjects with and without HLA-C\*16-KIR2DL3 association.

**257 Immunomodulatory Effects of Recreational Marijuana Use in Youth Living With HIV-1**Ashok Dinasarapu<sup>1</sup>; Carla Mavian<sup>2</sup>; Alberto Riva<sup>2</sup>; Sofia Appelberg<sup>3</sup>; Julie Williams<sup>2</sup>; John Sleasman<sup>4</sup>; Maureen Goodenow<sup>2</sup><sup>1</sup>Emory Univ, Atlanta, GA, USA; <sup>2</sup>Univ of Florida, Gainesville, FL, USA; <sup>3</sup>Karolinska Inst, Stockholm, Sweden; <sup>4</sup>Duke Univ Sch of Med, Durham, NC, USA

**Background:** While the effects on human health by marijuana, a widely used recreational substance, remain debatable among the public and policymakers, therapeutic benefits for some conditions are recognized. Significant changes in gene expression in immune cells are associated with alcohol or tobacco use, but the combined effects are unknown. Our study was designed to explore the immunomodulatory effects of recreational marijuana use alone, or in combination with tobacco and alcohol, in HIV-infected young adults.

**Methods:** Blood samples were collected in PaxGene tubes following IRBs protocols from a cross sectional cohort of HIV-seronegative [n=51] or HIV-infected, antiretroviral-treated [n=94] individuals with or without detectable viral loads [VL] matched for age [22 and 24 years, respectively] and demographics. Use of alcohol, marijuana or tobacco was assessed by self-reporting system and classified by frequency. Use of marijuana or tobacco was verified by plasma toxicology assays. RNA was amplified and hybridized to HG U133 Plus 2.0 Arrays. Raw probe signal values were normalized and differentially expressed genes were identified. Enrichment analysis was performed to identify the significance of regulated genes. Levels of secreted CXCL10 from THC-treated peripheral blood mononuclear cells (PBMC) were determined by ELISA.

**Results:** Expression of almost 4000 genes differed between HIV-seronegative and HIV-seropositive individuals. Within the HIV-seropositive group, significant overlap of differentially expressed genes was found between triple substance use (VL≤50) and no-substance use (VL>50) groups, suggesting that multiple substance use and viremia modulate similar gene expression. Pathway analysis revealed that the cytokine-cytokine receptor pathway was enriched in the HIV-seropositive, non-substance use group (VL>50) compared to other groups. Marijuana use in subjects with VL≤50 led to enrichment in the MAPK-signaling pathway and to normalization of IL-15 and CXCL10 expression compared with seronegative non-substance users. The inhibitory properties of marijuana in vivo were supported by ex vivo studies where THC completely inhibited IFNβ-stimulated CXCL10 release from PBMC in a dose dependent manner.

**Conclusions:** Recreational marijuana use normalized inflammatory and/or immune activation genes that were dysregulated even in virally suppressed HIV-infected individuals. Multi-substance use of marijuana with tobacco and alcohol reversed the immune suppression effects of marijuana alone.

**258LB T Regulatory Cell Depletion in Controller Macaques Reactivates SIV and Boosts CTLs**

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**Background:** T regulatory cells (Tregs) may play a critical role in formation of the latent reservoir, as they are susceptible to HIV/SIV infection and resting Tregs harbor higher levels of HIV/SIV than the general quiescent CD4<sup>+</sup> T cell population. During acute infection, Tregs decisively contribute to the establishment of HIV reservoir by reversing CD4<sup>+</sup> T cell immune activation status. During chronic infection, they contribute to the impairment of CTL responses, as Treg expansion correlates with loss of CTL function and their ex vivo depletion enhances T cell responses to HIV/SIV antigens. HLAB27<sup>+</sup> and B57<sup>+</sup> HIV-specific CD8<sup>+</sup> T cells from elite controllers evade Treg suppression. As such, we hypothesized that Treg depletion is a valid approach for HIV cure, in which a single intervention reduces the size of the reservoir, reactivates the virus and boosts cell-mediated immune responses.

**Methods:** Two SIVsub-infected RMs, in which spontaneous supercontrol of virus replication (<3 copies/ml plasma) associates complete control of immune activation, were depleted of Tregs by administration of Ontak (Denileukin diftitox), an engineered protein combining IL-2 and diphtheria toxin. Treg depletion was monitored by flow cytometry and immunohistochemistry; plasma viral load was measured by single copy assay; specific cellular immune responses to SIV antigens were monitored flow-cytometrically by intracellular cytokine staining after stimulation with SIVsub peptides.

**Results:** Ontak administration to SIVsub-infected RMs resulted in significant depletion (>75%) of the circulating Fox-P3<sup>+</sup> CD25<sup>+</sup> CD4<sup>+</sup> T cells. Up to 60% and >50% of Tregs were depleted from gut and the lymph nodes, respectively. Ontak impact on overall CD8<sup>+</sup> T cell counts was minimal. Treg depletion resulted in a major increase of the levels of CD4<sup>+</sup> T cell activation (Ki-67). In the absence of antiretroviral therapy, virus rebound to 10<sup>3</sup> vRNA copies/ml of plasma occurred after Ontak administration. Importantly, Treg depletion resulted in a significant boost of the SIV-specific CD8<sup>+</sup> T cells and rapid clearance of the reactivated virus.

**Conclusions:** Treg depletion in chronically SIV-infected superelite controller RMs resulted in both reactivation of latent virus and a boost of CTL responses. The overall Treg ability to control immune responses was significantly impaired despite the fact that Treg depletion was incomplete. As no latency reversing agent in development has such a dual activity, our strategy holds great promises for cure research.

**259LB Increased Effector CD8 Lymphocyte Trafficking to Lymph Nodes Induced by hetIL-15**George N. Pavlakis<sup>1</sup>; Antonio Valentin<sup>1</sup>; Cristina K. Bergamaschi<sup>1</sup>; Dionysios K. Watson<sup>1</sup>; Costantinos Petrosas<sup>2</sup>; Xintao K. Hu<sup>1</sup>; James I. Mullins<sup>3</sup>; Barbara K. Felber<sup>4</sup><sup>1</sup>NCI at Frederick, Frederick, MD, USA; <sup>2</sup>NIH, Bethesda, MD, USA; <sup>3</sup>Univ of Washington, Seattle, WA, USA; <sup>4</sup>NCI, Rockville, MD, USA

**Background:** IL-15 stimulates the growth activation and tissue dissemination of NK cells and cytotoxic lymphocytes. We have produced and characterized heterodimeric IL-15 (hetIL-15), the authentic form of IL-15 found in the circulation. This formulation is in clinical trials for cancer.

**Methods:** hetIL-15 was purified and its pharmacokinetic and pharmacodynamic properties were evaluated in macaques upon SC administration. Phenotype and functional changes in lymphocyte subsets were monitored by flow cytometry and multiplexed confocal imaging (MCI).

**Results:** Treatment of SIV or SHIV infected macaques with hetIL-15 resulted in a significant increase of circulating CD8<sup>+</sup> effector T cells and NK cells with activated cytotoxic phenotype (Granzyme<sup>+</sup>). This expanded T lymphocyte population was disseminated to effector sites, and was also present in secondary lymphoid organs where an increased frequency of total CD8 and of Ag-specific effector CD8 T cells could be observed by flow cytometry and imaging (MCI). A subset of CD8 T cells present in lymph nodes expresses CXCR5, indicating ability to migrate into germinal centers where chronically infected CD4<sup>+</sup>Tfh reside. MCI confirmed the presence of effector CD8 cells in germinal centers and showed that these cells are cytotoxic (GrzmB<sup>+</sup>) and actively proliferating (Ki67<sup>+</sup>) in response to hetIL-15.

These results strongly suggest the use of hetIL-15 in chronic HIV/SIV infection. An exciting possibility is the augmentation of immune responses in the areas of residual HIV/SIV persistence, and the combination with therapeutic vaccination, which has resulted in promising reduction in chronic viremia. We test new generation of therapeutic vaccines focusing immune responses on the conserved regions of HIV/SIV in combination with hetIL-15 in an effort to amplify novel cytotoxic responses with no possibility of immune escape.

**Conclusions:** We explore the potential of IL-15 as a viral reservoir reducing agent in ART-treated SIV infected macaques. hetIL-15 treatment enhances the number, activation and cytolytic potential of CD8 cells in LN and germinal centers, a known site of virus persistence. hetIL-15 treatment in combination with pDNA vaccine targeting the "Achilles' heel" of the virus, i.e., the highly conserved regions (CE), in virus sanctuary areas (germinal centers) is a promising HIV eradication strategy.

**260 Distinct Gut Microbiota Composition in Gay Men**Muntsa Rocafort<sup>1</sup>; Marc Noguera-Julian<sup>1</sup>; Yolanda Guillen<sup>2</sup>; Mariona Parera<sup>1</sup>; Piotr Nowak<sup>3</sup>; Falk Hildebrand<sup>4</sup>; Georg Zeller<sup>4</sup>; Anders Sönnnerborg<sup>3</sup>; Peer Bork<sup>4</sup>; Roger Paredes<sup>1</sup><sup>1</sup>IrsiCaixa Inst for AIDS Rsr, Badalona, Spain; <sup>2</sup>IrsiCaixa Inst for AIDS Rsr, Barcelona, Spain; <sup>3</sup>Karolinska Inst, Stockholm, Sweden; <sup>4</sup>Structural and Computational Biology, European Molecular Biology Lab, Heidelberg, Germany

**Background:** The precise effects of HIV-1 on the human microbiome are unclear. Initial cross-sectional studies provided contradictory associations between microbial richness and HIV status and suggested shifts from *Bacteroides* to *Prevotella* predominance following HIV-1 infection, which have not been found in animal models or in studies matched for HIV-1 risk groups.

**Methods:** This was a cross-sectional study where we first tested 129 HIV-1-infected subjects and 27 HIV-negative controls in Barcelona (BCN0). Findings were internally validated in 110 subjects from BCN0 providing a second fecal sample 1 month later (BCN1). External validation was obtained in 77 HIV-1-infected and 7 non-infected subjects from Stockholm (STK). In all study participants, we produced MiSeq™ 16S rRNA sequence data on fecal microbiomes and collected comprehensive metadata. Alpha and beta diversity analyses of the gut microbiota were performed. LASSO regression was used to quantify the strength of the association between sexual practice, HIV-1 status and global fecal microbiota composition.

**Results:** Men who have sex with men (MSM) consistently had a significantly richer and more diverse fecal microbiota than non-MSM individuals. After stratifying for sexual practice, HIV-1 infection remained consistently associated with reduced bacterial richness. The lowest microbial richness was observed in HIV-1-infected individuals with immunovirological discordant phenotype. Fecal microbiomes strongly clustered by sexual practice rather than by HIV-1 serostatus, with high concordance between BCN0 and BCN1 (Procrustes  $m^2=0.3475$ , PROTEST  $p=0.001$ ). The fecal microbiota composition in BCN and STK significantly differed by sexual practice, with MSM and non-MSM subjects mostly belonging to the *Prevotella* and *Bacteroides* enterotypes, respectively. Cross-validation accuracy of the LASSO model was very high for sexual practice (mean AUC=95%), confirming a different fecal microbiota composition in MSM and non-MSM individuals after excluding multiple other potential confounders. In contrast, HIV-1 status was not associated with consistent changes in the global fecal microbiota composition at genus level.

**Conclusions:** Gay men have a distinct gut microbiota composition, which is a potential confounder of all human fecal microbiome studies. Yet, HIV-1 infection remains independently associated with reduced bacterial richness, which offers new avenues for interventions to improve HIV immune dysfunction.

## 261 Impact of HIV-Associated Changes in the Gut Microbiome on Disease Progression

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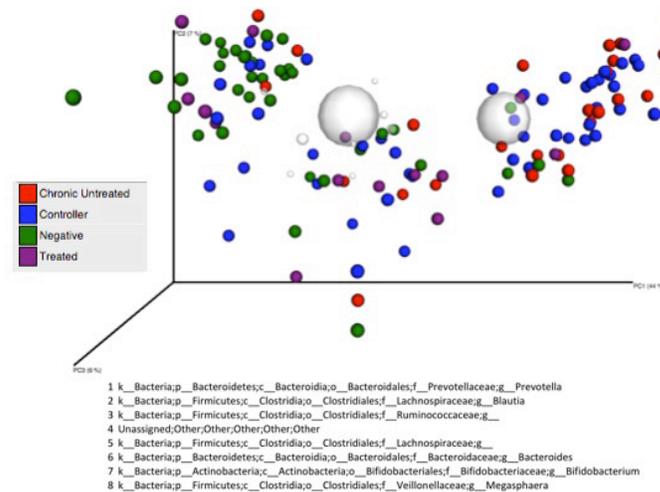
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**Background:** During early HIV infection, the intestinal mucosa is a critical reservoir of viral replication and profound CD4+ T cell depletion, with subsequent loss of epithelial barrier function. This is believed to result in translocation of luminal bacterial products, which may contribute to systemic immune activation and eventual immune exhaustion. We investigated the role of the enteric microbiome in HIV disease progression by using high-throughput sequencing to conduct a comprehensive survey of changes in gut bacterial populations using stool paired with patient plasma and clinical metadata.

**Methods:** A total of 124 stool samples with matching plasma and clinical data were analyzed; 90 of these subjects completed a comprehensive diet survey. Patient cohorts include HIV uninfected and infected (controllers, untreated, and ARV-treated) subjects. DNA extracted from fecal samples was used to perform 16S rRNA sequencing. QIIME was used to group viable sequences into Operational Taxon Units (OTUs) with >97% shared similarity, and PICRUST was used to infer gene expression of representative microbial communities. Soluble levels of sCD14, sCD163, CRP, and I-FABP/FABP2 in plasma were measured.

**Results:** At all taxonomic levels the HIV-uninfected subjects demonstrated more intra-subject bacterial community variability with greater levels of *Verrucomicrobia* and lower dominant levels of *Bacteroidetes* at the phyla level than HIV-infected subjects. Weighted Unifrac analysis of bacterial communities demonstrated significant clustering on a principal coordinate plane ( $p<0.01$  PERMANOVA testing via Adonis). Communities from HIV uninfected and HIV+ untreated patients clustered independently, but those from infected patients on ARVs were spread between the two groups. Relationships among bacterial communities, inferred microbial gene expression, and systemic markers were assessed.

**Conclusions:** We identified specific HIV-associated alterations in the gut bacterial communities and their inferred gene expression, and associations with soluble clinical markers. These alterations suggest that the enteric microbiome is significantly altered by HIV infection and may directly contribute to disease progression.



**Figure 1:** Principal Coordinate analysis of data from Weighted Unifrac comparison of gut lumen microbiota among Negative, ARV-Treated, Chronic Untreated, and Controller subgroups at operational taxonomic unit (OTU) level showing the top eight components of community structure listed below the plot. Gray spheres indicate specific taxa that drive sample clustering; volume is relative to the abundance of that taxa.

## 262 Altered Gut Microbes Enhance Mucosal CD4 T Cell Infection and Depletion Ex Vivo

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**Background:** Early HIV-1 infection is characterized by high levels of HIV-1 replication and CD4 T cell depletion in the gut mucosa as well as epithelial barrier breakdown and translocation of microbial products into the lamina propria (LP) and systemic circulation. HIV-induced disruption of gut homeostasis is associated with changes in the gut microbiome (dysbiosis) that are linked to mucosal and systemic immune activation. Mechanisms by which dysbiotic bacteria contribute to HIV-1 pathogenesis are poorly characterized. Here we investigated the impact of exposure of representative bacterial species previously shown to be in either high or low abundance in the colonic mucosa of viremic HIV-1 infected individuals (HIV-altered mucosal bacteria; HAMB) on LP CD4 T cell function, infection by HIV-1, and survival *in vitro*.

**Methods:** LP mononuclear cells isolated from normal human jejunums (n=11) were infected with CCR5-tropic HIV-1<sub>Bat</sub> or mock-infected and cultured with high abundance HAMB (3 gram-negative, GN) or low abundance HAMB (2 GN, 2 gram-positive, GP) or with control GN *Escherichia coli*. Flow cytometry was used to measure levels of CD4 T cell infection

(intracellular p24), activation (CD38/HLA-DR; CD25), proliferation (CFSE) and HIV-1 co-receptor expression (CCR5, CD4). Levels of CD4 T cell depletion were also determined. Non-parametric statistical analyses were performed.

**Results:** The majority of HAMB significantly ( $p < 0.05$ ) increased LP CD4 T cell infection and depletion, but GN HAMB enhanced T cell infection to a greater degree than GP HAMB. Most GN HAMB enhanced T cell infection and depletion similarly to *E. coli* despite lower HAMB stimulation of T cell activation and proliferation. GN HAMB and GN bacterial cell wall Lipopolysaccharide (LPS) induced upregulation of CCR5 expression on LP CD4 T cells ( $p < 0.05$ ), whereas GP cell wall lipoteichoic acid (LTA) did not ( $p = 0.3$ ). GN HAMB-induced CCR5 upregulation was largely abrogated in CD4 T cell-enriched cultures suggesting an indirect mode of stimulation.

**Conclusions:** GN commensal bacteria that are altered in abundance in the colonic mucosa of HIV-1 infected individuals have the capacity to enhance productive CCR5-tropic HIV-1 infection and depletion of LP CD4 T cells *in vitro*. Enhanced infection likely results from increased expression of the HIV-1 co-receptor CCR5 on LP CD4 T cells, mediated by indirect exposure to LPS. This represents a novel mechanism that potentially links intestinal dysbiosis to HIV-1 mucosal pathogenesis.

### 263 Enhancement of Microbiota in Macaques Leads to Beneficial Immune Function Modulation

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**Background:** With more than thirty million HIV-infected individuals worldwide, developing an effective vaccine to prevent new HIV infections remains a top priority in contemporary biomedical research. Given the critical role of mucosal surfaces in susceptibility to HIV, it is imperative that we induce effective mucosal responses. However, current approaches to enhance mucosal immunity have not been successful in preventing HIV acquisition. Modulating the microbiota in the GI tract is a safe and well-tolerated approach to enhance mucosal and overall health. We assessed the longitudinal impact of daily treatment with the VSL#3 probiotic (PBio) on cellular and humoral immunity and inflammation in healthy macaques.

**Methods:** Five macaques (SIV-) were treated with the PBio VSL#3. Colon, jejunum and LN were sampled prior to PBio treatment (PBio-tx) and at days 28 and 77/80 post-treatment. Indicators of cellular and humoral immunity and inflammation were assessed using multi-parameter flow cytometry.

**Results:** PBio-tx resulted in significantly increased frequencies of B cells expressing IgA in the colon ( $p = 0.0072$ ) and LN ( $p = 0.0151$ ) d80 post-PBio-tx, likely due to persistently increased LN T follicular helper cell (Tfh) frequencies (d28:  $p = 0.0085$ ; d80:  $p = 0.0173$ ). Increased frequencies of IL-23+ antigen presenting cells (APCs) in the colon ( $p = 0.0173$ ) and LN ( $p = 0.0475$ ) were found d28 post-PBio-tx, and colon IL-23+ APCs correlated with the frequency of LN Tfh ( $p = 0.0358$ ,  $r = 0.5446$ ). Increased frequencies of ILC3 in the jejunum were observed ( $p = 0.005$ ) d80 post-PBio-tx. The frequencies of activated (HLA-DR+) and proliferating (Ki-67+) CD4+ T cells were significantly decreased in the colon at d28 ( $p = 0.0310$  and  $0.0042$ , respectively) and d80 ( $p = 0.0612$  and  $0.0013$ , respectively) post-pBio-tx. Finally, VSL#3 significantly down-modulated the response of TLR2-, 3-, 4-, and 9-expressing HEK293 cells to stimulation with Pam3CSK4, Poly(I:C), LPS, and ODN2006, respectively ( $p < 0.0001$ ).

**Conclusions:** These data provide a mechanism for the beneficial impact of PBio on mucosal health and has potential implications for using PBio-tx to alter mucosal immunity in the context of vaccination or preventative approaches. In particular, the immunomodulatory properties of PBio-tx in conjunction with HIV vaccination may provide an opportunity for enhanced mucosal HIV vaccine responses that could improve protection from infection by improving immune defenses at the mucosal portal of entry and regulating the frequency of potential target cells.

### 264 Fecal Microbial Transplantation: Safety and Engraftment During Treated HIV Infection

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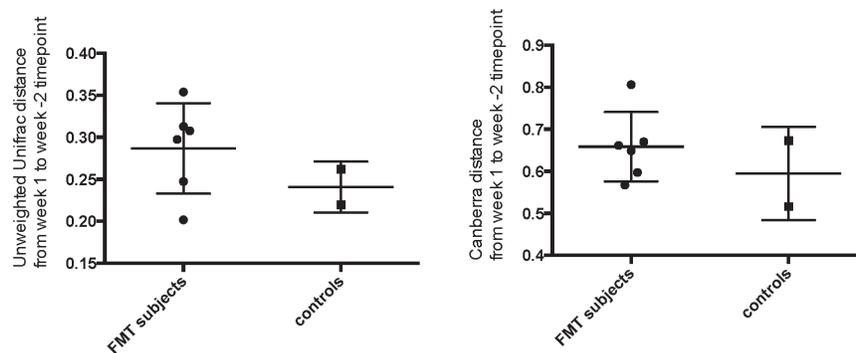
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**Background:** Despite antiretroviral therapy (ART), a substantial number of HIV-infected individuals exhibit chronic systemic inflammation. Many treated HIV-infected individuals exhibit marked alterations in the gut microbiome, and the degree of dysbiosis correlates positively with plasma markers of inflammation. Whether interventions to modulate the microbiome can affect systemic inflammation is not known.

**Methods:** A single-arm colonoscopically delivered fecal microbial transplantation (FMT) was employed. Participants with CD4 T cell count  $< 500$  cells/mL and CD4 to CD8 ratio  $< 1.0$  were preferentially recruited. Participants were screened for infectious pathogens in stool. OpenBiome provided donor material for FMT. Participants will be followed for 6 months post-FMT for adverse events, blood draws, dietary history, and stool samples. Stool was examined for engraftment of donor microbes and peripheral blood was assayed for biomarkers of immune activation at weeks -2, 1, and 8 relative to FMT. HIV-infected participants undergoing clinical colonoscopy served as controls. Appropriate regulatory approvals were obtained (FDA IND 15926, UCSF IRB 13-12675, NCT02256592).

**Results:** Nine individuals were screened and 6 were enrolled for FMT. One was excluded because of multiple comorbidities and two declined participation. All enrolled participants were men, median age was 61 (range 31-72), median CD4 was 431 (range 357-835), and median CD4 to CD8 ratio was 0.44 (range 0.33-1.36). All 6 who received FMT had at least 8 weeks of follow-up post-FMT with no serious adverse effects (range of follow-up, 8-24 weeks). One participant reported increased dizziness at week 8 however vital signs were stable and there were no episodes of syncope. Two participants with baseline loose stools reported that their stool quality improved (Bristol stool scale 5-6 to 4). Preliminary bacterial community sequence analyses indicated that FMT recipients exhibited greater compositional changes in their fecal microbiome beyond those observed over a comparable time period in control subjects although comparison with donor community is still pending. Kynurenine to tryptophan ratio showed no evidence for change pre- and post-FMT.

**Conclusions:** FMT was well tolerated in HIV-infected ART-suppressed individuals without significant adverse effects. The impact of FMT to alter the fecal and mucosal microbial communities and its effects on systemic inflammation is currently being investigated and will be discussed.



## 265 Effects of HAART Treatment on the Microbiome of HIV+ Patients

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**Background:** HIV depletes the mucosal immune system and leads to an imbalance of gut microbiota. Previous work demonstrates gut microbial dysbiosis in HIV patients. This study explores the effects of HAART treatments on the gut microbiome, and whether this treatment brings the community in line with HIV- individuals.

**Methods:** 33 treatment naïve HIV patients were enrolled and randomly assigned to treatment by maraviroc, efavirenz, or MRV+RAL. Blood draws plus colonic and duodenal biopsies were performed before treatment and at the 9 month time point. CD4 and CD8 T-cell counts were taken from peripheral blood, colonic and duodenal biopsies. Measurements for viral load, zonulin, CD14, and lipoteichoic acid (Lta) levels were also performed at both time points.

16S rRNA gene fragments were isolated from participant stool samples collected at both time points, and sequenced using Illumina's MiSeq. Sequence data was processed using QIIME v1.9 pipeline, including demultiplexing (default parameters) and OTU-picking (SortMeRNA, closed-reference against Greengenes 13\_8). Data was rarefied to 85,000 sequences/sample. Comparisons of alpha diversity (AD) use PD Whole Tree. Comparisons of beta diversity (BD) use unweighted UniFrac.

**Results:** 24 patients completed the 9 month follow up and had amplifiable samples. Of these patients, maraviroc (n=9), efavirenz (n=8), and MRV+RAL (n=7).

Preliminary analysis showed significant differences in microbial composition (using BD) between entry and 9 month samples ( $p=0.010$ ), however segmenting by treatment regimen ( $p=0.14$ ) and responders (defined as a CD4 increase of  $>100$ ) ( $p=0.70$ ) did not explain this change. Significant differences in BD were also found between HIV+ patients and HIV- controls ( $p=0.009$ ), suggesting the recovery was not towards an HIV- state.

AD was shown to weakly correlate with peripheral blood CD4% ( $r^2=0.068$ ,  $p=0.045$ ) and Lta readings ( $r^2=0.086$ ,  $p=0.037$ ), while changes in alpha diversity over the 9 months were shown to correlate with changes in activated CD8 T-cell counts from peripheral blood ( $r^2=0.74$ ,  $p=0.00072$ ).

**Conclusions:** HAART treatment changes correlate to CD8 T-cell count, suggesting a tie between gut microbial diversity and CD8 T-cell expansion. These changes do not drive the gut towards an HIV- like state, suggesting long-term perturbations from HIV cause a different equilibrium than HIV- individuals. These observations suggest larger studies are warranted to assess what organisms are involved in the CD8 interaction and HIV+ recovery state.

## 266 Diet Effects on the Gut Microbiome of People Living With HIV-1

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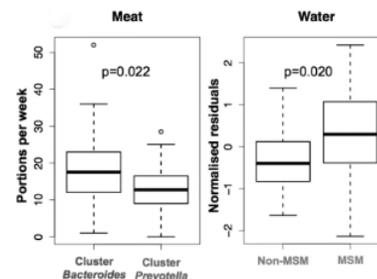
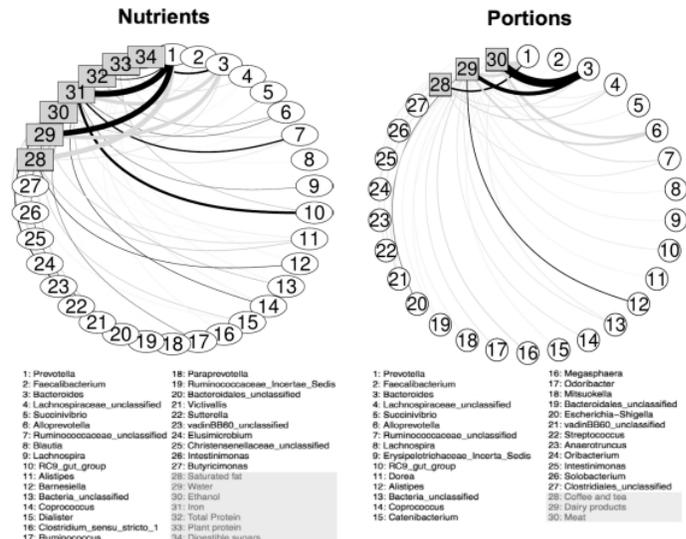
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**Background:** The human gut microbiome might be involved in HIV pathogenesis. In turn, long-term dietary patterns have been linked to human gut enterotypes. It is unknown how diet might affect the microbiome composition of people living with HIV

**Methods:** Diet information was collected from HIV-1-infected and HIV-negative controls with MiSeq 16S rRNA gut microbiome information available in Barcelona, Catalonia, Spain, using two independent dietary questionnaires: (a) a standardized prospective daily registry of food/drink consumption during 3-to-5 day consecutive days including at least one weekend day, which was then converted to amounts of nutrients; and (b) a recall questionnaire on the average number of portions of food/drinks taken per week during the prior year. Nutritional data was standardized by energy intake and was evaluated by HIV-1 infection status, enterotype (*Prevotella* vs. *Bacteroides*) and sexual practice (MSM/non-MSM). A Dirichlet-Multinomial (DM) regression model was used to detect associations between bacterial genera and diet components from both questionnaires. Wilcoxon Mann-Whitney test plus Benjamin-Hochberg correction was used to evaluate dietary differences between groups. A LASSO logistic regression model was fitted to analyze the global predictive power of dietary data to classify subjects according to enterotype.

**Results:** 127 (108 HIV-infected / 19 controls) and 116 subjects (99 HIV-infected / 17 controls) completed the prospective nutrient and the 1-year recall questionnaires, respectively. Energy intake was higher in MSM and in subjects in the *Prevotella* cluster. In the DM regression, the genus *Prevotella* was associated with decreased consumption of meat and dairy products, as well as decreased intake of saturated fat and digestible sugars and increased consumption of iron and dietary water; the exact opposite associations were found for the genus *Bacteroides*. Subjects in the *Bacteroides* cluster ate more meat and dairy products in line with reported associations. However, the *Prevotella* cluster had higher consumption of total protein which likely derived from the addition of different origins other than meat. However, none of the diet components, neither nutrients nor food portions, was selected by multivariate LASSO regression model as a consistent predictor of enterotype clustering and no associations were detected by HIV status.

**Conclusions:** Diet exerts a moderate influence on enterotype clustering in people living with HIV.



## 267 Tryptophan Metabolites Are Associated to Gut Microbiota in HIV-1 Infected Patients

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**Background:** The catabolism of tryptophan (TRP) into kynurenine (kyn) by indoleamine 2,3-dioxygenase-1 (IDO-1) contributes to the immune dysfunction during chronic HIV infection. An increased IDO-1 activity has been associated with microbial translocation (MT), gut microbiota dysbiosis and HIV progression. We aimed to investigate the association between TRP catabolites and gut microbiota during chronic HIV-1 infection. Additionally we studied the effect of antiretroviral therapy (ART).

**Methods:** We conducted an observational study including 29 HIV-1 infected patients and 9 uninfected controls. Peripheral blood and fecal samples were collected from ART naïve patients at baseline and for 16 patients during follow up (FU, after introduction of ART). Levels of the TRP metabolites were evaluated by HPLC assay and microbiota composition was determined by 16s rRNA sequencing. Soluble markers were analyzed by ELISA.

**Results:** We found significantly higher levels of KYNA in healthy controls compared to ART naïve patients,  $p < 0.05$ , and significantly increased levels of IDO-1 activity was detected during FU compared to healthy subjects,  $p < 0.05$ . Levels of kyn ( $p < 0.005$ ), IDO-1 ( $p < 0.0005$ ), quinolinic acid (QA,  $p < 0.0005$ ), 3-hydroxykynurenine (3-HK,  $p < 0.05$ ) and Nicotinamide (NAM,  $p < 0.005$ ) were reduced in HIV patients at FU. Levels of kyn and IDO-1 were correlated to CD8+ T cells (R-value=0.40;  $p < 0.05$ ). In ART naïve patients, kyn was associated to the genera: *Granulicatella* (0.48;  $p < 0.05$ ), *Butyrivimonas* (0.43;  $p < 0.05$ ) and *Escherichia* (0.51;  $p < 0.05$ ). IDO-1 was associated to *Granulicatella* (0.43;  $p < 0.05$ ) and *Lachnospirillum* (0.38;  $p < 0.05$ ), while negatively correlated to *Sutterella* (-0.38;  $p < 0.05$ ). *Butyrivimonas* was correlated to: HAA (0.61;  $p < 0.05$ ), XA (0.44;  $p < 0.05$ ), KA (0.42;  $p < 0.05$ ), QA (0.47;  $p < 0.05$ ) and NAM (0.43;  $p < 0.05$ ). After ART intro, new associations between gut microbiota composition and tryptophan catabolites were revealed. Thus, kyn was correlated to *Blautia* (0.63;  $p < 0.05$ ) and *Rothia* (0.59;  $p < 0.05$ ) and negatively associated to *Heamophilus* (-0.52;  $p < 0.05$ ) and *Bilophila* (-0.56;  $p < 0.05$ ). IDO-1 was correlated to *Blautia* (0.66;  $p < 0.05$ ), but negatively correlated to *Oscillospira* (-0.62;  $p < 0.05$ ) and *Oxalobacter* (-0.77;  $p < 0.05$ ).

**Conclusions:** We show that ART decreases the levels of TRP catabolites in viremic patients. Several significant associations between TRP catabolites and gut microbiota were found, and changed at FU. Our findings imply close interplay between gut microbiota and TRP pathway during HIV-1 infection.

## 268 Chronic Semen Exposure Reduces SIVmac251 Vaginal Infection in Rhesus Macaques

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**Background:** We tested the effects of prolonged intravaginal exposure to semen and/or defective SIV particles on a low-dose SIV challenge in nonhuman primates. We hypothesized that a decrease in infection would be documented based on cross-sectional studies of HIV negative human sexworker cohorts where chronic semen exposure was associated with an induced antiviral host response.

**Methods:** We exposed 46 randomized Indian Rhesus macaques (*Macaca mulatta*) to twice-weekly intravaginal inoculations with (n=23) or without (n=23) human semen with/without containing defective SIV particles for 20 weeks. A subset of animals (n=14) was euthanized and vaginal tissues taken to assess immune cell infiltrates by IHC. Remaining animals were given a low-dose 16-week intravaginal challenge with SIVmac251 while continuing respective semen exposures until infection. Peripheral immune cell status, mucosal activation, and SIVmac251 specific mucosal antibody were assessed at baseline and pre/post challenge. Differences between groups were tested using the Wilcoxon rank sum test,  $\alpha = 0.05$ . Log-rank test was used to determine the effect of semen with/without defective particle exposure on infection susceptibility.

**Results:** During SIVmac251 challenge, animals receiving semen were less susceptible to SIVmac251 infection (Log-rank  $p = 0.0332$ ) with 50% infection not occurring until week 15 of the 16-week challenge series as compared to week 7 for those animals not receiving semen. Exposure to defective particles had no effect on infection. Animals receiving semen had increased levels of RANTES upon exposure to SIVmac251 ( $p = 0.0280$ ). 20-week exposure to semen increased cervical Mx1 protein expression ( $p = 0.02$ ), CD4+ T-cell infiltrate ( $p = 0.0087$ ), and HLA-DR expression ( $p = 0.0027$ ). Reduced expression of CCR5 on circulating CD4+ T-Cells ( $p = 0.0167$ ) was noted in semen-exposed animals yet no difference in activation markers HLA-DR and CD69.

**Conclusions:** Chronic exposure to semen can modulate for reduced susceptibility to SIVmac251 in association with lower expression of CCR5 in peripheral CD4+ T-cells, an induction of RANTES, and an increase in Mx1. Chronic semen exposure may allow animal models to induce vaginal changes likely present in high-risk human cohorts otherwise not accounted for in pre-clinical studies.

## 269 Single-Cell Expression Profiling Reveals Immune Dysfunction in Acute SIV Infection

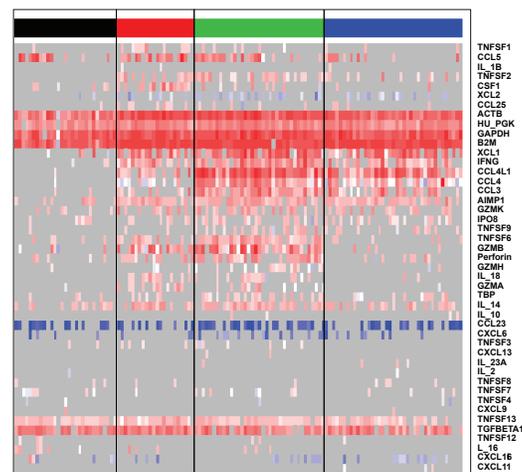
**Sama Adnan**; Premeela A. Rajakumar; James M. Billingsley; R. P. Johnson  
Emory Univ, Atlanta, GA, USA

**Background:** Despite the correlation between the HIV set-point and disease progression, little is known about the immunologic determinants of the viral set-point. During the first weeks of wild-type SIV (SIV-wt) infection, the rate of viral clearance decreases steadily, culminating in viral set-point by week 8, whereas animals infected with nonpathogenic SIV $\Delta$ nef maintain high virus clearance rates. We hypothesized that changes in the effector function of SIV-specific CD8 T cells would provide insights into the divergent virus clearance rates in the two models and applied a novel technique involving high-throughput expression profiling of SIV-specific CD8 T cells to address this hypothesis.

**Methods:** We isolated viable SIV-specific CD8 T cells sorted by expression of CD69 and CD137 after peptide stimulation followed by targeted qPCR transcriptional profiling. Cells were sorted from PBMC of 10 animals infected with wild-type SIV or SIV $\Delta$ nef at weeks 3, 8 and 20. We then profiled the expression of 85 effector molecules in SIV-specific CD8 T cells using the Fluidigm Biomark platform. Single cell expression analysis of the same 85 effector molecule panel was conducted on SIV-specific CD8 T cells from week 8 using the Fluidigm C1 System.

**Results:** At week 3, the expression profiles of CD8 T cell responses to SIV-wt and SIV $\Delta$ nef were largely similar, although TGF- $\beta$ 1 expression, which inversely correlated with the magnitude of the CD8 T cell response, was significantly higher in SIV-wt infection, corresponding to a lower initial response magnitude in SIV-wt infection despite higher viral loads. By week 8, the CD8 T cell response in SIV-wt infection significantly increased the expression of pro-inflammatory cytokines (IL-1 $\beta$ , IL-1 $\alpha$ , IL-18, IL-8) relative to week 3, even as responses in SIV $\Delta$ nef infection downregulated pro-inflammatory cytokine expression. Single cell expression profiling at week 8 revealed a unique subset of SIV-specific CD8 T cells in wild-type SIV, but not SIV $\Delta$ nef, infection (see Figure) that expressed CSF1 as well as lower levels of both chemotactic cytokines and cytolytic effector molecules.

**Conclusions:** Our results demonstrate that, as early as week 3, the CD8 T cell response to SIV-wt is dampened relative to SIV $\Delta$ nef, potentially due to TGF- $\beta$ 1 expression. By week 8, higher expression of pro-inflammatory cytokines, including CSF-1, in SIV-wt infection implicates myeloid derived suppressor cells, reportedly induced and recruited by persistent inflammation, in determining set-point viral load.



**Figure:** Single-cell expression profiling of CD8 T cell response. K-means clustering of SIV-specific CD8 T cells from SIV $\Delta$ nef (blue) and WT SIV (red) at week 8 reveals 4 distinct cell populations. The red cluster is largely composed of cells from WT SIV infection.

**270 CD169 Is a Marker of Immune Activation in SIV Infection but Not Rectal Susceptibility**

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**Background:** CD169 is an activation marker expressed by monocytes, macrophages, and dendritic cells (DCs) under inflammatory conditions. Expression is upregulated upon HIV infection and correlates with pathogenesis. Macaque monocytes and DCs also express CD169, and blood monocyte expression correlates with viral load during SIV infection. On monocyte-derived DCs (moDCs) matured with LPS or poly(I:C) (PIC), CD169 captures infectious HIV particles and delivers them to CD4<sup>+</sup> T cells at the infectious synapse. Clinical grade PIC, poly(I:CLC) (PICLC), is being pursued as a vaccine adjuvant, but the implications of *in vivo* CD169 upregulation for HIV acquisition are not defined.

**Methods:** We identified CD169 on rhesus macaque PBMCs by flow cytometry (anti-human CD169 mAb clones 7D2 and 7-239) and quantitative RT-PCR and within rectal tissue samples by qRT-PCR. Freshly isolated PBMCs were stained after overnight culture (2x10<sup>6</sup>/ml) in 10-25ug/ml PIC or PICLC vs. media. To gauge the *in vivo* impact of PICLC stimulation, we quantified CD169 mRNA in rectal tissues of macaques biopsied 4-24h after PICLC vs. PBS application. We also monitored rectal CD169 mRNA expression over time following wild type (WT) and attenuated (Delta Nef) SIV rectal challenge. Differences between groups were evaluated with the Mann Whitney or Wilcoxon signed rank tests.

**Results:** Overnight culture of rhesus macaque PBMCs with PIC or PICLC increased CD169 mRNA in the PBMCs and surface protein expression on Lin-HLA-DR+CD11c+ myeloid DCs. CD169 mRNA was similarly elevated in rectal tissue of PICLC-treated macaques and also in macaques infected with SIV. Comparison of WT and Delta Nef SIVmac239 infections revealed greater rectal CD169 expression in WT-infected animals in both acute and chronic infection. Baseline expression correlated with peak viremia, and expression over time correlated with plasma viral load. However, rectal CD169 level at baseline did not predict infection outcome in these animals.

**Conclusions:** We show that CD169, an inducible receptor for infectious HIV on human DCs and a marker of viral load in HIV infection, is induced in rhesus macaques rectally by PICLC as well as SIV and correlates with SIV replication. While we confirm CD169 is a biomarker of pathogenesis, we show it is not likely to direct virus transmission across the rectal mucosa.

**271 Adipose Tissue and HIV Infection**

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**Background:** Chronic immune activation/inflammation and viral persistence in reservoirs are important features of chronic HIV infection even in patients receiving ART. The aim of our work was to identify site that may combine viral persistence and inflammatory potential. We believed that adipose tissue was a very promising candidate because it included the major targets of HIV infection (CD4 T cells, and macrophages) and exhibited a highly pro-inflammatory potential. Although adipose tissue has been extensively studied as a target of antiretroviral toxicity, we readdress the role of adipose tissue as a reservoir and a site of inflammation.

**Methods:** Adult cynomolgus macaques (*Macaca fascicularis*) were infected with simian immunodeficiency virus (SIV) SIVmac251 for 15 months. Non-SIV-infected animals were used as controls. At sacrifice, blood samples, abdominal subcutaneous adipose tissue (SCAT) and visceral adipose tissue (VAT) were collected. Phenotypic characterization of macrophages and T lymphocytes from the stromal vascular fraction (SVF) of adipose tissue were performed. SIV DNA and RNA was measured in sorted adipose CD4+ T cells and macrophages. In the second part of this work, we analyzed adipose tissue from 13 anti-retroviral therapy-treated HIV-1-infected with undetectable viral load for over four years. SVF samples fractions were screened for HIV DNA. HIV RNA detection following *in vitro* reactivation assay was also performed.

**Results:** We analyzed the impact of SIV infection on abdominal SCAT and VAT in SIVmac251 infected macaques and found that both adipocytes and adipose tissue immune cells were affected. The adipocyte density was elevated and adipose tissue immune cells presented enhanced immune activation and/or pro-inflammatory profiles. We detected cell-associated SIV DNA and RNA in the SVF and in sorted CD4+ T cells and macrophages from adipose tissue. We demonstrated that SVF cells and more specifically CD4+ T cells are infected in ART-controlled HIV-infected patients. Importantly, the production of HIV RNA was detected after the *in vitro* reactivation of sorted CD4+ T cells from adipose tissue.

**Conclusions:** We identified adipose tissue as a crucial cofactor in both viral persistence and chronic immune activation/inflammation during HIV infection. These observations open up new therapeutic strategies for limiting the size of the viral reservoir and decreasing low-grade chronic inflammation via the modulation of adipose tissue-related pathways.

**272 α4-Integrin Antibody Blocks Monocyte Traffic and Decreases SIV Pathology in DRGs**

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**Background:** Infiltration of activated immune cells into dorsal root ganglia (DRG) is a critical mechanism of pathology in HIV peripheral neuropathy. Our recent work has shown that accumulation of recruited (BrdU<sup>+</sup>MAC387<sup>+</sup>) monocyte/macrophages is associated with severe DRG pathology and loss of intraepidermal nerve fiber density in SIV-infected macaques. Here, we sought to block leukocyte traffic by treating animals with natalizumab, which binds to α4-integrins, a subunit of VLA-4 expressed on leukocytes.

**Methods:** Sixteen rhesus macaques were SIV-infected and CD8-depleted. Eight animals were sacrificed early at 21 days post-infection (dpi) and eight animals were sacrificed late (49-77 dpi). 5/8 animals in the early group received natalizumab at the day of infection and 4/8 animals in the late group received natalizumab at 28 dpi. All animals received serial BrdU inoculations to label cells emigrating from the bone marrow to the tissue.

**Results:** Histopathology showed diminished DRG pathology including decreased inflammation, neuronophagia, and Nagoette nodules in natalizumab treated groups compared to control animals. Natalizumab treatment decreased the amount of BrdU<sup>+</sup> cell traffic in early treated animals and MAC387<sup>+</sup> monocyte influx in late-treated animals. The number of CD68<sup>+</sup> macrophages was decreased in both early and late treated groups. The number of SIVp28<sup>+</sup> cells in DRG tissues was also decreased in late treated animals compared to untreated controls. The number of CD3<sup>+</sup> T cells in DRGs was not altered by early or late natalizumab treatment. VCAM-1 was detected on blood vessels in DRGs of early and late untreated animals, but was significantly diminished in DRGs of all natalizumab-treated animals.

**Conclusions:** These data show that blocking monocyte, but not T cell traffic to the DRGs results in decreased pathology, further supporting a role for monocyte traffic and activation in HIV peripheral neuropathy. Additionally, natalizumab treatment significantly diminished the amount of SIVp28<sup>+</sup> cells in the DRG in late treated animals suggesting that targeting monocyte traffic may prevent formation of viral reservoirs. Additionally, we have demonstrated using natalizumab in a SIV-infection model reduces VCAM-expression in the DRGs.

**273 SIV-Associated Pathogenesis Modulation With Macrophage Targeted MGBG**

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**Background:** The role macrophages play as a target for HIV infection and in AIDS pathogenesis is controversial. This study used a novel oral form of a polyamine biosynthesis inhibitor MGBG (PA300), that exclusively targets activated and infected macrophages and not T cells, in a SIV infection associated pathogenesis study.

**Methods:** Two studies were performed using SIVmac251-infected CD8-depleted rhesus macaques. The first was a 30 day pK study (0, 3, 10 and 30mg/kg/day PA300 n=2/group), and the second was a blinded longitudinal study with daily dosing of 30mg/kg for 9 weeks (untreated, n=6 and PA300 n=9). Both studies began PA300 at 21 days post-infection

(dpi). Control and treated animals were grouped and sacrificed with AIDS or by 84 dpi. Blood studies were performed longitudinally. A full SIV necropsy was performed, including quantitative analysis of CNS and cardiac tissues.

**Results:** Histologic and blood chemical analyses revealed no signs of PA300 mediated pathology. pK analysis demonstrated that a biologically effective concentration of PA300 (0.7 $\mu$ M) was achieved at 30 mg/kg in plasma and all organs tested including brain. 7/8 (87.5%) of the untreated animals developed AIDS with 4/8 (50.0%) having SIV encephalitis. By contrast only 4/11 (36.3%) of the 30mg/kg dosed PA300 treated animals developed AIDS and 0/11 (0.0%) had SIVE. Levels of inflammatory CD14+CD16+ monocytes in the PA300 Rx group were significantly lower than in untreated animals. Plasma viral loads and CD4+ T cells did not differ. PA300 treated animals had significantly lower numbers of CD68+ and CD163+ macrophages in CNS cortex and CD68+ and MAC387+ macrophages in the left ventricle of the heart. There were reduced numbers of SIV-RNA positive macrophages in CNS with PA300 Rx. Aortic intima media thickness and cardiac ventricular fibrosis was decreased with PA300 Rx compared to controls and were correlated with decreased numbers of CD68+ and MAC387+ macrophages.

**Conclusions:** These data demonstrate that macrophage targeted therapy with PA300 resulted in prevention of AIDS and reversal of trends for the development of SIVE and cardiovascular disease in a SIV model. These data underscore the potential use of PA300 as an adjunctive therapy with ART to target activated and infected monocyte/macrophages in HIV-infected humans.

## 274 IL-7 and Chemokines Trigger Intestinal Cell Homing in SIV-Infected Macaques

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**Background:** SIV/HIV infection induces gut mucosa damages, where the ileum represents a major site for early viral replication. Therefore, understanding T-cell and macrophage anatomical distribution in the ileum mucosa along with local chemokine/cytokine network is warranted. In SIV-infected Chinese rhesus macaque (RM), we investigated SIV-induced modifications of T-cell and macrophage distribution in the ileum lamina propria (LP) and submucosa during the first 2 weeks of infection. In addition, we assessed mucosal expression of Interleukin-7 (IL-7) and chemokines involved in cell homing to the gut.

**Methods:** Simian gut tissues were analyzed after euthanasia at day 3, 7, 10, 14 in 9 SIV-infected RM and 2 uninfected controls. Four additional uninfected macaques were sacrificed at 0.5, 1 or 7 days following IL-7 injection (80 $\mu$ g/Kg). Viral DNA and RNA were quantified by qPCR. The levels of mRNA coding for IL-7 and 13 chemokines involved in lymphoid and myeloid cells homing were quantified by RT-qPCR. Confocal imaging after immunohistofluorescent staining was used to investigate immune cell counts and distribution in large ileum sections (25 mm<sup>2</sup>). The evolution of peripheral T-cell subsets and monocytes was assessed in circulating blood by flow cytometry.

**Results:** Concomitant with viral detection, IL-7 overexpression was observed by day 3 in the ileum (p<0.01). The expression of CCL2, CCL3, CCL4, CCL5, CCL19, CCL25 and CXCL10 was increased by day 10 and dropped at day 14. These local chemokine expressions correlated either to viral load or to IL-7 expression in the gut. CCL2, CCL4, CCL25, CCL28 and CXCL8 were also enhanced in the ileum of IL-7-treated uninfected macaques. The number of CD8 T-cell per tissue section reached a maximum at day 10 in the LP (p<0.01) and CD4 T-cell counts were not affected. CD4<sup>+</sup>PM-2K<sup>+</sup> macrophages significantly increased at day 3 in the LP (p<0.05) and inversely decreased in the submucosa (p<0.05). In the blood, T-cell subsets and monocyte counts transiently decreased up to day 10.

**Conclusions:** These observations demonstrate that IL-7 expression characterizes the ileum of acutely SIV-infected Chinese rhesus macaques. IL-7- and viral replication-driven local chemokine production leads to CD8 T-cell infiltration in the tissue as well as macrophage relocalization close to the epithelium. The rapid changes in the T and myeloid cell distribution in the ileum following SIV infection highlight the importance of early intervention to prevent mucosal pathogenesis.

## 275LB Apoptosis of Innate Lymphoid Cells Precedes CD4 T-Cell Death in Acute SIV Infection

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**Background:** Innate lymphoid cells (ILC) are a newly appreciated immune cell subset that are important in health and disease. Previous reports have shown NKp44+ type 3 ILC (ILC3) that are important sources of IL-17 and IL-22 are depleted in gut tissues in HIV and SIV infection, yet it is unclear how HIV infection affects the frequencies or function of ST2+ ILC2 that secrete IL-13 or ILC1 that secrete IFN- $\gamma$ . Finally, the role of microbial translocation on ILC survival and function is poorly understood.

**Methods:** In non-human primate models of HIV infection, we assessed the frequencies of ILC subsets in gut-draining mesenteric lymph nodes (MLN) by flow cytometry and IL-17, IL-13, and IFN- $\gamma$  production by PMA/ionomycin treatment. To determine the role of microbial translocation on ILC frequency and function, we treated healthy uninfected rhesus macaques (RM) with DSS to induce chronic gut barrier damage.

**Results:** Bulk CD4 T cells or IL-17+ CD4 T cells (Th17) in the MLN were not lost at early infection time points (day 10-14dpi), yet NKp44+ ILC3 in acutely infected RM (N=8) were significantly depleted (mean ILC3=0.03%) when compared to ILC3 of uninfected RM (N=10)(mean ILC3= 0.12%)(p=0.007). No significant differences in frequencies of ST2+ ILC2 or ILC1 were observed between acutely infected and uninfected RM. While caspase-3 expression of MLN CD4 T cells was unchanged, caspase-3 expression was elevated in ILC1 (mean caspase-3=12%), ILC2 (mean caspase-3=24%), and ILC3 (mean caspase-3=4%) subsets in acutely infected animals when compared to caspase-3 expression in these ILC subsets in MLN of healthy animals (p<sub>ILC1</sub>=0.001)(p<sub>ILC2</sub>=0.04)(p<sub>ILC3</sub>=0.002). ILC3 in chronic SIV+ MLN secreted more IL-17 (mean IL-17=30.5%) than NKp44+ ILC3 from uninfected RMs when stimulated (mean IL-17= 6.6%)(p=0.004). When healthy animals were treated with DSS to induce microbial translocation (N=4), frequencies of all ILC subsets remained unchanged yet ILC3 secreted more IL-17 (mean IL-17= 33%) than ILC3 from uninfected RM (mean IL-17=6.6%)(p=0.009).

**Conclusions:** Depletion of NKp44+ ILC3 and elevated apoptosis in all ILC subsets in the MLN is surprising given that this precedes MLN CD4 T cell death and ILCs are not permissive to SIV infection. Microbial products alone may not contribute to ILC loss, yet may augment IL-17 production in ILC3 as this is apparent in settings of microbial translocation with and without viral replication.

## 276 Immune Activation Profile Associated With Metabolic Syndrome in HIV-Treated Patients

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**Background:** Immune activation in HIV-1-infected patients persists under suppressive antiretroviral treatment and may fuel comorbidities such as atherothrombosis, osteoporosis, metabolic syndrome, neurocognitive disorders, and liver steatosis. Our hypothesis was that treated patients present with distinct profiles of immune activation and that each profile is linked to a specific comorbidity. We thus explored the profile of immune activation that was associated with the presence of a metabolic syndrome.

**Methods:** We measured by flow cytometry and ELISA the level of activation of CD4+ and CD8+ T cells, B cells, monocytes, NK cells, and endothelial cells as well as of inflammation with a total of 68 soluble and cell surface markers in 120 virologically suppressed patients and 20 healthy donors (aged  $\geq$  45 years). We used a hierarchical clustering analysis to classify the patients according to different markers of immune activation, and logistic regression with odds ratios (OR) and 95% CIs to measure the association between immune activation profiles and metabolic syndrome.

**Results:** We observed evidence of inflammation and immune activation in all the cell subpopulations analysed. Patients were clustered in 5 distinct immune activation profiles. Each one of these 5 profiles could be characterized by a marker of CD8+ T cell, NK cell, monocyte, endothelial cell activation or of inflammation, respectively, and could be

distinguished between the other profiles by a signature of 8 biomarkers. Only one of these immune profiles was significantly associated with marks of metabolic syndrome: hypertriglyceridemia (OR 4.18 [95% CI 1.08-16.19],  $p = 0.038$ ), hyperinsulinemia (OR 12.17 [95% CI 1.79-82.86],  $p = 0.011$ ), and lipodystrophy (OR 4.87 [95% CI 1.36-17.39],  $p = 0.015$ ).

**Conclusions:** We have uncovered an immune signature that might be useful for the prevention and early diagnosis of metabolic syndrome in HIV-infected patients. A better knowledge of the links between immune activation profiles and their consequences might highlight biomarkers predictive of comorbidities, as well as new therapeutic targets in HIV-induced immune activation or other situations of chronic hyperactivity of the immune system including aging.

## 277 Immune Activation, Cell Turnover, and Exhaustion in HIV+ Women With Heavy Alcohol Use

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**Background:** Heavy alcohol use in HIV disease is associated with accelerated HIV disease progression. Immune activation is a hallmark of HIV disease. Chronic T cell activation results in proliferation/cell turnover and immune exhaustion. We hypothesize that chronic heavy drinking through biological pathways of immune activation will contribute to immunosuppression and HIV disease progression.

**Methods:** Women's Interagency HIV Study (WIHS) hepatitis C seronegative participants were categorized in 4 groups of HIV+ and HIV- individuals with chronic heavy alcohol consumption (> 7 drinks/week) and abstainers, matched on age, race, education and history of IDU,  $n=25$ /group. Markers of immune activation (HLADR+CD38+), cell turnover (Ki67+), and immune exhaustion (PD-1+) were measured on CD4 and CD8 T cells using 10 color flow-cytometry at 4 time points from 2001-11. We tested baseline group differences (ANOVA/t-test); changes in markers over time (random regression models); and relationships of markers with log10 viral load (partial correlations).

**Results:** The mean % of CD8 HLADR+CD38+ cells in HIV+ chronic drinkers was significantly higher than among HIV+ abstainers at baseline ( $p=0.02$ ); this difference increased significantly over time ( $\beta=3.39$ ,  $p=0.03$ ). The mean % of CD8 Ki67+ cells in HIV+ chronic drinkers was also significantly higher than among HIV+ abstainers at baseline ( $p=0.02$ ), and over time ( $\beta=1.49$ ,  $p<0.01$ ). The mean % of CD8 PD1+ cells was higher in HIV+ chronic drinkers than HIV+ abstainers at baseline ( $p=0.05$ ); but did not significantly increase over time. In contrast, % CD4 HLADR+CD38+, CD4 Ki67+, and CD4PD-1+ cells in HIV+ chronic drinkers did not differ at baseline compared to HIV+ abstainers but did increase significantly over time ( $\beta=0.67$ ,  $p=0.01$ ), ( $\beta=0.81$ ,  $p=0.01$ ), ( $\beta=3.82$ ,  $p<0.01$ ), respectively. Adjusting for time and HAART adherence, % immune activation (CD4 & CD8 HLADR+CD38+) correlated positively ( $p<0.05$ ) with viral load in HIV+ drinkers but not in abstainers. There were no significant differences in either CD8 or CD4 T-cell markers among HIV- women by drinking status at baseline or over time.

**Conclusions:** CD8 T cell activation, cell turnover, and exhaustion, were associated with chronic heavy drinking in HIV+ women. Chronic heavy drinking in HIV+ women was also associated with increases in activated and cycling CD4 T cells which has implication to HIV replication. Thus, alcohol related immune dysregulation could contribute to accelerated HIV disease progression.

## 278 Oral Bovine Immunoglobulin Reduces Immune Activation in HIV+ Immune Nonresponders

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**Background:** A multi-center trial in patients (pts) with HIV-enteropathy showed that oral administration of serum-derived bovine immunoglobulin/ protein isolate (SBI) led to increases in peripheral and mucosal CD4+ T-cells after 4 and 24 weeks among pts in the baseline (BL) CD4 lowest quartile (LQ;  $\leq 418$  cells/mL). Here we report on analyses of immune activation and gastrointestinal (GI) biomarkers in plasma samples in LQ pts that provides insight into the restoration of peripheral and mucosal immunity seen in previous SBI studies.

**Methods:** A total of 103 HIV+ pts receiving suppressive ART (median 19 cp/mL and 8.3 years, respectively) and a history of HIV enteropathy were enrolled. Median CD4 T-cell counts were 637 cells/mL (189-1754). Pts were randomized to receive blinded SBI 2.5 vs 5.0 grams (g) BID or placebo during a 4-week lead-in phase followed by SBI 2.5 vs 5.0 g BID for 20 weeks. Earlier analysis showed no change among all study pts but significant increases in median peripheral CD4 counts from BL to week 24 (311 to 366 cells/mL,  $p=0.002$ ) in pts in the LQ at BL. Additionally, a separate duodenal biopsy sub-study at week 0 and 24 was performed in 8 pts with duodenal CD4 densities increasing from 217 to 329 cells/mm<sup>2</sup> ( $p=0.02$ ). Therefore, plasma biomarker evaluations were conducted on the CD4 LQ subgroup and data from the 2.5 and 5.0 g cohorts combined for analysis. Plasma biomarkers included: zonulin, intestinal FABP (I-FABP), sCD14, IL-6, and bacterial flagellin. Pearson correlations were conducted between CD4 cells and plasma biomarkers.

**Results:** Mean plasma IL-6 levels decreased from 2.4 ( $\pm 3.0$ ) to 0.9 pg/mL ( $\pm 0.9$ ) ( $p<0.001$ ) for LQ pts receiving SBI through 24 weeks. Peripheral CD4/CD8 ratio was inversely correlated with plasma IL-6 at week 24 ( $p=0.02$ ). Change in plasma I-FABP, a marker of enterocyte damage, was correlated to change in plasma flagellin, a marker of microbial translocation, at week 8 ( $p=0.03$ ) and week 24 ( $p=0.04$ ). Correlations were also observed with changes in plasma IL-6 and plasma zonulin levels. Mucosal CD4/CD8 levels at week 24 inversely correlated with plasma flagellin ( $p=0.01$ ).

**Conclusions:** Oral SBI may help decrease translocation of immunogenic substances like flagellin and in turn decrease systemic immune activation and improve CD4/CD8 ratios. SBI may be a novel therapy for reducing immune activation and support mucosal and systemic immune restitution among pts who have not achieved normal CD4 counts despite prolonged suppressive ART.

## 279 Proteomic Profiles Associated With an Inflammaging Phenotype During HIV-1 Infection

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**Background:** We previously described a novel inflammaging phenotype (IP) in older, HIV-uninfected persons that incorporated plasma biomarkers of intestinal epithelial barrier damage (intestinal fatty acid binding protein), microbial translocation and monocyte activation (soluble (s)CD14), immune activation (sCD27), and inflammation (C-reactive protein). When this classification model was applied to HIV-infected (HIV+) study participants on anti-retroviral therapy (ART), IP+ participants were of a similar age, but had increased levels of plasma IL-6 and LPS and were at higher risk of cardiovascular disease based on Framingham index scores compared to IP- participants. To understand mechanistic pathways involved in inflammaging, we performed a pilot study comparing the plasma proteome in IP+ versus IP- ART-treated HIV+ persons.

**Methods:** Stored plasma samples were obtained from age (60-67yrs) and sex-matched ART-treated HIV+ participants identified in our previous study as IP+ ( $n=6$ ) and IP- ( $n=6$ ). Participants gave informed consent. SOMAscan proteomics technology was used to identify and quantify plasma proteins. Unpaired t tests were used to compare protein levels between the two groups. Pathway and network analysis was performed using a modified Fisher's exact test as applied by the Ingenuity Pathway Analysis (IPA) platform.

**Results:** 274 of 1128 proteins measured were significantly different ( $P<0.05$ ) between IP+ and IP- participants with 105 (38%) significantly higher in IP+. Predicted canonical pathways increased in IP+ participants were NRF2-mediated Oxidative Stress Responses ( $p=0.04$ , activation score=2.3) and Apoptosis Signaling ( $p=0.07$ , activation score=1.9). 37 canonical pathways were significantly ( $p<0.05$ ) decreased. Of the significantly altered proteins, IPA identified 90 with either a direct or indirect biological relationship to IL-6. Of these IL-6-connected proteins, 85 (94%) associated with biological processes of cell proliferation, 79 (88%) with apoptosis and 78 (87%) with necrosis.

**Conclusions:** Using systems biology, we identified proteome signatures linked to inflammaging in ART-treated, virally suppressed HIV<sup>+</sup> persons. Inflammaging was associated with proteins involved in a number of different biological networks including oxidative stress and cell death. Understanding the mechanistic pathways driving inflammaging may provide new targets for anti-inflammatory treatments and identify novel biomarkers to further interrogate the effects of chronic HIV infection and aging on inflammation.

## 280 Inflammasome and Th17 Activation in HIV+ Immunological Nonresponders

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**Background:** Inflammasomes are multimeric protein platforms involved in the regulation of inflammatory responses. Inflammasome activity results in the production of proinflammatory cytokines and in Th17 activity (Mills K.H.G. et al, JLB 2013; Peelen E. et al, Molecular Immunology 2015); its role in immune reconstitution in HIV+ patients is nevertheless unclear. We analyzed possible associations between inflammasomes or Th17 activation and the degree of immune reconstitution in HIV+ patients.

**Methods:** Cross-sectional, single-site study enrolling HIV-infected patients on antiretroviral therapy for ≥24 months and plasma HIV-RNA <50cp/mL for ≥12 months. Exclusion criteria: presence of actual opportunistic AIDS-related diseases, HBV or HCV coinfection, chronic inflammatory disorders, ongoing immunosuppressive therapy. Patients were classified as immunological responders (IR) or non responders (INR) if CD4 count was ≥500 or ≤350 cells/μL, respectively. Immune activation markers (HLA-DRII, CD38 and Ki-67), Th17 activity and expression of genes involved in the inflammasome pathway were measured in unstimulated or LPS- and AT2-stimulated cells.

**Results:** Thirty-nine patients (22 IR and 17 INR, 76.9% males, medians: age 47 years, time from HIV diagnosis 10 years, time with HIV-RNA <50cp/mL 57 months) were enrolled. INR patients were older (median 60 vs. 43 years,  $p < 0.001$ ) and had a higher prevalence of past AIDS-defining illnesses (76.5% vs. 18.2%,  $p < 0.001$ ) compared to IR patients. Median CD4 count was 840 (IQR 718-1131) cells/μL in IR vs. 295 (IQR 256-343) cells/μL in INR. LPS-stimulated inflammasomes (NLRP3 and NLRP1) and pro-inflammatory cytokines expression (IL-1β, IL-18, TNFα, type-I IFNs, CCL3, IL-6) were significantly increased in INR patients. Higher median levels of Th17 lymphocytes (CD4/IL17A/RORγT) were also seen in INR in unstimulated (0.32% vs. 0.19%,  $p = 0.045$ ), as well as in LPS- (0.60% vs 0.22%,  $p = 0.011$ ), and AT2- (0.67% vs 0.17%,  $p = 0.006$ ) stimulated conditions. Finally, HLADR/II/CD4 (12.48% vs. 5.86%,  $p = 0.025$ ) were significantly increased in unstimulated cell cultures of INR. Immune recovery was independently associated with lower Th17 levels (mean change -0.42%,  $p = 0.005$ ) after adjustment for age and past AIDS.

**Conclusions:** Higher levels of inflammasome factors, an increased percentage of Th17 and immune activation characterize the immune scenario of ART-treated INR patients. These alterations are likely to contribute to the lack of CD4 recovery seen in INR.

## 281 Derangement in Protein S and C4b Binding Protein Levels in HIV-Infected Adults

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**Background:** The introduction of highly active antiretroviral therapy (HAART) has significantly improved survival among HIV infected persons. Chronic complications of the disease and long term toxicities of the antiretroviral therapy are now important issues that confront the health care providers managing HIV infected patients. HIV infected patients have an increased risk of developing thrombotic disorders. Protein S is a vitamin K-dependent plasma glycoprotein that exists in a free form and one bound to complement protein (C4BP). Protein S is critical in the anticoagulation pathway functioning with Protein C in the inactivation of Factors Va and VIIIa. We evaluated this with the Euglobin clot lysis test and measured serum levels of free protein S and C4BP (an acute phase reactant that serves as carrier protein for Protein-S in plasma), to determine their role in the increased risk of thrombosis.

**Methods:** The study population consisted of 61 HIV infected adults on HAART that had achieved virological suppression, 58 HIV infected adults not yet on treatment with ART and 59 HIV negative healthy adults as controls. Blood samples were collected and serum levels of free protein S, C4b binding protein beta and the Euglobin clot lysis time were determined.

**Results:** There was a significant decrease in serum free Protein S level in untreated HIV infected adults (75.7%±27.3) compared to the control group (94.9%±7.9). ( $p = 0.000$ ). There was no statistically significant difference between Protein S levels in HIV infected subjects on ART (86.9%±25.8) and the control group (94.9%±7.9) ( $p = 0.119$ ).

C4b Binding Protein beta was significantly higher in untreated HIV infected subjects (mean: 85.1mg/dl±52.6) compared to those on ART (mean: 43.8mg/dl±42.4) and the control group (22.4mg/dl±18.4). ( $p = 0.000$ ). There was a statistically significant difference between C4BP levels in HIV infected subjects on ART and the control group ( $p = 0.012$ ).

Of the 24 HIV infected subjects with low protein-S levels, 19 (79.16%) had elevated C4B binding proteins. whereas in 95 HIV infected subjects with normal protein-S levels, 51 (53.68%) had elevated C4B binding proteins. Protein S deficiency is more prevalent among the subjects with elevated C4b Binding Protein beta ( $p = 0.023$ ).

Mean Euglobin Clot Lysis Time (ECLT) was significantly longer among the (n=47) untreated HIV infected subjects (241.9secs±34.7) compared to the HIV uninfected control group (n=59) (189.5±40.7). ( $p = 0.000$ ). ECLT in HIV infected subjects on ART (210±61.9 sec n=50) was not significantly different from the control group. ( $p = 0.064$ ).

**Conclusions:** HIV infection is associated with derangement in the levels of C4b Binding Protein beta and serum free Protein S. This may be responsible for the increased risk of thrombosis associated with HIV infection as demonstrated by the prolonged ECLT in treatment naïve HIV subjects. The risk of thrombosis as measured by these biomarkers was reduced with the use ARVs.

## 282 Monocytes From Treated HIV1 Patients Release ROS Causing DNA Damage and CD4 Cell Loss

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**Background:** Immune activation is a hallmark of HIV infection. Antiretroviral therapy reduces but doesn't abolish it. Immune activation is linked to sub optimal immune response and to non-AIDS comorbidities, including various types of cancers. The aim of this project was to explore how immune activation could favor impaired CD4+ T cell restoration and oncogenesis.

**Methods:** We tested the capability of various populations of peripheral blood mononuclear cells (PBMC), isolated by cell sorting from 70 HIV-1 aviremic patients under antiretroviral therapy for at least two years to induce DNA damage in bystander primary fibroblasts (BJ cells) or PBMC. DNA damage was measured in immunofluorescence by looking for the formation of γH2AX, 53BP1, and 8-hydroxy-2-deoxyguanosine foci. Reactive oxygen species (ROS) production in patients monocytes and apoptosis induced in CD4+ T cells was quantified in flow cytometry after annexin-V and 2',7' -dichlorofluorescein diacetate (DCFDA) labeling, respectively.

**Results:** We observed that the PBMC from 38 out of 70 (54%) virologic responders induced γH2AX foci in bystander BJ cells. DNA double-strand breaks and DNA oxidation were detected in these BJ cells using anti-53BP1 and anti-8-hydroxy-2-deoxyguanosine, respectively. Positive and negative cell sorting provided evidence that among the HIV patients PBMC, the monocytes were responsible for this DNA damage. They cause DNA damage by secreting reactive oxygen species (ROS), since this effect was eliminated by the pretreatment of the monocytes with NADPH oxidase inhibitor Diphenyleneiodonium (DPI), or by the ROS scavenger N-acetylcysteine (NAC). Moreover, the intensity of the DNA damage induced by the monocytes in coculture was linked to the amount of ROS detected in these cells *ex vivo* by DCFDA staining ( $r = 0.12 ± 0.05$ ,  $p ≤ 0.05$ ). Monocytes also caused DNA damage in primary CD4+ T cells, thereby inducing apoptosis. Finally, the capability of the monocytes of HIV+ patient to damage DNA was inversely correlated with its CD4 slope during treatment.

**Conclusions:** This study uncovers the genomic instability mediated by monocytes-derived ROS in about half of the aviremic patients under treatment. This genomic instability can lead to poor immune recovery and oncogenesis.

### 283 Low ART Adherence Is Associated With Higher Inflammation Despite HIV Suppression

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**Background:** A significant proportion of virologically-suppressed HIV-infected individuals on antiretroviral therapy (ART) exhibit residual inflammation. It is unclear whether variable ART adherence, which could result in intermittent viral replication, partially explains this phenomenon. We aimed to determine if suboptimal ART adherence is associated with increased inflammation despite HIV suppression.

**Methods:** Longitudinal adherence data (4-day self-report) and serum concentrations of 24 biomarkers of inflammation and immune activation were measured at the same study visit in virologically-suppressed (<50 copies/ml) HIV-infected men in the Multicenter AIDS Cohort Study (MACS) from 1998 to 2009. We used generalized gamma regression to estimate the effect of <100% ART adherence on the concentrations of these biomarkers. We also categorized adherence using the clinically meaningful cutoffs of <85%, 85-99%, and 100%. Data are presented as percentage differences in biomarker concentrations across groups; we adjusted for multiple testing by controlling the false discovery rate at 5% using the Benjamini-Hochberg procedure.

**Results:** We studied 927 men (225 African American; 143 Hispanic) for a total of 2901 person-visits at which HIV viral suppression was documented. Median (range) age was 48 (21-81) years. ART included PI-based (50%), NNRTI-based (46%) and NRTI-only (3%) regimens. Models were adjusted for covariates associated with both adherence and biomarker concentrations in univariate analyses: age, race, HCV co-infection, and depression. Individuals reporting <100% adherence had higher concentrations of pro-inflammatory cytokines and C-reactive protein compared to men reporting 100% adherence (Table, shaded column). Differences were significant at  $p < 0.05$  for six biomarkers, but only for TNF- $\alpha$  and IFN- $\gamma$  after adjusting for multiple tests. Further evaluation of adherence sub-categories showed that, after controlling for multiple testing, significantly higher concentrations of four biomarkers were identified in individuals reporting <85% adherence (Table).

**Conclusions:** Suboptimal self-reported adherence was associated with higher concentrations of inflammatory biomarkers in virally-suppressed HIV-infected men. This suggests that adherence variations could have significant biological consequences beyond plasma HIV RNA suppression, possibly due to residual viral replication (i.e., below the limit of detection). Maximizing ART adherence could improve chronic inflammation and its deleterious long-term consequences.

**Table.** Percent difference in inflammatory biomarker concentrations across groups (by adherence category, adjusted for age, race, HCV infection, and depression).

Biomarker	<100% vs. 100%		<85% vs. 100%		85-99% vs. 100%	
	Estimate	<i>p</i> value	Estimate	<i>p</i> value	Estimate	<i>p</i> value
TNF- $\alpha$	10.8%	<b>&lt;0.001</b>	7.7%	0.042	9.5%	0.017
IFN- $\gamma$	15.6%	<b>0.004</b>	18.6%	<b>0.002</b>	6.6%	0.413
CRP	18.2%	0.019	22.2%	<b>0.006</b>	14.3%	0.277
IL-2	14.2%	0.020	20.0%	<b>0.003</b>	-7.3%	0.463
IL-10	11.2%	0.022	11.6%	0.030	6.7%	0.376
IL-6	10.0%	0.029	17.9%	<b>0.001</b>	2.1%	0.787
sIL-6R	0.6%	0.763	-0.4%	0.830	6.2%	0.042

Significant *p* values when controlling the false discovery rate at 5% appear in bold.

Not significant: BAFF, CCL11, CCL13, CCL17, CCL2, CCL4, CXCL10, CXCL13, GM-CSF, IL-12p70, IL-1 $\beta$ , IL-8, sCD14, sCD27, sGP130, sIL-2R $\alpha$ , sTNFR2

### 284 Progesterone (P4) Indirectly Increases Susceptibility to HIV Within Genital Mucosa

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**Background:** HIV risk is linked to use of P4-based hormonal contraceptives. However, the molecular basis of how P4 increases susceptibility to HIV transmission in women is not well understood. Because the endometrium is a P4-responsive tissue in the female reproductive tract (FRT) and serves as a potential portal of entry for HIV, we hypothesized that P4 may increase vulnerability to HIV infection in the endometrium. Here, we assessed how P4 impacts the dominant cell types of this tissue, the endometrial epithelial cells (eEC) and underlying stromal fibroblasts (eSF), in the context of how they affect HIV infection of permissive cells.

**Methods:** Primary eSF and eEC were co-cultured in a transwell system, and the effects of estradiol (E2) alone or in the presence of P4 (E2P4) on permeability of eEC monolayers was assessed. Infection of eSF, eEC, and patient-matched PBMCs with HIV, with each type of cell on its own or upon co-culture, were conducted.

**Results:** Compared to E2, E2P4 increased permeability of eEC, suggesting that luminal contents have increased access to underlying eSF during the secretory phase when P4 levels are high. HIV did not infect eEC or eSF. However, eEC and eSF both markedly affected infection of co-cultured CD4+ T cells. Whereas eEC decreased HIV infection of CD4+ T cells by 80% (compared to CD4+ T cells alone), eSF increased infection rates up to 81-fold. Comparison of eEC and eSF by microarray revealed anti-HIV factors SLPI and b-defensin as the top differentially expressed genes, and SLPI production by eEC and not eSF was confirmed at the protein level. Mechanistic analysis of how eSF promote HIV infection of CD4+ T cells revealed two mechanisms: trans-infection of CD4+ T cells, and increasing permissivity of the co-cultured cells. Of significance for prevention strategies, eSF-mediated enhancement of HIV infection increases the IC50 of tenofovir.

**Conclusions:** These data lead to a model whereby in comparison to the lower FRT, which is subject to increased HIV susceptibility induced by sexual intercourse or ulcerative STDs, the upper FRT is prone to natural "cyclical susceptibility" via E2P4-induced permeability. Under conditions where P4 is high, the eEC layer is more permeable and HIV gains access to eSF, which promotes HIV infection of resident mucosal CD4+ T cells. This model may help explain why P4-based contraceptives are associated with higher HIV transmission risk in women, and suggest targeting the ability of eSF to enhance HIV infection as a new prevention approach.

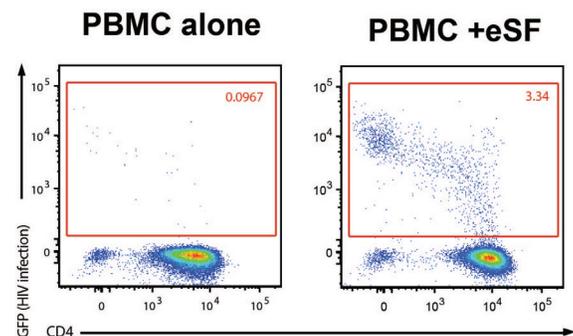


Figure Legend. Primary endometrial stromal fibroblasts (eSF) enhance HIV infection of CD4+ T cells. PHA/IL2-activated PBMCs were cultured alone or in the presence of eSF, and infected with 50 ng/ml p24 of an HIV GFP reporter virus. Infection was monitored three days later by flow cytometric quantitation of GFP-expressing cells. CD4 down-regulation of infected cells was also monitored. Results are gated on viable, CD3+CD8- lymphocytes. Similar experiments using endometrial epithelial cells (eEC) instead of eSF resulted in the opposite phenotype whereby HIV infection of CD4+ T cells was inhibited (data not shown).

## 285 Seminal Plasma Induces Inflammation and Enhances HIV-1 Infection in Cervical Explants

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**Background:** Exposure of the female genital mucosa (FGM) to semen induces a local inflammatory response, characterized by increased expression of pro-inflammatory cytokines and leukocyte infiltration. Although this phenomenon has long been reported in humans, its mechanisms and implications for susceptibility to HIV remain elusive. Here we validated an *ex vivo* model of coitus to study the effect of seminal plasma (SP) on HIV transmission to the FGM.

**Methods:** Specimens of cervix were obtained from hysterectomized patients (age 35-50). Samples of SP from healthy individuals (n=12) were pooled to replicate experiments. Polarized explants of ectocervical mucosa were exposed to SP diluted 1:1 or 1:3, or medium only, for 2, 4 or 12 hours, with or without the anti-inflammatory drug indomethacin. After washing, explants were cultured with medium for additional 12 hours. *In situ* apoptosis was evaluated using a TUNEL assay. Levels of protein and gene expression of pro-inflammatory and growth factors were measured in medium and tissue respectively. The chemotactic effect of explant-conditioned medium on peripheral blood leukocytes was assessed with a transwell assay and flow cytometry. Explants exposed to SP were infected with the CCR5-tropic variant HIV-1<sub>BAL</sub> and viral replication was measured as p24<sub>gag</sub> concentration in culture medium.

**Results:** In comparison to control explants, exposure to SP resulted in: the absence or similar levels of apoptosis (n=3); increased levels of IL-1α, IL-6, TNF-α, CXCL1, CXCL8, CCL20, TGF-β1, CSF2, IL-7, and PTGS2 either as protein concentration in CM or gene expression, or both (n=7, p<0.05); 2-fold and 3-fold increased transmigration of neutrophils and monocytes respectively (n=4, p<0.05). Treatment with indomethacin did not affect the SP-induced changes in protein and gene expression. Explants exposed to SP productively supported HIV-1 replication, with a 2-fold increase in cumulative p24<sub>gag</sub> production over 18 days of culture compared to donor-matched untreated explants (n=5, p=0.06).

**Conclusions:** Exposure of human cervical explants to SP recapitulates the main features of the response occurring in the FGM upon coitus. The inflammatory nature of this response may explain the observed enhancement of HIV-1 replication. Our model can be implemented to investigate further the early molecular events underlying HIV-1 transmission to the FGM, in order to develop prevention strategies that target other determinants of infection in addition to the virus.

## 286 The Effect of Condomless Receptive Anal Intercourse on the Rectal Mucosa in MSM

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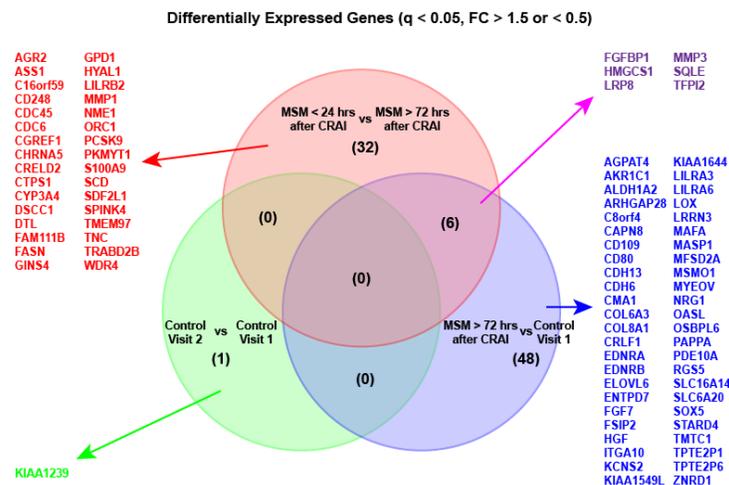
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**Background:** Rectal HIV transmission is considerably more efficient than penile or vaginal exposure. However, it is not known how condomless receptive anal intercourse (CRAI) alters the rectal mucosal immune environment in HIV negative men who have sex with men (MSM).

**Methods:** HIV negative MSM (n=41; median age 28) engaging in CRAI with an HIV negative partner underwent rectal biopsy via rigid sigmoidoscopy at two time points separated by at least 8 weeks: 1. After abstaining from CRAI for > 72 hours and 2. After engaging in CRAI < 24 hours prior. Men who had never engaged in anal intercourse (n=21; median age 24) were enrolled as controls. Peripheral blood mononuclear cells (PBMCs) and rectal mucosal mononuclear cells (RMMCs) were isolated for immunophenotyping and mitogen (PMA/ionomycin) stimulation experiments. For flow cytometry data, differences between groups were analyzed with repeated measures analysis using linear mixed effects modeling. For a subset of MSM and controls, RNA was extracted from tissue biopsies for Illumina RNA-Seq and differential expression was assessed using DESeq2 algorithm.

**Results:** In RMMCs, there were no differences between MSM and controls in the percentage of memory CD4+ cells that expressed CCR5 or Ki67; however MSM had lower overall mean (±SEM) expression of CD38 on CD4+ cells (38.0±2.5 vs. 49.4±3.5%; p=0.01). MSM had elevated levels of Ki67 on memory CD8+ cells (ln 1.4±0.1 vs. 1.0±0.2; p=0.03) and stimulated CD8+ cells had greater production of IFNγ (55.6±2.8 vs. 41.3±4.0; p=0.005) and TNFα (40.0±2.4 vs. 31.9±2.9; p=0.04) compared to controls. By RNA-Seq, 54 genes were significantly different between MSM who abstained for >72 hours and controls; an additional 32 unique genes were differentially expressed in MSM <24 hours after CRAI compared to >72 hours (0.5>Fold-change<1.5; q<0.05). Genes important in cell proliferation, tissue remodeling, and immune activation were upregulated among MSM.

**Conclusions:** The rectal mucosa of MSM engaging in CRAI did not harbor higher levels of activated CD4+ cells (CD38+ or Ki67+) or CCR5 expression indicating no increase in HIV target cell availability. However, elevated levels of CD8+ cell proliferation (Ki67+) and production of IFNγ and TNFα upon mitogen stimulation support a pro-inflammatory mucosal environment. Our data suggests that repeated exposure to gut microbes, lubricants, douching, and/or semen during CRAI is associated with a pro-inflammatory mucosal phenotype that may influence rectal HIV transmission.



## 287 Reduced Peripheral α4β7+ CD4+ T Cells Correlate With Mucosal CD4+ T Cell Loss in AHI

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**Background:** Intestinal CD4+CCR5+ T cells are rapidly and profoundly depleted early during acute HIV infection (AHI) contributing to persistent systemic immune activation. The integrin homing receptor α4β7 is thought to play a major role in the propagation and dissemination of HIV-1. CD4+ T cells expressing high levels of α4β7 (α4β7<sup>hi</sup>) are CD45RO+ and also express high levels of CCR5 and activation markers. Due to the difficulty monitoring intestinal CD4+ T cells we evaluated α4β7 as a predictive marker for the loss of intestinal CD4+CCR5+ T cells during early AHI.

**Methods:** Thirty-three subjects underwent phlebotomy and sigmoid biopsy at the time of AHI diagnosis. AHI was grouped using the fourth generation (4thG) immunoassay (IA) staging, with all stages being HIV RNA+ (Stage I: 4thGIA+/3rdGIA; II: 4thGIA+/3rdG; III: 4thGIA+/3rdGIA+). Of the 33 subjects 14 were 4thGI and 19 4thGIII. Multiparameter flow-cytometry was performed to determine the frequency of mucosal CD4<sup>+</sup>CCR5<sup>+</sup> T cells and the frequency of peripheral  $\beta 7^{\text{high}}$  CD4<sup>+</sup> T cell subsets at the time of AHI diagnosis.

**Results:** During 4thGI the frequency of mucosal CD4<sup>+</sup>CCR5<sup>+</sup> T cells and peripheral  $\beta 7^{\text{high}}$  CD4<sup>+</sup> T cells remained comparable to HIV-uninfected subjects, however with progression of 4thG stage a significant decrease in frequency was observed (Table 1). The frequency of peripheral  $\beta 7^{\text{high}}$  CD4<sup>+</sup> T cells correlated inversely with plasma ( $r=-0.52$ ,  $p<0.001$ ) and sigmoid ( $r=-0.36$ ,  $p=0.03$ ) HIV RNA and directly with the loss of mucosal CD4<sup>+</sup>CCR5<sup>+</sup> T cells ( $r=0.36$ ,  $p=0.02$ ). Additionally, we observed a loss of peripheral central memory (CM; CD27<sup>+</sup>CD45RO<sup>+</sup>) CD4<sup>+</sup> T cells expressing CCR5 and  $\beta 7^{\text{high}}$  during progression of AHI (Table 1). The frequency of CCR5<sup>+</sup>  $\beta 7^{\text{high}}$  CM CD4<sup>+</sup> T cells showed a strong direct correlation with the loss of mucosal CD4<sup>+</sup>CCR5<sup>+</sup> T cells ( $r=0.44$ ,  $p=0.008$ ) and correlated inversely with plasma HIV RNA ( $r=-0.51$ ,  $p=0.002$ ).

**Conclusions:** The loss of peripheral  $\beta 7^{\text{high}}$  CD4<sup>+</sup> and CCR5<sup>+</sup>  $\beta 7^{\text{high}}$  CM CD4<sup>+</sup> T cell subsets closely paralleled the loss of mucosal CD4<sup>+</sup>CCR5<sup>+</sup> T cells during early AHI. This is consistent with the hypothesis that  $\alpha 4\beta 7$  has a role in the selective depletion of mucosal CD4<sup>+</sup> T cells, and indicates that monitoring  $\beta 7^{\text{high}}$  expression on peripheral blood CD4<sup>+</sup> T cells could be a useful surrogate marker to estimate mucosal CD4<sup>+</sup>CCR5<sup>+</sup> T cell loss.

Table 1: Frequencies (%) of mucosal CD4<sup>+</sup>CCR5<sup>+</sup> T cells and peripheral CD4<sup>+</sup>  $\beta 7^{\text{high}}$  and CM CD4<sup>+</sup> T cells expressing CCR5 and  $\beta 7^{\text{high}}$  using 4thG staging

T Cell Population	4thGI	4thGIII	HIV-	CHI
%CD4 <sup>+</sup> CCR5 <sup>+</sup> (MMC)	67.3	22.1**	69.1	13.5**
%CD4 <sup>+</sup> $\beta 7^{\text{high}}$ (PBMC)	4.5	2.8*	4.8	1.6*
%CD4 <sup>+</sup> CM CCR5 <sup>+</sup> $\beta 7^{\text{high}}$ (PBMC)	2.5	1.6*	3.0	0.8**

All data are median (interquartile range); All comparisons were made to 4thGI: \* $p<0.05$ , \*\* $p<0.01$ ; MMC: mucosal mononuclear cells; PBMC: peripheral blood mononuclear cells

## 288 HIV Infections in Novel CD1a Cells From Human Vaginal Mucosa

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**Background:** Transfer across mucosal surfaces, such as the vaginal mucosa, account for the majority of new HIV-1 infections in the world. The identity and susceptibility of potential target cells in the vaginal mucosa remain unclear.

**Methods:** Skin and vaginal cells were obtained from women undergoing elective reduction mammoplasties and vaginal repairs respectively. Discontinuous density gradients were used to isolate epithelial and subepithelial cells, and anti-human CD1a magnetic beads were used to further purify the epithelial cells. Isolated cells were characterized using flow cytometry, electron-microscopy (EM) and Western blotting. Ability of different CCR5 (R5) or CXCR4-using (X4) HIV-1 to fuse, integrate, and replicate in these cells was examined by using the BlaM-Vpr fusion assay, Alu-PCR integration, and growth curves respectively.

**Results:** Similar to skin derived Langerhans cells (LCs), vaginal epithelium based cells expressed CD1a, CD1c and langerin but not DC-SIGN or CD14. In contrast to skin LCs, vaginal epithelial cells, termed CD1a+ VDCs, either contained low numbers or no Birbeck Granules, a hallmark of all LCs. HIV-1 YU-2 (R5) but not NL4-3 (X4) replicated in CD1a+ VDCs. The CD1a+ VDCs expressed the CXCR4 receptor, and NL4-3 both fused and integrated in the CD1a+ VDCs. Vaginal subepithelial tissue resident lymphocytes were CD69+ and displayed a memory phenotype. Both YU-2 and NL4-3 replicated in vaginal lymphocyte cultures. Interestingly, both NL4-3 and YU-2 replicated in CD1a+ VDCs exposed to virus, washed and co-incubated with vaginal sub-epithelial lymphocytes 3 days post-exposure.

**Conclusions:** CD1a+ VDCs differentially support replication of CCR5- but not CXCR4-using HIV-1. These unique CD1a+ VDCs are located at the portal of virus entry and are only susceptible to types of HIV-1 strains that initiate most infections, which suggests that CD1a+ VDCs are likely the earliest target cells for HIV-1 in the vaginal mucosa.

## 289 Influx of Th1 Cells in the Gut Mucosa Impairs the Homing of Th17 Cells Under cART

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**Background:** During HIV-1 infection, the integrity of the intestinal immune barrier is disrupted due to a deep depletion of CD4<sup>+</sup> T cells in the gut, including Th17 cells, a T cell subset exerting a major role in antimicrobial immunity. Th17 cells traffick to the gut mainly along the CCR6-CCL20 axis. We previously established that despite effective cART, Th17 cells homing to the small intestine mucosa is altered due to a reduced CCL20 production by enterocytes. A regulating loop occurs between IL-17 secretion, CCL20 production, and Th17 cells gut homing. The persistence of various inflammatory stimuli in the gut mucosa despite cART could contribute to a disequilibrium in the gut mucosa homeostasis. We thus assessed how Th1 cells could influence the homing of Th17 to the gut along the CCR6-CCL20 axis in this setting.

**Methods:** Duodeno-jejunal biopsies were obtained by endoscopy in 10 HIV-1-infected individuals and uninfected controls. HIV-1-infected individuals had plasma HIV-1 RNA <50 copies/mL under cART for about 5 years. Their median blood CD4<sup>+</sup> T cells count was of 668 cells/ $\mu$ L. Gut Th1 cells frequency was evaluated by flow cytometry and CXCR3<sup>+</sup> cells frequency was determined by immunohistochemistry. The effect of IL-2 and IFN- $\gamma$  on CCL20 expression was assessed in an *ex-vivo* model of human primary enterocytes cultured as a tight monolayer on transwells. To explore the impact of Th1 cells on CCL20 production, cocultures were done between the enterocytes and various proportions of Th1 and Th17 cells placed in the bottom chamber of the transwells to mimic *lamina propria*-epithelium interactions. Th1 and Th17 cells were sorted from PBMCs by flow cytometry on the basis of their CD3<sup>+</sup>CD4<sup>+</sup>CXCR3<sup>+</sup>CCR4<sup>+</sup>CCR6<sup>-</sup> and CD3<sup>+</sup>CD4<sup>+</sup>CXCR3<sup>+</sup>CCR4<sup>+</sup>CCR6<sup>+</sup>CD161<sup>+</sup> phenotype respectively. The expression of CCL20 by the enterocytes was evaluated by qRT-PCR and ELISA.

**Results:** In small intestine mucosa of HIV-1-infected individuals under effective cART, Th1 and CXCR3<sup>+</sup> cells frequencies were significantly increased compared to uninfected controls ( $P<0.05$ ). *Ex-vivo* on human primary enterocytes, both IL-2 and IFN- $\gamma$  decrease CCL20 expression, by 2-fold and 6-fold, respectively. In coculture experiments, CCL20 expression decreases as the Th1/Th17 ratio skews from Th17 to Th1 cells.

**Conclusions:** An influx of Th1 cells in the *lamina propria* of HIV-1-infected individuals despite effective cART could contribute to the reduced production of CCL20 by the enterocytes, then blunting the homing of Th17 cells to the gut.

## 290 Reduced Rectal HIV RNA After Peg-IFN-a2b Added to ART Together With ART Interruption

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**Background:** The effects of Peg-IFN-a2b immunotherapy on gut tissue HIV-1 RNA remain unknown. We hypothesized that in antiretroviral therapy (ART) chronically suppressed HIV-1+ individuals addition of Peg-IFN-a2b to ART, including of a short ART interruption (to induce HIV reactivation), would decrease rectal tissue HIV levels by triggering anti-HIV mechanisms.

**Methods:** 20 ART chronically suppressed (<50 copies/ml) HIV-1+ subjects received 20 weeks of Peg-IFN-a2b. ART was continued for the first 5 weeks, followed by a 4-week interruption, and resumption from week 9-20. Viral load (VL) and CD4<sup>+</sup> T cell levels were monitored throughout the treatment. Parameters assessed at baseline (BL) and after 20 weeks of treatment (EoT) included: a) immune activation markers (e.g. CD38, CD69, NKG2A, NKG2C, KIR) and IFN-responsive molecules (e.g. CD169, Tetherin) on cryopreserved

peripheral blood mononuclear cells (PBMC) by flow cytometry, b) soluble markers of immune activation (CD14, CRP, D-dimer, IL-6, CD163) in plasma by ELISA, and c) HIV RNA in rectal mucosa biopsies by in situ hybridization (confirmed by reverse transcriptase-ddPCR). Differences between BL and EoT were tested, using non-parametric Wilcoxon Sign-Rank test or paired t-tests, depending on data distribution. A  $p < 0.05$  was considered significant.

**Results:** 17/20 subjects completed the treatment; 3 subjects were withdrawn prematurely due to adverse event (AEs; neutropenia). 9/17 subjects had VL > 50 copies/ml during ART interruption, returning to undetectable upon ART re-initiation. Coupled (BL-EoT) rectal biopsies were available for 15/17 subjects with 7 of them having detectable tissue in situ HIV RNA at BL. Of these, 7/7 had a decrease in tissue HIV RNA at EoT, with a complete loss of detectable tissue HIV RNA in 5/7. 1 subject had detectable tissue HIV RNA at EoT, but not at BL. We observed a decrease in CD4<sup>+</sup> T cell count and an increase in CD4<sup>+</sup> and CD8<sup>+</sup> T cell activation (CD38, HLA-DR, PD1) and Tetherin expression between BL and EoT. Higher frequencies of CD56<sup>bright</sup> and lower of CD56<sup>dim</sup>CD16<sup>+</sup> NKs at EoT were also found, as well as an increase in CD38 and NKG2A in both NK subsets. In the myeloid compartment, we noted an increase in CD169 and Tetherin, and in plasma levels of CD163. No changes in plasma CD14, CRP, D-dimer, or IL-6 were found.

**Conclusions:** 20 weeks of combined Peg-IFN- $\alpha$ 2b+ART, including a 4-week ART interruption, result in a decrease in tissue HIV RNA and an increase in peripheral innate and adaptive immune activation.

## 291 Effect of Acute HIV Infection on CD4/CD8 Normalization After ART Initiation

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**Background:** Low CD4/CD8 ratios may increase risk of clinical events, and earlier antiretroviral therapy (ART) may lead to a higher likelihood of CD4/CD8 normalization. We estimated time to CD4/CD8 normalization following ART initiation and risk of mortality and serious clinical events among acutely and chronically infected patients.

**Methods:** Patients who achieved HIV RNA suppression within 1 year of ART initiation between 2000 and 2014, were included from the UNC CFAR HIV Clinical Cohort Study (chronic HIV infection, CHI) and the Acute HIV Infection Study (acute HIV infection, AHI). Normalization was defined as a ratio  $\geq 1$ . Kaplan-Meier and multivariable Cox proportional hazards methods were used to model time from ART initiation to normalization and outcomes of interest, adjusting for baseline covariates.

**Results:** AHI patients (n=102) were younger and more likely men than CHI patients (n=545), (median 28 vs. 38 years old; 89% vs. 73%; both  $p < 0.001$ ). At ART initiation AHI versus CHI patients had higher CD4 (median 489 vs. 203 cells/mm<sup>3</sup>;  $p < 0.001$ ) and were more likely to have a normal CD4/CD8 ratio, 14% and 2%, respectively. 57% and 14% of AHI and CHI patients had normal ratios at 1 year of suppressive ART, respectively. Median times to normalization were 6, 16, 43, 51 and >125 months among AHI and CHI patients with ART initiation CD4 > 500, 350, 200-350 and < 200 cells/mm<sup>3</sup>, respectively (log-rank  $p < 0.001$ ). In multivariable analyses AHI patients had shorter time to normalization (Hazard Ratio [HR]=2.7, 95% Confidence Interval [CI] 2.0-3.7), as did older patients and those with a higher CD4 (HR=1.14, 1.0-1.3 per 10 years; HR=1.3, 1.3-1.4 per 100 CD4). Among patients initiating ART with CD4 > 500, AHI patients also had shorter time to normalization with adjusted HR of 1.9 (1.2, 3.1). During 4,097 person-years of follow-up, 92 patients died. Having a normalized CD4/CD8 ratio at 1 year post-ART initiation had no apparent effect on all-cause mortality or a composite endpoint of serious non-AIDS clinical events and mortality (HR=0.8, 0.4-1.8; HR=1.1, 0.6-2.0; respectively).

**Conclusions:** CD4/CD8 normalization occurred earlier among AHI versus CHI patients but early normalization did not appear to predict mortality and serious non-AIDS events using a stringent criterion for normalization. The effect of longitudinal CD4/CD8 ratios on serious clinical events among patients on suppressive ART is important to evaluate given proposed hypotheses of an association with immune activation and immunosenescence.

## 292 Acute HIV Treatment Reverses CD8 T Cell Activation, Exhaustion, and Apoptosis

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**Background:** Despite viral suppression with cART, persistent inflammation contributes to pathogenesis and morbidity. We hypothesized that treatment during acute HIV infection (AHI) with an integrase inhibitor based regimen would decrease cellular activation, exhaustion, and apoptosis compared with subjects treated in chronic infection.

**Methods:** Subjects identified in AHI (n=18, Fiebig stages I-IV), were treated with tenofovir/emtricitabine and either raltegravir (RAL) or efavirenz (EFV). Matched chronically HIV infected subjects (CHI) (n=25) served as controls. Using flow cytometry, we measured surface expression of markers of activation (CD38), exhaustion (PD-1, CD160, CD244), senescence (CD57), pro-survival (Bcl-2) and apoptosis (cleaved-caspase-3) on CD8 T cells pre- and post-treatment. Comparisons were made within groups (Wilcoxon rank) and between groups (Mann-Whitney test),  $p < 0.05$  denoted by \*.

**Results:** Treatment in both AHI and CHI cohorts significantly reduced the frequency of activated CD38+ CD8 T cells (acute pre ART and virally suppressed (VS): 28% vs 6.3%,\*, chronic 24% vs 17%,\*), and, VS AHI had lower frequency compared with VS CHI (6.3% vs 17%,\*). Treatment also reduced some markers of exhaustion, reducing the frequency of PD-1hi CD8 T cells in both groups (AHI 5.6% vs 2.3%,\*, CHI 11% vs 7.1%,\*), with greater reduction in VS AHI (VS AHI 2.3% vs VS CHI 7.1%,\*). Treatment in both AHI and CHI reduced the frequency of apoptotic CD8+ T cells (CC-3hi/Bcl-2lo) (AHI 8.7% vs 2.9%,\*, CHI 8.3% vs 5.2%,\*), with greater reduction in VS AHI (VS AHI 2.9% vs VS CHI 5.2%,\*). Despite early treatment, viral suppression in AHI did not impact expression of CD160, CD57, or CD244. Enumeration of memory markers (naïve, central (CM) and effector memory (EM)) demonstrated that AHI treatment resulted in fewer CD8 T EM populations when compared to CHI (4.9% vs 32%,\*), but did not impact naïve or CM.

There was a trend to faster time to undetectable VL in the RAL vs EFV arm. No differences were observed in immune parameters between RAL vs EFV groups. Treatment in AHI resulted in more subjects achieving normal CD4% (AHI 85% vs CHI 22%,\*) and CD4/CD8 ratios of >1 (AHI 95% vs CHI 60%,\*).

**Conclusions:** Treatment during acute HIV reverses some measures of immune inflammation, including CD8 T cell activation (CD38), exhaustion (PD-1), and apoptosis (CC-3hi/Bcl-2lo) and results in more robust immune recovery. These data reinforce that early identification and treatment are warranted to improve clinical outcomes.

## 293 Inflammatory Biomarker Changes After Antiretroviral Treatment (ART) Initiation

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**Background:** Inflammatory cytokine levels pre- and one year post-ART were associated with incident non-AIDS events after one year in a nested case-control study of ACTG A5001. We examine changes in biomarkers after ART initiation.

**Methods:** Stored plasma from pre-ART (Y0), year 1 (Y1) and at the visit preceding an event was tested for inflammatory biomarkers (IL-6, sTNFR-I, sTNFR-II, sCD14, D-dimer) in 142 cases (non-accidental non-AIDS death, MI, stroke, non-AIDS malignancy, serious bacterial infection) and 302 matched controls. Unadjusted and partial Spearman correlations evaluated among controls the associations between levels and changes of biomarkers with pre-ART factors: HIV-1 RNA (VL), CD4, CD4:CD8 ratio, CD8, age, waist-hip ratio (WHR). Associations of biomarker changes with non-AIDS events were evaluated by conditional logistic regression.

**Results:** Median pre-ART age was 44 years; 85% were male; median Y0 CD4 215 cells/mm<sup>3</sup> and VL 64,371 copies/mL. Among controls: Higher biomarker levels at Y0 and Y1 (except for Y1 D-dimer) correlated with higher Y0 VL ( $p < 0.05$ ) and lower Y0 CD4 ( $p < 0.05$ ). All biomarkers declined from Y0 to Y1 ( $p < 0.0001$ ). Declines between Y0 and Y1 were greater for high Y0 VL ( $p < 0.002$ ) (Table), low Y0 CD4 ( $p < 0.001$ ) [even after Y0 VL adjustment] and low Y0 CD4:CD8 ( $p < 0.001$ ) [even after Y0 VL adjustment]. Older participants had greater declines for sTNFR-II, sCD14 and D-dimer from Y0 to Y1 ( $p < 0.04$ ). Those with higher WHR had greater declines in sCD14 and D-dimer from Y0 to Y1 ( $p < 0.01$ ). Though higher Y1 levels for all biomarkers were significantly associated with incident non-AIDS events (cases vs controls), greater declines in biomarkers at Y1 showed no significant associations. Cases, however, had significantly greater increases in all biomarkers from Y1 to the visit preceding the event ( $p < 0.02$ ).

**Conclusions:** Soluble inflammatory biomarkers decreased after 1 year of ART and subsequently remained mostly stable in controls; greater increases in cases prior to a non-AIDS event may reflect subclinical disease processes. Controls with greater pre-ART VL levels or lower pre-ART CD4 levels had higher biomarker levels at year 1 after ART initiation while also experiencing the greater biomarker declines in the first year of ART. Results support earlier initiation of ART in HIV+ infected adults.

**Table: Correlations (p-values) of pre-ART Factors with Changes of Inflammatory Biomarkers**

Pre-ART factors	Changes of Inflammatory Biomarkers: Y1 – Y0 (controls)				
	IL-6	sTNFR-I	sTNFR-II	sCD14	D-dimer
HIV-1 RNA	-0.17 (0.002)	-0.32 (<0.001)	-0.36 (<0.001)	-0.42 (<0.001)	-0.28 (<0.001)
CD4	0.27 (<0.001)	0.40 (<0.001)	0.39 (<0.001)	0.43 (<0.001)	0.41 (<0.001)
CD4:CD8 ratio	0.22 (<0.001)	0.39 (<0.001)	0.43 (<0.001)	0.41 (<0.001)	0.40 (<0.001)
CD8	0.10 (0.08)	0.08 (0.16)	-0.04 (0.44)	0.06 (0.30)	0.06 (0.30)
Age	-0.06 (0.30)	-0.07 (0.26)	-0.13 (0.02)	-0.12 (0.04)	-0.12 (0.03)
Waist-hip ratio	-0.01 (0.88)	-0.10 (0.11)	-0.06 (0.32)	-0.20 (0.002)	-0.17 (0.01)

## 294 Increased Innate Function Following HIV-1 Suppression Is Linked to Epigenetic Changes

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**Background:** HIV-1 infection leads to both profound immune suppression and pathologic inflammation. While adaptive immune dysfunction has been extensively studied, less is known about innate immunity in the context of HIV-1. Based on the broad pathogen susceptibility in progressive HIV-1, we hypothesized that there would be aberrant innate responses. We tested innate function and investigated the underlying molecular mechanisms, analyzing subset distribution, transcription factor translocation, and epigenetic modifications.

**Methods:** Pre and post-antiretroviral therapy (ART) peripheral blood mononuclear cells from HIV-1 infected subjects were tested for *ex vivo* responses to a panel of innate stimuli (TLR2, TLR4, Dectin-1, and NOD) by culture supernatant analysis and intracellular cytokine staining (ICS). Cell activation and subsets and plasma levels of cytokines, sCD163, LPS, and sCD14 were measured. Associations between innate responses and plasma factors were assessed using resampled Spearman correlations and generalized estimating equations to account for repeated sampling. Monocytes were tested for NFκB nuclear translocation, and epigenetic modifications at the TNFα promoter were investigated by chromatin immunoprecipitation (ChIP) qPCR.

**Results:** Declining viral load correlated with increasing magnitude of monocyte and mDC responses to innate stimuli. This inverse relationship with viral load was most striking for TLR4(LPS) stimulation; culture supernatant: TNFα r=-0.37 (p<0.01), ICS: monocyte TNFα r=-0.57, mDC TNFα r=-0.54, mDC IL-1β r=-0.58, (p<0.001 for all). There was no difference in monocyte subset distribution or in baseline or LPS-induced nuclear translocation of NFκB between the pre and post-ART samples. In contrast, histone 4 acetylation and H3K4 trimethylation increased after viral suppression, connoting a poised transcriptional status.

**Conclusions:** Suppression of HIV-1 viral load with ART correlated with increased *ex vivo* innate immune responses. The altered monocyte function was not associated with subset redistribution or changes in NFκB signaling, but was associated with epigenetic modifications at the TNFα promoter. The high viral load environment conditions an epigenetic program associated with decreased innate function that is reversed with virus suppression. Further work is warranted to elucidate additional epigenetic determinants of innate immune deficiency and excessive inflammation in HIV-1 infection and their functional recovery following viral control.

## 295 Inhibition of P2X7 Enhances T-Cell Potential of CD34 in HIV+ c-ART Nonresponders

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**Background:** Although many factors are known to limit CD4 T-cell recovery during HIV infection, the impact of alteration in T-cell lymphopoiesis remains poorly understood. We hypothesize that deficient T-cell differentiation from CD34 hematopoietic progenitors (HP) could, at least partially, explain poor CD4 levels restoration under c-ART in HIV+ patients.

**Methods:** PBMC were obtained from HIV+ immune responders under c-ART (IR, n=16, CD4=1086/mm<sup>3</sup>, CD4/CD8=1.7) and HIV+ c-ART nonresponders (INR, n=16, CD4=380/mm<sup>3</sup>, CD4/CD8=0.58) followed in CHU Henri Mondor (Creteil, France) and from HIV- controls (n=18, CTS, Creteil). Ethical committee approval and written informed consent from all subjects were obtained before study initiation. T-cell precursor's frequency was determined using limiting dilution assay (LDA) on coculture of HP on OP9-DLL1 cell line. LDA results were generated using ELDA webtool. Flow cytometry analysis was performed on LSR2 cytometer, and FlowJo v8.2. Statistical analyses were performed using appropriate test in Prism software.

**Results:** We have previously shown a specific T-cell, but not B-cell differentiation impairment in CD34 HP from INR patients (see table 1). Now we extended our knowledge by demonstrating that CD34 HP from INR appeared extremely sensitive in regard of extracellular ATP known to induce caspase-1 mediated pyroptosis. We found abnormally high P2X7 levels (ATP recognition) and absent CD73 (ATP hydrolysis) expression. Importantly, caspase-1 inhibitor, VX-765, disturbed normal lymphopoiesis *in vitro*, thus P2X7 antagonist, PPAD, restored T-cell lymphopoiesis in INR: T-cell frequency of 1/314.7 (1/186.3-532.3) without PPAD vs 1/145.3 (1/98.3-214.7) with PPAD (p<0.05). Transcriptomic analysis revealed a general alteration in cellular death pathway in INR as compared with IR individuals.

**Conclusions:** Our results suggest that CD34 HP from INR are more prone to cellular death via extracellular ATP that affects normal T-cell lymphopoiesis and impacts CD4 T-cell restoration under c-ART. Currently we are evaluating reasons of this dysregulation and possible ways to interfere with it in therapeutic perspective. Such complementary to c-ART strategies might dramatically improve outcomes in treated immune nonresponders.

Table 1	Cell frequency (1/)			P		
	HIV-	HIV+ IR	HIV+ INR	HIV- vs IR	IR vs INR	HIV- vs INR
<b>T-cell potential</b>	71.9 (54.8-94.5)	86.3 (67.3-111)	240.6 (162.1-806.6)	NS	***	**
<b>B-cell potential</b>	63.1 (42.5-94.1)	47 (32.5-68.2)	64 (42.04-100.1)	NS	NS	NS

296 **Maraviroc and Immune Recovery in Advanced AIDS (CD4 <100)**

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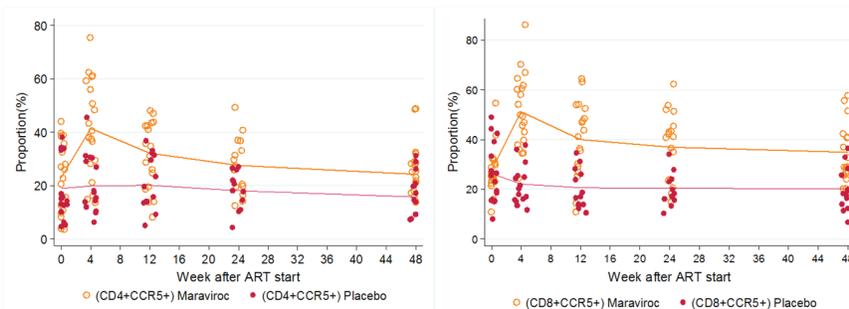
**Background:** In the CADIRIS trial 276 ART naïve patients with CD4 <100/uL were randomized to receive maraviroc (MVC) or placebo added to a standard ART regimen to evaluate the effects of CCR5 antagonism on occurrence of IRIS. No effect was demonstrable (Sierra-Madero Lancet HIV 2014). This substudy reports dynamics of phenotypic and functional cellular restoration upon viral suppression with or without CCR5 blockade.

**Methods:** Flow cytometry was used to characterize the maturation phenotype, CCR5 expression and activation status of circulating T cells at weeks 0,4,12,24 and 48. CD4 and CD8 T cell reactivity with peptides of CMV, MTb and Staphylococcal enterotoxin B was assessed by intracellular expression of IFN $\gamma$ , TNF $\alpha$  and CD40 ligand at weeks 0, 4 and 12.

**Results:** Forty patients were studied (MVC=22; placebo=18). MVC selectively retained CCR5+ CD4 and CD8 T cells in circulation as these proportions increased significantly during the first 4 weeks of MVC but not placebo (18.4% vs 1%,  $p<0.001$  and 23.7% vs -4.3%,  $p<0.001$ ). This increase was transient, and after week 12 the proportions of CCR5-expressing cells decreased in both arms, but subjects in the MVC group retained significantly higher rates of CCR5+ CD4 and CD8 cells at later time points. CD4 and CD8 T cell maturation subset numbers were similar in the treatment arms. The proportion of activated (CD38+ and HLA-DR+) CD4 and CD8 lymphocytes increased more at Week 4 in patients receiving MVC (10.6% and 6%) versus those receiving placebo (1.3% and -7.6%,  $p=0.04$  and  $p=0.007$ ). These proportions fell in both treatment arms thereafter. Mycobacterial responses were negligible throughout the study, but subjects on MVC had marginally CD8 cellular responses to SEB at weeks 4 (9.5% CD8 cells expressed IFN $\alpha$  in placebo and 17.4% in MVC,  $p=0.032$ ) and 12 (9.2% CD8 cells expressed IFN $\alpha$  in placebo and 17.8% in MVC,  $p=0.032$ ).

**Conclusions:** During ART administration in advanced AIDS, MVC selectively retained in circulation CCR5+ CD4+ and CD8+ T cells without affecting antigen-specific T-cell responses. Sustained increases in circulating CD8 T cell counts were observed in the MVC arm but treatment had no effect on the occurrence of IRIS

**Figure.** Proportion of CD4+ and CD8+ T lymphocyte expressing CCR5 at baseline, week 4, 8, 24 and 48 and rate of change by treatment arm.

297 **Transcriptomic Signature of Atorvastatin Response Among ART-Treated Adults in Africa**

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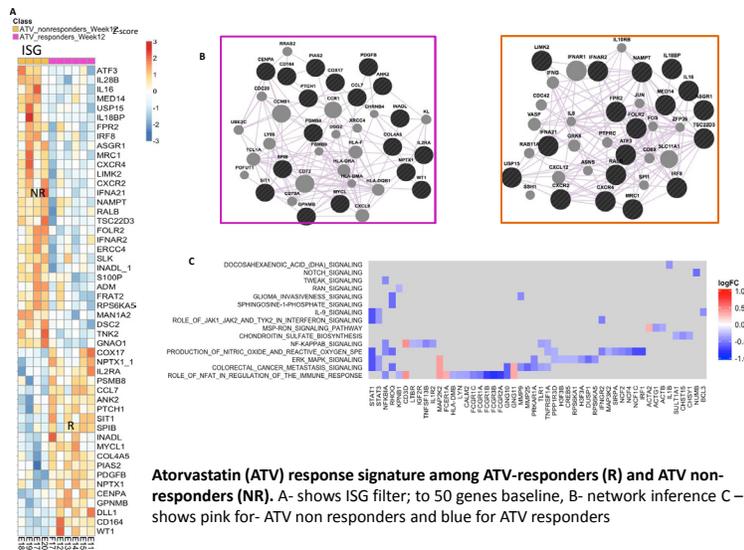
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**Background:** Inflammation is a hallmark of chronic HIV disease. Persistent immune activation was associated with suboptimal CD4 count recovery despite four years of suppressive antiretroviral therapy (ART), and has been associated with increased risk of cardiovascular disease and mortality among ART-treated HIV-infected adults. To understand the pathways affected by atorvastatin (ATV) adjunct therapy, which has immune activation properties, we examined the effect of ATV on gene expression among ART suboptimal immune responders.

**Methods:** Within a randomized, double-blind cross-over trial of atorvastatin adjunct therapy among suboptimal immune responders to ART (NCT01766076), paxgene samples were collected from suboptimal immune responders (SO-IR): patients with CD4 count increase <300 cells/ $\mu$ L (difference between pre-ART CD4 count and CD4 count after seven years of suppressive ART) before and after use of atorvastatin 80mg daily for 12 weeks. Using microarrays, atorvastatin responders (ATV-R): patients that had the highest reduction in immune activation [CD4+HLA+CD38+ expression] from week 0 to week 12 of atorvastatin in the parent study were compared with atorvastatin non-responders (ATV-NR): patients with the least reduction in immune activation after week 12. DEG analysis of the top 50 genes and top 50 ISG genes associated with interferon were analyzed. Network inference was done using geneMania algorithm, followed by linear regression analysis using clinical parameters and pathway analysis.

**Results:** Atorvastatin downregulated the inflammatory genes IL18, IL16 IFN, ATF3, MED14 and IFNA21 among six ATV-R which remained up-regulated in four ATV-NR. Pathways that were down-regulated by ATV-responders include: NF-KAPPAB signaling, NOTCH signaling, TWEAK signaling, RAN signaling, IL-9 signaling, role of Jak1, Jak2, tyk2 in interferon signaling, and role of NFAT in regulating immune response. We demonstrated that NF kappa B genes were significantly upregulated among 17 SO-IR to ART when compared with 19 ART-responders (patients with CD4 increase >500 cells after seven years of ART). Upregulation of inflammatory pathways was significantly associated CD4 and CD8 activation levels.

**Conclusions:** Atorvastatin downregulates inflammatory genes among suboptimal immune responders to suppressive antiretroviral therapy. Transcriptomic signature of atorvastatin response could be used to assess the effects of atorvastatin when used as adjunct therapy for ART-treated HIV-infected individuals.



**Atorvastatin (ATV) response signature among ATV-responders (R) and ATV non-responders (NR).** A- shows ISG filter; to 50 genes baseline, B- network inference C – shows pink for- ATV non responders and blue for ATV responders

**298 ACE-Inhibitors to Decrease Lymphoid Fibrosis and the Size of the Latent Reservoir**

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**Background:** In HIV infection, lymphoid architecture is disrupted by fibrosis and this persists during antiretroviral therapy (ART). The role of anti-fibrotic agents in HIV-infection is therefore an area of active investigation. Studies have shown that ACE inhibitors have anti-fibrotic properties through inhibition of transforming growth factor-β1. We completed a randomized, placebo-controlled, pilot study to assess whether the addition of lisinopril to ART reverses fibrosis in gut-associated lymphoid tissue (GALT), and whether this leads to more effective host immune responses and reduction of the latent reservoir.

**Methods:** Thirty HIV-infected individuals on ART (<75 copies/mL) for ≥1 year were randomized to lisinopril 20mg daily or matching placebo for 24 weeks. All subjects were male. Enrollment was stratified by CD4<sup>+</sup> T cell counts of <350 cells/mm<sup>3</sup> (non-responders, NRs) and ≥ 350 cells/mm<sup>3</sup> (immunologic responders, IRs). The primary end point was the change in collagen turnover in rectal biopsies from baseline. Heavy water labeling was used to quantify a fractional synthesis rate of collagen in GALT using liquid-chromatography-mass spectrometry. Secondary outcomes included the change in: 1) CD4<sup>+</sup> and CD8<sup>+</sup> total Gag-specific responses (IFN-γ, IL-2, TNF-α, or CD107a) in GALT and peripheral blood mononuclear cells (PBMCs); 2) the percentage of CD38<sup>+</sup>HLA-DR<sup>+</sup> CD4<sup>+</sup> and CD8<sup>+</sup> T cells in GALT and PBMCs; and 3) HIV RNA and DNA levels in GALT and PBMCs. Levels were compared using the Wilcoxon rank sum test.

**Results:** Collagen turnover rates did not differ significantly between the two treatment arms. The addition of lisinopril did not have a significant effect on the change in CD4<sup>+</sup> and CD8<sup>+</sup> Gag-specific responses, T cell activation, or in the levels of HIV RNA or DNA in either GALT or PBMCs. Likewise, no difference was observed between IRs and NRs in the response to lisinopril. NRs did have increased levels of CD38<sup>+</sup>HLA-DR<sup>+</sup> CD4<sup>+</sup> and CD8<sup>+</sup> T cells in GALT at baseline compared to IRs (CD4: 14.8% vs 8.2%, p=0.008; CD8: 28.6% vs 19.7%, p=0.09). CD4<sup>+</sup> T cell activation in GALT correlated negatively with peripheral CD4<sup>+</sup> T cell count (rho= -0.47, p=0.009).

**Conclusions:** Treatment with lisinopril for 24 weeks in HIV-infected adults did not result in a significant change in collagen turnover, HIV-specific responses, T cell activation or HIV RNA or DNA in either GALT or PBMCs. Further study is needed to see if anti-fibrotic agents may be useful in reversing lymphoid fibrosis and reducing the size of the HIV reservoir.

**299 Distinctive Dynamics of Phenotypic Cellular Restoration in IRIS**

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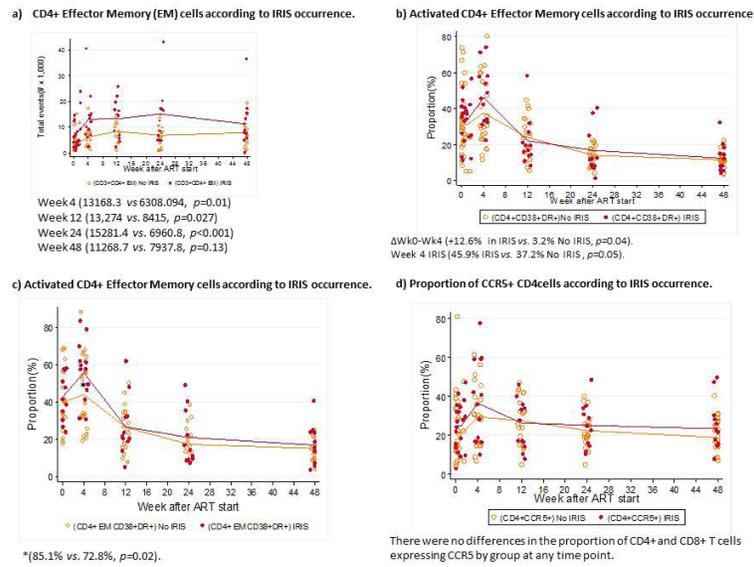
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**Background:** IRIS is a common and potentially fatal complication of ART initiation in advanced AIDS; its immunopathogenesis remains poorly understood. While the clinical manifestations and underlying pathogenic mechanisms might vary for each IRIS-associated-pathogen, some studies suggest common inflammatory mechanisms. Here, we characterize the dynamics of lymphocyte maturation phenotypes, CCR5 expression and activation status of circulating T cells in patients who did and didn't develop IRIS.

**Methods:** Lymphocyte phenotypes were monitored at baseline and weeks 4, 12, 24 and 48 by flow cytometry on cryopreserved PBMC obtained from 40 patients initiating ART plus maraviroc or placebo in a substudy of the CADIRIS trial.

**Results:** Randomization to maraviroc had no effect on occurrence of IRIS. Twelve (30%) subjects developed IRIS. Patients with IRIS had a significantly greater CD4 cell increase at Weeks 4, 12 and 24 that were mostly effector memory (EM) cells. EM numbers were greater in IRIS patients than those without IRIS at weeks 4 (13168/mL vs 6308, p = 0.01), 12 (13,274 vs. 8415, p = 0.027) and 24 (15281.4 vs. 6960.8, p < 0.001). Thereafter EM numbers fell and were comparable in the groups at week 48. No differences were observed in naïve (N), Central Memory (CM) and Terminally Differentiated (TD) CD4 cells. The proportion of activated (CD38+DR+) CD4+ cells also increased more during the first 4 weeks in patients with IRIS than in patients without (+12.6% vs. 3.2%, p = 0.04), and were significantly different at week 4 (45.9% vs. 37.2%, p=0.05). Also, a higher proportion of the CD4+ EM cells in patients with IRIS expressed activation markers (CD38+DR+) than was seen in patients without IRIS (+12.9 vs. +3.03%, p=0.08), but no differences were observed for other CD4 maturation subtypes. There were no differences in the proportion of CD4+ and CD8+ T cells expressing CCR5 by group at any time point perhaps reflecting the failure of maraviroc to affect IRIS occurrence. Absolute CD8 cells, CD8 maturation subtypes and activated maturation subtypes declined throughout the study in both groups similarly.

**Conclusions:** These results suggest that that activated CD4 effector memory cells might participate in the immunopathogenesis of IRIS.



**300LB Only T-Cell Responses to Nef/Tat/Rev Correlate With Infected Cell Frequencies on ART**

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**Background:** HIV-specific CD8+ T-cell responses limit viral replication in untreated infection. After initiation of antiretroviral therapy (ART), these responses decay, and the viral reservoir that persists is commonly considered to be invisible to CD8+ T-cells. We hypothesized that HIV antigen recognition may persist in ART-treated individuals, due to low-level or episodic protein expression. We reasoned that the 'early' HIV gene products Nef, Tat, and Rev would have lower barriers to expression than the Rev-dependent late gene products. Ongoing recognition of HIV antigens in ART-treated individuals could manifest as associations between T-cell responses and either numbers of infected cells (cell associated HIV DNA), or residual HIV RNA expression (cell-associated or cell-free).

**Methods:** We performed IFN-γ ELISPOT assays with peptide pools spanning: i) Gag ii) Env iii) Pol iv) Nef,Tat,Rev v) Vif,Vpr,Vpu vi) CMV-pp65 in cross-section on PBMC from a cohort of 99 individuals suppressed on ART (<50cp/mL HIV RNA), for a median of 6.8 years (ACTG HIV Reservoirs Cohort Study, A5321). Cell associated HIV DNA and RNA, and plasma HIV RNA were measured by qPCR.

**Results:** HIV-specific T-cell responses were primarily directed against Gag, Pol, and Nef/Tat/Rev with mean±SD values of 171±271, 295±282, and 124±205 SFU/10<sup>6</sup> cells, respectively. We observed a modest but significant correlation between the magnitude of the Nef/Tat/Rev-specific T-cell response and cell-associated HIV DNA (Spearman p=0.026, r=0.23). No correlations were observed between T-cell responses to other HIV antigens and HIV DNA. Nef/Tat/Rev-specific T-cell responses did not correlate with pre-ART plasma HIV RNA or CD4 counts. T-cell responses to HIV antigens did not correlate with cell-associated or plasma HIV RNA.

**Conclusions:** These results suggest that ongoing Nef, Tat, and/or Rev expression in ART-treated individuals drives preferential maintenance of T-cells reactive to these proteins. We did not observe evidence for interactions between T-cells targeting other HIV gene products and the viral reservoir. The direct correlation between Nef/Tat/Rev-specific T-cell responses and proviral DNA levels suggests that ongoing recognition of infected cells by these T-cells may occur in ART-treated individuals, but that this does not result in efficient elimination. Strategies to enhance the functionality of Nef/Tat/Rev-specific T-cell responses may facilitate reductions in the reservoir even without the addition of latency reversing agents.

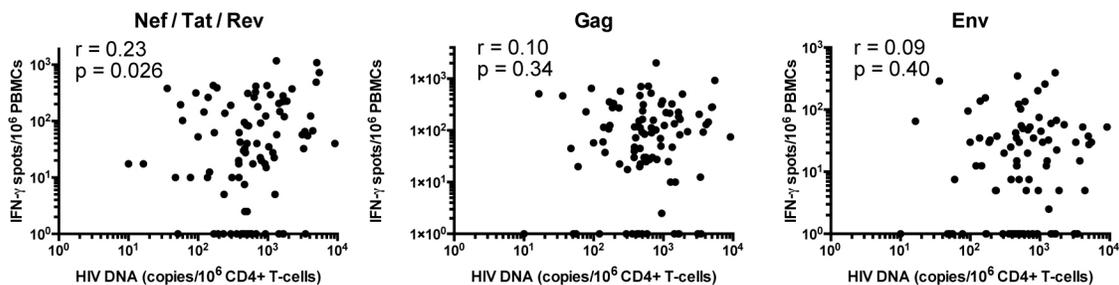


Fig. 1. Testing for correlations between HIV-specific T-cell responses to the indicated gene products (IFN-γ ELISPOT) and frequencies of HIV-infected CD4+ T-cells.

**301 Oral PrEP Enhances Genital HIV-Neutralizing IgA in HIV-1 Exposed Seronegative Women**

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**Background:** Several clinical trials have reported efficacy of pre-exposure prophylaxis (PrEP) with antiretroviral drugs against sexual HIV-1 transmission. Non-human primate studies have found that SIV-1 exposure during PrEP can induce adaptive immunity in the absence of productive infection. We evaluated cervicovaginal IgA in female study participants on PrEP and placebo, and examined whether responses persist or wane after discontinuation of PrEP.

**Methods:** Cervicovaginal swab samples were collected from a subset of 99 HIV-1 uninfected women participating in the Partners PrEP Study (a clinical trial of tenofovir-DF based oral PrEP versus placebo in HIV-1 uninfected East Africans). An HIV-1 exposure risk score was calculated from longitudinal data including unprotected sex frequency and plasma HIV-1 RNA level of the HIV-infected stable partner; a 1-unit increase in exposure score indicates a 2.7-fold increased risk of infection. Total IgA1 was extracted from swabs, and tested for anti-HIV-1 functional activity in a PBMC-based HIV-1 neutralizing assay. Positive IgA neutralization was defined as neutralization at  $\geq 90\%$  in duplicate wells.

**Results:** Among 99 samples processed, 40 were from women on PrEP, 45 at 2 months post-PrEP (32 of these paired with on treatment), and 14 on placebo. PrEP and placebo recipients were comparable by age and HIV-1 exposure. Twenty percent of women on PrEP had HIV-1-neutralizing IgA, versus 9% in the post-treatment group, and 0% in the placebo group. Women on PrEP had a 29%-point increase in level of neutralization compared to those on placebo ( $p=0.006$ ). Women off-PrEP also had a significant though smaller increase of 22%-point compared to placebo ( $p=0.03$ ). We also examined the effect of HIV-1 exposure on the level and presence of HIV-neutralizing IgA: each unit increase in the HIV-1 exposure risk score was associated with a 5% increase in level of HIV-neutralization ( $p=0.04$ ), or with 47% higher odds of neutralizing both wells at  $\geq 90\%$  (OR = 1.47 (95% CI: 1.01-2.13),  $p=0.04$ ).

**Conclusions:** We found an enhanced IgA-mediated HIV-1 neutralizing activity in genital samples of women on PrEP compared to placebo. These observations are reminiscent of data from studies on HIV-exposed uninfected individuals who can harbor mucosal HIV-1 specific immune responses in the absence of productive HIV-1 infection. Further studies in humans and non-human primate models are needed to elucidate whether induced mucosal immune responses could contribute to PrEP efficacy.

**302 Potent Broadly Neutralising Antibody Responses in Slow-Progressing Pediatric HIV**

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**Background:** In the absence of anti-retroviral therapy (ART), ~50% of HIV-infected children have died by 2yrs. However, ~5% of ART-naïve HIV-infected children maintain normal CD4 counts through childhood. Unlike non-progressing adults, where high CD4 counts are associated with low viremia and protective HLA class I allele expression, non-progressing pediatric infection features high viral loads and a minimal role for HLA I. We here tested the hypothesis that non-progressing children present a similar phenotype to that observed in natural SIV infection; and make broadly neutralising antibody (bNAb) responses in association with persistent high viremia.

**Methods:** We studied 276 ART-naïve HIV-infected children aged >5yrs recruited in South Africa. Phenotypic characterisation of T-cells was undertaken using mAbs specific for CD3, CD4, CD8, HLA-DR, CD38, CD45RA, CCR7, PD-1, CCR5, IL-2, IFN $\gamma$ , and TNF $\alpha$ . Plasma sCD14 and iFABP levels were quantified via Luminex and ELISA. Antibody neutralization was measured in TZM-bl cells using a multi-subtype panel of 16 Env-pseudotyped viruses, with breadth defined as neutralization of  $\geq 50\%$  of heterologous viruses.

**Results:** Low immune activation was strongly associated with high absolute CD4 count in ART-naïve children. Low levels of plasma sCD14 and iFABP were consistent with limited microbial translocation in slow-progressing children. Immune factors associated with non-progression included high naïve CD4 T cells, low effector memory CD4 cells; upon antigen stimulation, high IL-2 and low IFN $\gamma$  production; and low expression of T-cell exhaustion markers. LASSO and generalized linear models identified CD4 T-cell immune activation as the primary driver of CD4 decline among 16 parameters analyzed; notably, viral load had no significant impact. bNAbs were detected in 70% of slow-progressing pediatric subjects ( $n=89$ ) compared to 15% of C clade infected adults ( $n=48$ ) ( $p=1 \times 10^{-12}$ ). The NAb titres were unusually high in the children often exceeding 1:10,000.

**Conclusions:** Slow-progressing pediatric HIV infection shows features in common with natural SIV infection. HIV-specific immune responses in these children included high frequency bNAb responses in 85% of subjects. Further evaluation of this non-progressing pediatric group may be of value both in defining mechanisms limiting immune activation in the face of persistent viremia, and also in isolating highly potent bNAbs to facilitate future HIV prevention and cure strategies.

**303 Broadly Reactive Neutralizing Activity Within the First 6 Months of HIV-1 Infection**

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**Background:** According to the present knowledge, a fraction of HIV-1 infected individuals are able to generate broadly reactive neutralizing activity (brNA) after 2-4 years of infection and, in very rare occasions, close to the first year post-seroconversion. However, brNA has not been described in acute/recent HIV-1 infection.

**Methods:** In this study, serum samples from 157 antiretroviral-naïve individuals within the first year of documented HIV-1 infection were tested in triplicate at a 1/200 dilution against a minipanel of 6 recombinant viruses from 5 different subtypes and different tropisms. The epitopes recognized by the antibodies present in these sera have been characterized by ELISAs, neutralization and competition assays. Samples with cross-reactive neutralization profile, within the first 6 months of infection, were then tested against an extended panel of 20 pseudoviruses (2 tier 3, 14 tier 2 and 4 tier 1) from 4 different subtypes and recombinant forms to confirm brNA.

**Results:** Detectable brNA was observed in sera from 21 patients (13%) with less than one year of infection with no significant neutralization of the vesicular stomatitis virus (VSV) pseudotyped control. In these patients, neutralization breadth was positively associated with time post-infection ( $p=0.0001$ ) but, contrary to what has been reported for chronic HIV-1 infection, no association with the level of viremia was observed. The characterization of the epitopes recognized by sera from patients with brNA in recent infection showed a predominant targeting of envelope epitopes within the V2 glycan dependent region. Remarkably, we were able to identify 5 individuals, within the first six months of infection (2 as early as 77 and 96 days post-infection), capable to neutralize viruses from 4 different subtypes and 2 circulating recombinant forms with a geometric mean ID50 titer between 100 and 800.

**Conclusions:** These results indicate that the induction of broadly reactivity neutralizing responses, despite being rare, is feasible in recently HIV-1 infected individuals. This data should encourage the search for immunogens able to elicit this kind of responses in a preventive HIV-1 vaccine.

**304 New Family of Neutralizing Antibodies in HIV Asymptomatic Long-Term Nonprogressors**

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**Background:** Through a large serie of studies, we previously characterized the pathogenic effect of the highly conserved 3S epitope of gp41 during HIV infection. By analyzing the humoral immune response induced in asymptomatic long-term non-progressor (ALT) patients, we recently observed that up to 25% patients elicited efficient neutralizing responses directed against a point-mutated form of 3S (W614A-3S). Here we extensively characterized the neutralizing profile of this new family of neutralizing Abs.

**Methods:** Abs binding to 3S-WT (wild-type) or W614A-3S mutants were isolated from the sera of ALT patients. Abs were purified from heat-inactivated plasma of ALT patients by immunoprecipitation with synthetic W614A-3S peptides immobilized onto an amine-reactive agarose support, concentrated with Centicon filter, and dialyzed against PBS. The

functional inhibitory profile of these Abs was defined using the well-standardized TZM-bl neutralization assay, the conventional neutralization assay on PBMCs, or the Fc-mediated inhibitory assay on macrophages.

**Results:** We found that the anti-W614A-3S purified Abs display efficient and broad neutralizing activity. They inhibit transmitted founder Tier 2 viruses, neutralize primary isolates on primary cells and display Fc-mediated inhibitory functions at ng to µg/ml concentrations. The detection of anti-W614A-3S Abs was specifically correlated both with lower viral DNA ( $p < 0.0001$ ), viral load ( $p < 0.0001$ ), and other clinical parameters ( $CD4^+$  T cells, HLA protective alleles, ...) suggesting that anti-W614A-3S neutralizing Abs participate in the control of HIV replication in ALT patients.

**Conclusions:** These results demonstrate that ALT patients develop efficient neutralizing Abs that can be purified using W614A-3S mutant protein capture assays. These Abs are distinct to that recently isolated from ELITE neutralizer patients. Abs directed against W614A-3S may therefore be considered as a new family of broadly neutralizing Abs, which need to be further characterized, considering their potential role on viral load and viral DNA.

### 305 WITHDRAWN

### 306 Neutralization Differences Between CCR5 and CXCR4 Tropic Viruses in HIV-1B Infection

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**Background:** The mechanism for emergence of CXCR4 tropic HIV-1 variants remains poorly understood. We hypothesized that neutralizing antibodies (nAb) generated within an infected host potentially influence emergence of CXCR4-utilizing variants. We examined neutralization susceptibility among co-existing and inter-subject CCR5- and CXCR4-using viruses.

**Methods:** Full-length envelopes (envs) were isolated from 21 individuals from ACTG study A5095 using single genome amplification. Envs were incorporated into an NL4-3 backbone to generate replication competent recombinant viruses. Viruses were examined for co-receptor usage and neutralization sensitivity using TZM-bl cells. Neutralization was assessed by measure of area under the curve (AUC), which assesses average neutralization within the range of two concentrations. Chimeric envelopes with swapped V3 loops were generated to determine the viral determinant influencing observed neutralization susceptibility differences. Comparisons were done using the Wilcoxon rank sum test.

**Results:** A median of 13 envs (range 3-27) was isolated from 21 individuals. Exclusively CCR5-using envs (R5) were isolated from 12 individuals, envs able to utilize both CCR5 and CXCR4 (R5X4) and or only CXCR4 (X4) were isolated from 7 subjects, and a combination of R5, X4, and R5X4 envs were amplified from 2 individuals. Sequence analysis showed that all envs were subtype B. None of the R5 or R5X4 envs ( $n = 52$ ), but all the X4 variants ( $n = 19$ ) had a 2-3 amino acid insertion prior to the V3 loop GPGR crown. In the 2 subjects with co-circulating X4 and R5 variants, X4 envs were less neutralization sensitive to autologous contemporaneous plasma compared to the R5 envs (subject 1: R5 median AUC-0.46 vs. X4 AUC-0.19; subject 2: R5 median AUC-0.19 vs. X4 AUC-0.0). In inter-subject comparisons, X4 as compared to R5 variants were more neutralization resistant to heterologous pooled plasma ( $p = 0.03$ ).

**Conclusions:** Similar to previously described subtype C HIV-1, subtype B env X4 variants possess a unique V3 loop genotypic signature. In some individuals, X4 variants potentially emerge as neutralization escape variants against the host generated nAbs. Chimeric envelope results will elucidate the role of the unique V3 amino acid insertion in conferring neutralization resistance.

### 307 Humoral Immune Pressure Selects for HIV-1 CXCR4-Using Variants

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**Background:** The emergence of a dual-mixed (DM) population (mixture of CCR5-using (R5), exclusively CXCR4-utilizing (X4), or R5X4 viruses) as opposed to presence of only R5 variants is associated with accelerated  $CD4^+$  T cells decline. Previous studies have not clarified if CXCR4-utilizing variants accelerate disease progression or if faster  $CD4^+$  T cell decline facilitates the emergence of DM virus populations.

**Methods:** Seven individuals enrolled in the Mashi study with documented DM populations and not on treatment were selected for this study. Envelopes (envs) were isolated using bulk PCR cloning. Envs were used to generate pseudovirions, replication competent viruses, or codon optimized gp120s. Infectious viruses were examined for neutralization sensitivity using TZM-bl assay, coreceptor usage using U87 cells, and evolution using in-vitro passage in the presence or absence of autologous plasma. Envs using different coreceptors were compared using the Wilcoxon rank-sum test.

**Results:** A median of 11 subtype C env clones (range 9-18) were examined from each individual. The X4 envs as compared to co-circulating CCR5-utilizing variants were less sensitive to neutralization by both contemporaneous autologous plasma ( $p = 0.03$ ) and plasma pools from individuals that harbor only R5 HIV-1 ( $p = 0.12$ ). The X4 and co-existing R5 envs, however, had similar sensitivity to two different broadly neutralizing antibodies (bNab), VRC01 and 10E8 ( $p > 0.05$ ). Among 2 individuals with variants susceptible to env V3 directed bNab, PGT128, X4 as compared to co-circulating R5 variants were more neutralization resistant in 1 case ( $p = 0.003$ ). Chimeric envs with X4 as compared to R5 V3 domains were less sensitive to pooled R5 plasma and PGT128, although the differences were relatively small. Chimeric gp120s with X4 as compared to R5 V3 segments also showed decreased binding to autologous plasma and V3 directed monoclonal antibody, 447-52D. In-vitro passage in activated  $CD4^+$  T cell cultures of a neutralization sensitive CCR5-using virus led to the emergence of a CXCR4-utilizing virus stock in 1 of 3 cases in the presence but not the absence of autologous plasma.

**Conclusions:** In some individuals, X4 as compared to co-circulating R5 variants are relatively neutralization resistant to host generated antibodies that potentially target the env V3 loop. In these individuals, CXCR4-utilizing viruses likely emerge as a consequence of humoral immune pressure.

**308 Systematic Analysis of Glycan Heterogeneity Guides Rational Design of HIV Immunogens**

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**Background:** Glycans account for more than half of the HIV envelope gp120 protein mass, thought originally to disguise virus from the host immune response and to drive viral evasion. However, mounting evidence suggests highly variable glycans are shown to be antigenic determinants in epitope recognition for many of the most potent broadly neutralizing antibodies (bNAbs). Thus, vaccine design approaches, which harness the antigenic nature of the envelope glycan to guide the vaccine-induced humoral immune response to the sites of vulnerability for effective neutralization, may significantly advance the field. Yet, efforts to exploit the natural antigenicity of the gp120 glycan shield have only shown limited success, because the focus was only on the proximal glycans directly impacting bNAb recognition, which is not enough to appreciate the complexity of global bNAb-glycan interactions.

**Methods:** Here we conducted a unique approach to systematically and computationally deconvolute the complexity of the global HIV glycan shield heterogeneity and link this heterogeneity to differential bNAb recognition fingerprints. Moreover, we developed a *de novo* immunogen design program which integrated all systematic data as well as gp120 structure information to engineer gp120 improving the bNAb recognitions.

**Results:** Mass spectrometry-based glycoproteomics defined glycan occupancy across the gp120 proteome isolated from 94 distinct HIV viral isolates. Remarkably, highly diverse glycan occupancy profiles were observed at most potential N-linked glycosylation sites, including sites known to be targeted by bNAbs. Using an unsupervised machine learning algorithm, unique bNAb-glycan binding interaction signatures were identified, directed by both agonistic and antagonistic glycans that cooperatively facilitate antibody recognition. Moreover, these signatures were utilized to design glycan-optimized immunogens, which were able to selectively improve or alter different bNAb recognitions.

**Conclusions:** This approach provides a novel rational design strategy to improve HIV envelope antigenicity, particularly to glycan-dependent bNAbs, via selective glycosylation profiles tailored to enhance bNAb binding.

**309LB Optimal Combinations of bnAbs for Prevention and Treatment of HIV-1 Clade C Infection**

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**Background:** The identification of a new generation of potent broadly neutralizing HIV-1 antibodies (bnAbs) has generated substantial interest in their potential use for the prevention and/or treatment of HIV-1 infection. While combinations of bnAbs targeting distinct epitopes on the viral envelope (Env) will likely be required to overcome the extraordinary diversity of HIV-1, a key outstanding question is which bnAbs, and how many, will be needed to achieve optimal clinical benefit.

**Methods:** We assessed the neutralizing activity of 15 bnAbs targeting four distinct epitopes of Env, including the CD4-binding site (CD4bs), the V1/V2-glycan region, the V3-glycan region, and the gp41 membrane proximal external region (MPER), against a panel of 200 acute/early clade C HIV-1 Env pseudoviruses. A mathematical model was developed that predicted neutralization by a subset of experimentally evaluated bnAb combinations with high accuracy. Using this model, we performed a comprehensive and systematic comparison of the predicted neutralizing activity of over 1,600 possible double, triple, and quadruple bnAb combinations.

**Results:** Best-in-class single bnAbs for potency and breadth were CAP256-VRC26.25 (V2-glycan), 10-1074V (V3-glycan), VRC07-523 (CD4bs) and 10E8 (MPER). Optimal bnAb combinations were identified based on breadth and potency of neutralization, as well as other relevant measures such as the extent of complete neutralization and instantaneous inhibitory potential. By these criteria, VRC07-523 + CAP256-VRC26.25, VRC07-523 + CAP256-VRC26.25 + 10-1074V and VRC07-523 + CAP256-VRC26.25 + 10-1074V + 10E8 were identified as the best 2, 3, and 4 bnAb combinations, respectively. Triple and quadruple combinations of bnAbs were significantly more effective than the best double combinations. Quadruple bnAb combinations were predicted to be similar to triple combinations by some metrics, and significantly better by others. Importantly, increasing the total number of bnAbs in combinations improved the probability of having multiple bnAbs simultaneously active against a given virus, a requirement that may be critical for countering escape *in vivo*.

**Conclusions:** Combinations with higher numbers of bnAbs are advantageous in providing increased potency, breadth, complete neutralization, and active coverage against HIV-1 clade C. These results provide a rationale for advancing bnAb combinations with the best *in vitro* predictors of success into clinical trials for both the prevention and treatment of HIV-1 infection.

**310LB Informatics-Based Improvement of HIV-1 Neutralizing Antibody 10E8**

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**Background:** Passive delivery of antibody may provide prophylaxis against and treatment of HIV-1. One antibody of potential promise is human antibody 10E8, which targets the membrane-proximal external region (MPER) and is capable of neutralizing ~98% of HIV-1 isolates. The extraordinary breadth of 10E8 is tempered, however, by its less-than-optimal solubility, which complicates manufacture, and the need to dose with large amounts antibody.

**Methods:** We employed technologies that provide information-rich output including X-ray crystallography, next-generation sequencing and surface-matrix screening to improve 10E8 solubility and potency. Specifically, we combined a structure-based approach with natural variants obtained through sequencing of immunoglobulin transcripts from donor N152, the source of antibody 10E8. The surface-matrix approach involved the creation of hundreds of individual surface mutants, to probe the effect of hydrophobic protrusion (Phe or Trp), hydrophilic protrusion (Arg), large hydrophilic protrusion (N-linked glycan), and side-chain removal (poly-glycine). Solubility was assessed through measurements of turbidity, and potency was assessed through *in vitro* neutralization of virus panels: 9-isolates for screening and up to 200-isolates for detailed comparison.

**Results:** By combining a structure-based approach with natural variation in potency and solubility from the 10E8 lineage, we created variants of 10E8, which retained the potency and extraordinary neutralization breadth of the parent 10E8. Of these, 10E8v4 with 26 changes versus the parent 10E8 was most soluble, with potency on a panel of 200-HIV-1 isolates similar to that of the parent 10E8 and half-life in rhesus macaques of ~10 days. Incorporation into 10E8v4 of select alterations from the surface-matrix approach yielded variants such as antibody 10E8v4-5R-100cF, with acceptable solubility, low poly-reactivity, and an IC80 potency of ~0.03 ug/ml, approximately 50-fold better than the parent 10E8.

**Conclusions:** The application of information-rich technologies to antibody optimization allows for the enhancement of manufacturing characteristics and therapeutic properties. We propose that these technologies might serve as a general means to enhance the clinical utility of naturally occurring antibodies. The increased solubility and potency of the 10E8v4-5R-100cF variant opens the possibility for use of a MPER-directed specificity in prophylaxis or immunotherapy at a modest dose (1-10 mg/every 2-3 months per typical adult).

**311LB Effect of Infusion of Broadly Neutralizing Antibody VRC01 on HIV Plasma Rebound**

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**Background:** Recent advances in immunogen and antibody cloning technologies have led to the isolation of several highly potent and broadly neutralizing HIV-specific antibodies (bNAb) from B cells of infected individuals. Among these, VRC01 has proven to be effective in neutralizing diverse strains of HIV *in vitro* and in animal models and has the capacity

to suppress plasma viremia in infected individuals. However, the ability of VRC01 to suppress plasma viral rebound in HIV-infected patients following cessation of antiretroviral therapy (ART) remains unclear.

**Methods:** An exploratory, open-label clinical trial was conducted to examine the effect of passive transfer of VRC01 on plasma viral rebound following discontinuation of ART in HIV-infected individuals who initiated ART during the chronic phase of infection and who suppressed plasma viremia >3 years with CD4<sup>+</sup> T cell count > 450 cells/mm<sup>3</sup> at enrollment. Subjects received VRC01 (40mg/kg) 3 days prior to and 14 and 28 days following interruption of ART, and monthly thereafter for up to 6 months. Levels of plasma viremia and VRC01 were measured at day -7, -3, 0, 3, 7, 14, 21, and 28 and biweekly thereafter. In addition, the capacity of VRC01 and other bNAbs to neutralize autologous infectious HIV prior to and following infusions of the antibody was examined.

**Results:** 10 subjects were enrolled in the study. Mean duration of ART was 10.6 years. Mean CD4<sup>+</sup> and CD8<sup>+</sup> T cell counts at baseline were 796 and 768 per mm<sup>3</sup>, respectively. Multiple infusions of VRC01 were safe and well tolerated. 9/10 subjects experienced plasma viral rebound (>40 copies/ml) between 11-54 days (median 39) following cessation of ART; 8 subjects reinitiated ART per protocol. Plasma concentration of VRC01 ranged between 142-352 µg/ml (median 160) at time of first detectable plasma viremia. Preliminary analyses of autologous replication-competent viral isolates revealed the existence of VRC01-resistant virus prior to infusion of antibody in several subjects; further assessment of prior and post-treatment resistance is ongoing.

**Conclusions:** While multiple infusions of VRC01 were safe and well-tolerated, the majority of patients experienced plasma viral rebound despite adequate levels of antibody in plasma. Therefore, therapeutic strategies involving passive transfer of bNAbs may require a combination (s) of Abs and/or resistance prescreening in order to achieve sustained virologic control in HIV-infected individuals upon withdrawal of ART.

### 312 CD8 T Cells From HIV Controllers Recognize and Kill HIV+ Non-Activated CD4 T Cells

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**Background:** Understanding the underlying immunological mechanisms of spontaneous HIV control by HIV+ controllers remains an important step in the development of efficient HIV vaccines and functional cures.

**Methods:** To understand control mechanisms triggered by CD8<sup>+</sup> T cells we established a system in non-activated peripheral blood mononuclear cells (PBMC) using infectious HIV (NL4.3 IRES GFP) carrying the fusion protein Vpr-Beta lactamase (to assess viral entry) and a staining strategy to study the CD8<sup>+</sup> T cell response by flow cytometry. The ability of the CD8 T cells from HIV controllers to form immunological synapses with HIV+ non-activated CD4 T cells was studied by confocal microscopy and the killing of the target cells was assessed by chromium release assay. In the study we included 10 HIV controllers and 10 HIV chronic progressors under antiretroviral therapy for less than 4 years. The statistical differences were calculated using PRISM software and an unpaired T test.

**Results:** We showed that non-activated CD4<sup>+</sup> T cells are permissive for HIV entry but this leads mostly to non-productive infection (b-lac+ GFP-). CD8<sup>+</sup> T cells from HIV controllers were significantly more efficient than CD8 T cells from progressors to recognize these HIV+ cells, became activated (IFNγ+ p=0.0068, MIP1b+ p=0.0162) and underwent degranulation (CD107a+ p=0.0038).

This activation was initiated by the presentation of HIV peptides from incoming viruses since viral entry but not reverse transcription was necessary and HLA-I blockade abolished this response. This CD8 T cell response was allowed by the formation of functional immunological synapses with HIV+ non-activated CD4+ targets and blocking adhesion molecules like LFA-1 disturbed the CD8 T cell degranulation. We confirmed that the CD8 T cell response lead to the killing of the target cells by chromium release assay. Finally we showed by flow cytometry that the TCR-beta chain may play an important role on this recognition.

**Conclusions:** In conclusion, recognition of HIV+ non-activated CD4+ T cells directly after HIV entry by CD8+ T cells before the establishment of latency or abortive infection participates to HIV control and should be further investigated for new vaccine strategies.

### 313 HIV-1-Specific CD8 T Cells Are Poorly Cross-Reactive During Acute Infection

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**Background:** HIV-1 has a high capacity to mutate so elucidating HIV-1 epitope-specific CD8 T cells that can efficiently target epitope variants is important for effective vaccine development. Prior studies indicating the capacity for CD8 T cells to cross-recognize epitope variants mainly used chronically infected samples, whereby presence of multiple viral quasispecies makes it difficult to ascertain the HIV-1 strain inducing the CD8 T-cell response *in-vivo*. To overcome this limitation, we evaluated the extent of T-cell cross-reactivity by analyzing samples from acutely HIV-1 infected patients with known identity of their transmitted founder virus (TFV) sequence.

**Methods:** Cross-reactive CD8 T-cell responses were assessed using PBMC obtained from 11 acute clade B infected patients. Autologous epitopes were designed based on each patient's TFV and HLA-I sequence. Commonly occurring variants of the autologous epitopes (>10% circulating clade B virus) were selected from the LANL database. CD8 T cells targeting autologous epitopes (N=18) were evaluated for cross-reactivity by stimulating PBMC with their cognate epitope variants in an IFN-γ ELISPOT assay. Cytotoxicity of cross-reactive T cells was determined by co-culturing epitope pulsed target cells with effector CD8 T-cell lines in a 7-AAD flow-based killing assay. In addition, effector/cytokine polyfunctionality, HLA-I binding affinity (predicted from NetMHC), T-cell avidity, epitope/HLA structural analysis and TCR clonotyping assays were performed.

**Results:** Only 5 of the 18 immunogenic autologous epitopes elicited T cells that cross-recognized one or more variants. Cross-reactive CD8 T cells exhibited poor target cell killing. This impaired killing was not driven by polyfunctionality; neither was HLA-I binding affinity, which was lower only for non-immunogenic epitope variants. Structural and TCR clonotype analyses of an autologous/variant epitope pair revealed that amino-acid changes in the TCR contact site and recruitment of dominant T-cell repertoires, respectively, affect epitope variant detection as well as the cytotoxicity of a cross-reactive response.

**Conclusions:** Cross-reactive CD8 T cells appear compromised during primary HIV-1 infection, associated with reduced HLA-I binding affinity and TCR recognition of epitope variants. These results suggest that for an efficacious CTL-based HIV vaccine, a mosaic or polyvalent approach aimed at inducing broad autologous responses would be a more promising vaccine strategy.

### 314 Deleterious Effect of KIR-HLA-Associated Gag Sites on Clinical Outcome in CRF01\_AE

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<sup>1</sup>Univ of Oxford, Oxford, UK; <sup>2</sup>Ministry of PH, Nonthaburi, Thailand; <sup>3</sup>Inst of Trop Med, Nagasaki Univ, Nagasaki, Japan; <sup>4</sup>Lampang Hosp, Lampang, Thailand

**Background:** Class I HLA molecule contributes to the immune control of HIV through antigen presentation to both CTLs and NK cells. Contribution of CTLs to HIV control through antigen presentation of Gag peptides by protective HLA alleles, such as HLA-B\*57, B\*58:01 and B\*27, has been well studied previously. However, reports on the NK cell contribution in HIV control, with the exception of the protective effect of HLA-Bw4 and its receptor, killer immunoglobulin-like receptor (KIR) 3DL1 and 3DS1, are limited. In particular, studies about viral adaptation to NK cell-induced immune pressure remain sparse.

**Methods:** 208 HIV-infected treatment-naïve patients were recruited from Thailand, and the effects of HLA-KIR-associated sites in Gag on clinical outcomes were analyzed. To avoid the overestimation of HLA-associated site detection derived from CTLs, HLA-KIR-associated site detection between subjects with or without KIR in the context of their ligand HLA alleles was introduced (e.g. mutation frequency difference at Gag residue 93E between HLA-C1+KIR2DL2+ and HLA-C1+KIR2DL2-).

**Results:** 26 HLA-KIR-associated sites were identified; 4 of them showed significant viral load differences between KIR+ vs KIR- subjects (5.5 vs 5.0 log copies/ml (p=0.03) at E93X vs 93E restricted by HLA-C1+KIR2DL2+, 4.8 vs 5.4 log copies/ml (p=0.02) at T122X vs 122T by HLA-C1+KIR2DL2+, 4.7 vs 5.3 log copies/ml (p=0.02) at M31X vs 31M by HLA-

C1+KIR2DS2+, and 5.4 vs 4.7 log copies/ml ( $p=0.006$ ) at I104X vs 104I by HLA-Bw4+KIR3DS1+). In longitudinal analysis, higher number of the mutations associated with higher viremia (E93X and I104X) among subjects expressing relevant HLA-KIR combination also had a higher mortality rate ( $p=0.02$ ) (Figure) and remained significant in multivariate analysis (adjusted HR 1.5 [95%CI 1.1-2.1],  $p=0.03$ ), independent of sex (2.5 [1.6-3.9],  $p<0.001$  and age (1.0 [0.9-1.0],  $p=0.3$ ). The number of mutations associated with lower viremia (T122X and M31X) did not have a significant effect on mortality rate ( $p=0.4$ ).

**Conclusions:** In this study we identified several predominant sites of NK cell-induced immune pressure, and viral adaptation to it, especially with deleterious effects on clinical outcome. These findings will impact the existence of the unique anti-HIV innate immune pressure and viral adaptation to it in each endemic area.

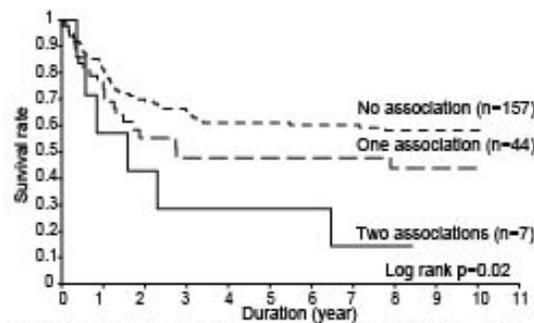


Figure. Kaplan-Meier curves showing the difference in survival rate among the subjects with susceptible HLA-KIR-restricted mutations (E93X or I and I104X) and without it.

### 315 Blockade of KIR2DL1/3 Significantly Improves the Anti-HIV-1 Activity of KIR+ NK Cells

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**Background:** NK-cell function is controlled by inhibitory KIR receptors through their interactions with HLA class I molecules, a process termed "licensing". However, it remains unclear whether the increased functional competence of licensed NK cells directly translates into increased antiviral activity in HIV-1 infection. We determined the antiviral capacity of KIR2DL1<sup>+</sup> and KIR2DL3<sup>+</sup> NK cells in the context of the cognate HLA class I ligand HLA-C.

**Methods:** Primary NK cells derived from HIV-1(-) individuals (N=32) were used to quantify the ability of NK cells to inhibit HIV-1 replication *in vitro*. HIV-1-infected autologous CD4<sup>+</sup> T cells were co-cultured with either sorted KIR2DL1<sup>+</sup>, KIR2DL3<sup>+</sup> or bulk NK cells in the presence or absence of KIR-blocking antibodies for 7 days. Viral RNA was quantified in the supernatant to monitor NK-cell-mediated inhibition of HIV-1 replication. In addition, KIR binding to infected and uninfected CD4<sup>+</sup> T cells was assessed using KIR-Fc constructs.

**Results:** NK cells expressing the self-inhibitory receptors KIR2DL1 and KIR2DL3 displayed a reduced ability to inhibit HIV-1 replication as compared to bulk NK cells ( $p=0.02$ ). Although infection of CD4<sup>+</sup> T cells was associated with significantly reduced KIR binding ( $p=0.002$  for both KIR2DL1-Fc and KIR2DL3-Fc), infected CD4<sup>+</sup> T cells displayed residual KIR binding (KIR2DL1 RFI, median: HIV(-):135 vs. HIV(+):28; KIR2DL3 MFI: HIV(-):15 vs. HIV(+):4; n=24). Blockade of self-inhibitory KIRs was associated with improved antiviral capacity ( $p<0.0001$ ) while blockade with KIR antibodies not matching donor HLA class I molecules did not affect inhibition of HIV-1 replication ( $p=0.8$ , n=14).

**Conclusions:** Our results show that the ability of NK cells to inhibit HIV-1 replication was limited by expression of self-inhibitory KIR2DL molecules, potentially due to residual KIR binding to infected CD4<sup>+</sup> T cells and subsequent inhibition of KIR2DL<sup>+</sup> NK cells. Blocking of inhibitory KIRs on NK cells unleashed the antiviral activity of these cells. These data provide a rationale for therapeutic blocking of specific inhibitory KIRs in HIV-1 cure approaches, as already investigated in clinical trials in the setting of tumor therapies.

### 316 IFN- $\alpha$ Augments NK-Mediated ADCC Lysis via VRC01 or HIV-1 Infected Subject Plasma

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**Background:** Interferon-alpha (IFN-alpha) is a potent clinical immuno-stimulatory agent able to limit viral replication and increase NK activity against HIV-1 infected target cells. We have previously shown that IFN-alpha stimulation augments traditional NK lysis of autologous HIV-1 infected CD4<sup>+</sup> primary T cells through the NK activating receptors NKp46 and NKG2D. Here, we investigated if IFN-alpha stimulation could also independently increase NK-mediated Antibody-Dependent Cellular Cytotoxicity (ADCC) of autologous HIV-1 infected CD4<sup>+</sup> primary T cells in the presence of antibodies against the HIV-1 viral envelope protein, gp120.

**Methods:** ADCC was triggered with a monoclonal antibody to CD4-binding site of gp120 (VRC01) or plasma from HIV-1 infected subjects (whole or IgG depleted). Autologous CD4<sup>+</sup> primary T cells infected *in vitro* with a panel of lab adapted or primary HIV-1 isolates (IIB, NL4-3, TYBE) were used as ADCC targets in chromium release assays. In parallel, uninfected CD4<sup>+</sup> T cells coated with recombinant gp120 were used as ADCC targets in CD107a degranulation assays. Autologous PBMC (Total or NK-depleted) were used as effectors both ADCC assays in the presence or absence of IFN-alpha pre-stimulation (5000 U/ml).

**Results:** The combination of IFN-alpha pre-stimulation and ADCC antibodies against gp120 including VRC01 or HIV-1 infected subject plasma significantly increased NK cell-mediated cytotoxicity against autologous HIV-1 infected CD4<sup>+</sup> primary T cells ( $p<0.05$ ) when compared to either therapy alone. IFN-alpha stimulation also significantly increased ( $p<0.01$ , n=6) CD107a degranulation of NK cells against gp120-coated CD4<sup>+</sup> T cells over uncoated background (median=27.5%, Inter-quartile range=8) compared to unstimulated NK cells (median=18.5%, Inter-quartile range=6).

**Conclusions:** In addition to increasing traditional NK cytotoxicity of HIV-1 infected targets, IFN-alpha also independently augments NK-mediated ADCC lysis of HIV-1 infected autologous CD4<sup>+</sup> primary T cells. Our data supports that an increase in ADCC by IFN-alpha can lead to the greater eradication of HIV-1 infected target cells following IFN-alpha immunotherapy alone, or in combination with the anti-gp120 broadly neutralizing monoclonal antibody, VRC01.

### 317 Immunogenicity and Efficacy of ALVAC-HIV/gp120-Clade C in Alum Regimen in Macaques

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**Background:** The RV144, a phase 3 trial held in Thailand, reduced the risk of HIV infection by 31.2%. In our lab we replicated the results of this study in a SIV<sub>mac251</sub> models using an ALVAC/SIV-gp120/Alum Alhydrogel vaccine (44% vaccine efficacy). A trial has begun in South Africa (SA) to test a similar vaccine using clade C immunogens. We design a study in which we tested the ALVAC HIV/gp120 Clade C Alum in macaques model

**Methods:** 27 all-female Indian rhesus macaques received at weeks (wk) 0, 4, 12 and 24 intramuscularly  $10^{10}$  PFU of both ALVAC vCP172 expressing SIV gag-pol and another and ALVAC vCP1521 expressing ZM96 Clade C HIV gp120 env trans-membrane anchor and the Clade B gag, pro. 200µg of each gp120 (TV1 and 1086) in alum hydroxide (proprietary formulation) were given at weeks 12 and 24. 20 controls received only Alum. 4 wk after last immunization, all the animals were challenged with repeated low doses SHIV1157ipd3N4 via vaginal route 17 times

**Results:** Plasmablasts (PBs) frequency increased 1 wk after the last vaccination ( $p=0.0005$ ) while the  $\alpha\beta7$  subset decreased ( $p=0.0010$ ) and the CXCR3<sup>+</sup> did not change. Serum antibodies titer to 1086 peaked at week 26 ( $>10^6$ ) in vaccinated animals that did not become infected and maintained a titer  $>10^4$  up to 48 wks. Plasma Abs elicited were able to neutralize only tier 1 Clade C viruses. Mucosal antibodies to V2 were higher in the vaginal mucosa than in the rectal. PBs CXCR3<sup>+</sup> correlated with antibodies to V1/V2 antigens. Surprisingly we observed no reduction in the risk of SHIV acquisition in the vaccinated animals, nor protection from high viral load, nor CD4 loss. However animals that were infected later had higher titer of Ab titer in plasma at wk 28 to 1086

**Conclusions:** The lack of vaccine effect was surprising given the efficacy observed in a similar vaccine tested in the SIVmac251 model. However the  $\alpha\beta7$  plasmablasts frequency differed in the two vaccine regimens. This difference might be due to differences in the adjuvants used in the two vaccine regimens that might also influence differences in vaccine efficacy.

### 318 Specific IgG Subclasses Induced in RV305: A Late Boost Vaccination of RV144 Subjects

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**Background:** While RV144 vaccine-induced IgG3 to HIV-1 Env variable loops 1 and 2 (V1V2) correlated with decreased risk of HIV-1 acquisition, the responses were not durable. We studied IgG subclasses (IgG1-IgG4) in HIV-uninfected RV144 volunteers who completed their primary vaccination series 6-8 years earlier and were randomized to receive booster vaccinations with either ALVAC-HIV/AIDSVAX B/E (Gr1), AIDS VAX B/E (Gr2) or ALVAC-HIV (Gr3) at 0 and 6 months (RV305).

**Methods:** ELISA IgG1-IgG4 to HIV gp120 A244gD, and gp70V1V2 scaffold proteins 92TH023 (subtype AE) and CaseA2 (subtype B) were assessed in plasma and cervico-vaginal mucus (CVM) at study entry, 2 weeks post 1<sup>st</sup> and 2<sup>nd</sup> boosts, and 6 and 12 months post 2<sup>nd</sup> boost.

**Results:** At study entry, geometric mean titers (GMT) of IgG to gp120 A244gD in plasma ranged from 50-67 in all groups. We did not detect a boosting effect of any IgG subclasses in plasma and CVM in Gr3. IgG1 GMT increased 2 weeks post 1<sup>st</sup> boost in Gr1 and Gr2, but significantly declined ( $p<0.01$ ) 6 months post 2<sup>nd</sup> boost (Gr1=4850 and 255; Gr2=5279 and 252, respectively). They were significantly higher than those in RV144 at peak immunogenicity and 6 months post last injection (492 and 88,  $p<0.01$ ). IgG3 GMT increased 2 weeks post 1<sup>st</sup> boost (Gr1=168, Gr2=159), but not after the 2<sup>nd</sup> boost, and were significantly lower than RV144 at peak immunogenicity (532,  $p<0.01$ ). While IgG4 was undetected in RV144, it increased to 951 and 611 and to 800 and 588 2 weeks post 1<sup>st</sup> and 2<sup>nd</sup> boosts in Gr1 and Gr2, respectively. IgG1-IgG4 to gp70V1V2 scaffolds was not detected at study entry. IgG1 GMT to AE and B V1V2 were similar in Gr1 and Gr2 after the 1<sup>st</sup> boost (Gr1-Gr2/AE=528-524 and Gr1-Gr2/B=50-43) but lower after the 2<sup>nd</sup> boost (Gr1-Gr2/AE=146-147 and Gr1-Gr2/B=28-24). IgG4 to AE and B V1V2 were detected in Gr1 and Gr2 but not detected in RV144. RV144 IgG3 to V1V2 scaffolds were not boosted in Gr1 and Gr2, and IgG2 remained weak for all antigens tested. In CVM, most responses to gp120 A244gD were IgG1 and IgG4, while to scaffolds were mostly IgG1.

**Conclusions:** Late boosts with AIDS VAX B/E, with or without ALVAC-HIV, induced plasma HIV-specific IgG responses that were predominantly IgG1 and IgG4, were significantly higher than RV144 responses, but did not improve magnitude nor durability of IgG3, and were similar to subtypes in CVM. These studies highlight the need for new strategies to durably boost HIV-specific, protective antibody responses observed in RV144.

### 319 Vaccine Targeting Protease Cleavage Sites Protects Cynomolgus Monkeys Against SIV

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**Background:** With the sobering results of the STEP and HVTN 505 HIV vaccine clinical trials, novel approaches to HIV vaccine development must be explored. The HIV protease is a 99-amino acid aspartic enzyme that mediates the cleavage of Gag, Gag-Pol and Nef precursor polyproteins. This process is a highly specific, temporally regulated and essential for the production of infectious viral particles. A total of 12 proteolytic reactions are required to generate an infectious virion, and a single impaired cleavage reaction can render the virus noninfectious. Thus, HIV vaccine-elicited responses targeting the protease cleavage sites (PCS) could be highly efficacious.

**Methods:** We assess vaccine immunogenicity elicited against PCS immunogens using a modified *Vesicular stomatitis virus* (VSV) vector combined with a nanodelivery system. We then evaluate protective efficacy to disrupt SIV acquisition and disease progression using a Cynomolgus macaque (*Macaca fascicularis*, Philippines) and SIV<sub>MAC239</sub> intrarectal challenge model. To examine whether the immune driven viral mutations surrounding the PCS were detrimental to the virus, we amplified and sequenced the plasma viruses by 454-pyrosequencing and correlated the amino acid mutations surrounding PCS with alterations in viral load and CD4 count.

**Results:** PCS peptides expressed by rVSVs and packaged in nanoparticles are able to generate both antibody and T cell responses in macaques. The ability of macaques to withstand high dose SIV<sub>MAC239</sub> challenge was significantly correlated with the antibody and antibody/T cell responses to the number of PCS peptides ( $p=0.0005$ ,  $R=0.8005$ ). This combination imparted resistance to all but the higher SIV doses that were required to infect vaccinees ( $p=0.01$ ). The vaccine group maintains significantly higher CD4 counts ( $p=0.0002$ ). Amino acid mutations (SN or NS) surrounding PCS correlated significantly with reduced viral RNA levels ( $p<0.0001$ ).

**Conclusions:** We show here in a Cynomolgus macaque/SIV model that a candidate HIV vaccine focusing immunologic responses to the regions surrounding the SIV protease cleavage sites can force viral mutation resulting in impaired SIV fitness. Focused immune response to the PCS region enables the macaques to withstand multiple high dose pathogenic SIV<sub>MAC239</sub> intrarectal challenges. Virus recovered from vaccinees harbored mutations surrounding the protease cleavage region that correlated significantly with reduced viral load, and the maintenance of CD4+ T cells *in vivo*.

### 320 Shaping CTL Immunodominance With Conserved HIV Vaccines After Early Treatment (BCN01)

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**Background:** Therapeutic T-cell vaccines targeting the most conserved regions of the HIV-1 proteome have the potential to enhance host immune control and facilitate clearance of the latent reservoir.

**Methods:** BCN01 (NCT01712425) is a phase I, multicenter trial to evaluate the safety, immunogenicity and impact on the latent reservoir of a combined ChAdV63-MVA.HIVcons vaccine in early-treated individuals ( $<6$  months from HIV-1 infection,  $n=24$ ) who initiated TDF/FTC/RAL 1 wk after diagnosis. Individuals received ChAdV63.HIVcons ( $5 \times 10^{10}$  vp, im) and MVA.HIVcons ( $2 \times 10^9$  pfu, im) 8 or 24 wk after (Short vs Long regimen) and were followed for 6 months. Immunogenicity to the HIVcons vaccine insert and the rest of the HIV-1 proteome was assessed by IFNg ELISPOT in cryopreserved PBMC. 24 unvaccinated controls were included to compare viral reservoir decay during 1st year of early-treatment initiation. Proviral DNA was quantified in purified CD4+ T-cells by droplet digital PCR. Single-copy assay was performed to investigate potential viral reactivation during vaccinations.

**Results:** 22 individuals (92%) induced HIVcons de-novo Tcell responses during vaccination schedule (not detectable before cART initiation). Responses were increased in 50% participants after ChAdV63 prime and in 100% of participants after MVA booster vaccination (median of  $938$  SFC/ $10^6$  PBMC, range 73-6,805 at peak,  $p=0.0001$ , Wilcoxon t-test compared to pre-vaccination). No significant expansion of T-cells targeting HIV-1 regions outside the vaccine insert was noted, reflective of an effective shift of CTL immunity towards conserved regions (48% of total HIV-1 T-cells being HIVcons-specific). No significant differences in peak or longevity of induced T-cells were observed between Short and

Long vaccination regimens. Levels and decay of proviral DNA after cART were not associated with vaccine-induced immunogenicity nor differed from non-vaccinated individuals. Viral reactivation was not observed during vaccinations.

**Conclusions:** Heterologous vaccination with ChAdV63 and MVA.HIVconsv was a safe strategy to induce new and shift pre-existing immune response towards conserved regions of HIV-1 in a cohort of early-treated individuals. Reservoir decay during first year of early-treatment was not further impacted by vaccinations. This is the first therapeutic vaccine trial able to demonstrate a refocusing of the CTL immunodominance pattern towards conserved regions of HIV-1 and may provide the base for effective kick and kill strategies.

### 321 DNA/rTV/Protein Vaccination Protects Against Acquisition of SHIV162P3 Challenges

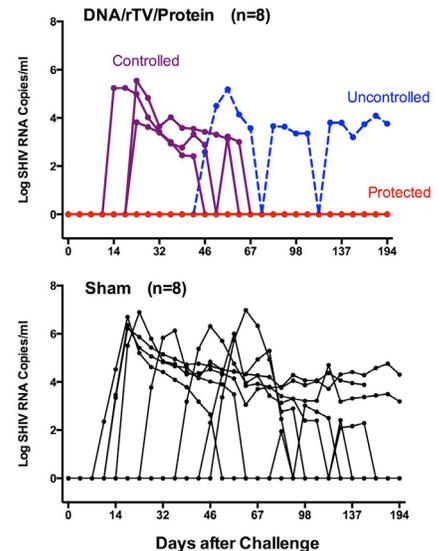
**Xuan He;** Zhou Zhang; Ying Liu; Yanling Hao; Li Ren; Kunxue Hong; Yiming Shao  
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**Background:** We have previously reported that replication-competent Tiantan vaccinia (rTV) is a promising vaccine vector, and rTV-based HIV-1 vaccine with DNA primer provided protection from SHIV challenge. To further improve vaccine-induced immunity and protection, we combined DNA vaccine with boosts of both rTV and proteins in the recent study.

**Methods:** Eight Chinese rhesus macaques were intramuscularly primed with DNA vectors expressing HIV Env/Gag/Pol/Nef and SIV Gag, followed by intradermal boost with rTV vectors expressing HIV gp145 and SIV Gag, and final intramuscular boost with proteins HIV gp140 and SIV Gag. Another eight macaques received sham vaccines. Animals were then challenged twelve times by intrarectal route with low dose of heterologous, neutralization-resistant virus SHIV162P3.

**Results:** DNA/rTV/protein vaccination induced high-titer HIV Env-specific antibodies, and modest cytotoxic CD8 T-cell responses. In the vaccinated group, half macaques were completely protected from SHIV acquisition, whereas three macaques exhibited transient acute viremia and later became elite controllers with undetectable viral loads. Chronic viremia was observed in one vaccinated macaque. In contrast, all 8 sham controls were systemically infected (setpoint viral loads: 2.00-4.44 log SHIV RNA copies/ml), with peak plasma viremia levels ranging from 6.00 to 6.98 log SHIV RNA copies/ml, which were significantly higher than that in the vaccinated group that became infected, with peak viremia ranging from 3.82 to 5.54 log SHIV RNA copies/ml ( $p=0.004$ ). No significant differences were observed among all immunized animals in terms of vaccine-induced neutralizing antibodies and cytotoxic CD8 T-cell responses, though the protected macaques and elite controllers showed modestly higher HIV Env-specific humoral response than viremia uncontrolled one. Dominantly higher titer of vaccine-induced binding antibodies to variable regions 1 and 2 (V1V2) of HIV-1 Env was observed in the protected animals (V1V2 titer: 4.40-5.01 log) and elite controllers (V1V2 titer: 4.10-5.01 log) compared with viremia uncontrolled macaque (V1V2 titer: 3.8 log).

**Conclusions:** The protective efficacy of DNA/rTV/protein vaccines against acquisition of neutralization-resistant SHIV infection in rhesus macaques in the study has important implications for HIV-1 vaccine development. Moreover, our immunologic correlates analyses suggest that Env-specific V1V2 antibodies may be critical for blocking acquisition of SHIV challenges.



### 322 Poly-ICLC, a TLR Agonist, Is Safe and Tolerable in HIV-Infected Individuals

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**Background:** Safe and effective adjuvants will likely be a key component of successful therapeutic vaccines for HIV. Toll-like receptor (TLR) agonists are promising investigational adjuvants that may enhance vaccine immunogenicity, and based on preclinical data may stimulate viral expression from latent HIV reservoirs. Poly-ICLC is a synthetic dsRNA complex that is a potent agonist of TLR3, as well as cytoplasmic sensors including MDA5.

**Methods:** We have undertaken a phase I/II, randomized, placebo-controlled, double-blinded, trial in cART-suppressed subjects with HIV infection (NCT02071095). Participants were randomized 3:1 to receive 2 consecutive daily doses of Poly-ICLC (1.4mg SQ) versus placebo. The primary outcome is safety and tolerability, in addition to multiple secondary immune and virologic endpoints. Transcriptional analysis of subjects' PBMCs was performed using NanoString Technologies (nCounter<sup>®</sup> gene expression panel, human inflammation kit).

**Results:** Fifteen participants have received both injections and have completed  $\geq 4$  weeks of follow up. Enrollment has closed, though the study remains blinded. Thus far, the injections have been safe and well-tolerated with only Grade 1/2 adverse events (AEs) attributed to the study agent, with the exception of one Grade 3 transient neutropenia without clinical sequelae. Injection site reactions (ISR) have been the most frequent AE, all of which were Grade 1. Pain has been the most common ISR, occurring at 66% of injection sites. Erythema was present at 16% of injection sites. Fever has also been common (33%), lasting 24-48 hours. Other frequently reported AEs include chills, myalgias, fatigue, malaise, and headache. Plasma viral control has been maintained in these cART-treated individuals. Transcriptional analyses have revealed that in subjects with injection site reactions, multiple innate immune pathways are significantly upregulated in subjects PBMCs, including strong interferon (IFN) signaling. These responses generally peaked  $\sim 24$  hours after injection and returned to baseline by day 8.

**Conclusions:** Poly-ICLC injections appear safe and tolerable, are associated with mild injection site reactions and transiently stimulate innate immune responses during treated HIV infection, indicating promise as an adjuvant for HIV therapeutic vaccines. Additional assays currently underway will better define the immunologic and virologic effects of TLR stimulation during HIV infection.

### 323 TLR-9 Signaling Rescues Defects in B-Cell Response in Needle-Free Immunization

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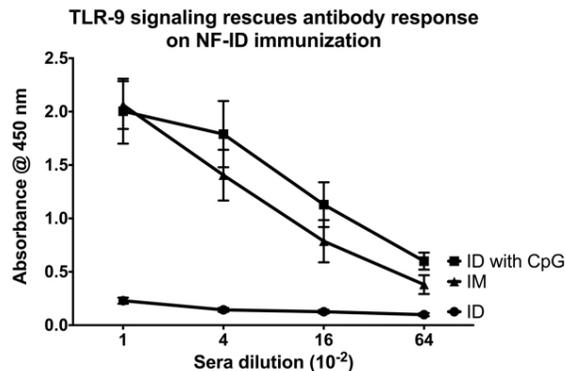
**Background:** The intradermal immunization (ID), unlike the intramuscular immunization (IM), can target diverse subsets of dendritic cells (DC) in the skin offering a great advantage especially with the DNA vaccines. We, therefore, compared in mice the ID immunization using a needle-free (NF) device with the traditional IM administration. The NF administration is also safe as it is a non-invasive technique.

**Methods:** BALB/c and C57BL/6 mice were immunized through the IM or ID routes using comparable quantities of plasmid vectors encoding HIV-1 Gag, Tat, Firefly or Gaussia luciferase under the CMV promoter. The T- and B-cell responses were measured from the splenocytes/PBMC and sera, respectively. The frequencies of the antigen-specific and activated T-cells expressing IFN- $\gamma$  and TNF- $\alpha$ , manifesting the proliferation or degranulation phenotypes were determined using the multi-parametric flow analysis. The in vivo antigen expression was evaluated using luciferase assay and confocal microscopy.

**Results:** The IM and ID immunizations elicited comparable T-cell responses with  $0.55 \pm 0.12$  and  $0.52 \pm 0.12\%$  CD8 T-cells manifesting the p24-specific IFN- $\gamma$ +TNF- $\alpha$  double positive phenotype, respectively. At a dose as low as 2  $\mu$ g, the ID route was superior to the IM route eliciting a 3 fold higher magnitude of the double positive cells. Surprisingly, although the IgM antibody titres were comparable between the routes of immunization, the IgG response was significantly diminished in the ID immunization. The mean IgG

titre was 6,500 on the IM immunization whereas 100 or lesser in the ID group. A rapid elimination of the expressed antigens at the site of ID immunization underlied the defect in isotype switching as determined by in vivo luciferase analysis and fluorescent microscopy. The antibody response was restored by additional booster immunizations or the co-administration of the TLR-9 inducing CpG ODN 2395 among many TLR agonists examined.

**Conclusions:** Despite the advantages of DC subset targeting and safety, the efficacy of ID immunization may be limited by the rapid removal of the expressed antigen from the skin. Unlike the previous reports, we, for the first time identified the antigen instability underlying the restricted antibody immune response in NF immunization although the T-cell responses were not affected. We additionally suggest ways to restore the compromised B-cell response. Our work has important implication for the optimization of the emerging NF technology.



### 324 Distinct B-Cell Gene Signatures Define Ability to Respond to H1N1 Vaccine in HIV+ Children

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**Background:** In HIV+ children, the immune response following seasonal or routine vaccinations is often inadequate, thus pointing to the need for predictive biomarkers of response. In this study we hypothesized that gene expression (gExp) of sorted B cell subsets may predict the ability of ART treated, virally suppressed HIV+ children to develop protective immunity to H1N1 following influenza vaccination (TIV).

**Methods:** Blood was collected before (T0) and 21 days after (T1) TIV. HIV+ patients (HIV, n=23) were classified as TIV-responders (R, n=11) based on Ab response to H1N1 (T1/T0 = >4 fold increase), and H1N1-memory B cell ELISpot (>80 spots/10<sup>6</sup> cells at T1) and TIV non-responder (NR, n=12). Healthy Controls (HC, n=10) were all responders. NR and R were similar in terms of viral load, CD4 (# and %), ART regimen and duration. Pre-vaccination samples, sorted by flowcytometry in aliquots of 500 cells each (PBMCs; total B; and B-cell subsets: Naïve, **Na**: CD19+CD10-CD27-IgD+; Double Negative, **DN**: CD19+CD10-CD27-IgD-; Resting Memory, **Rem**: CD19+CD10-CD27+IgD-CD21+; Activated Memory, **AM**: CD19+CD10-CD27+IgD-CD21-) were analyzed by multiplexed qPCR for 96 genes (Fluidigm, Biomark).

**Results:** Analysis of variance (ANOVA) among the 4 B cell subsets (NA, DN, Rem, AM) showed higher number of Differentially Expressed Genes (DEGs) (p<0.05) in HIV compared to HC. Collectively 258 DEGs in HIV versus 122 DEGs in HC were found when pairs of B cell subsets were compared. This could be in part due to the largest differences between AM and Rem and the rest of B cell subsets in patients suffering from chronic HIV stimulation. In line with this, a distinct signature of AM was found between HIV and HC (28 DEGs). Although this result was confirmed when R and NR were compared to HC, gExp of AM wasn't able to discriminate between R and NR (3 DEGs). Conversely, ANOVA of Rem showed 25 DEGs between R and NR. Higher expression of genes involved in adaptive immune response (*APRIL*, *BTK*), leukocyte activation and BCR signaling (*MTOR*, *FYN*, *CD86*), JAK/STAT signaling (*STAT4*, *IL6R*, *IFNAR*) and response to IFN (*IFNAR2*, *MX1*) was found in R when compared to NR.

**Conclusions:** Collectively, these results show that Rem, crucial for a potent and specific adaptive response, exhibits a distinct gene signature in HIV who are able to mount a response to TIV not present in NR. Future studies based on sorted subsets are needed to define a multiparameter gExp score able to predict the ability of immune compromised patients to respond to the vaccinations.

### 325 NextGen Sequencing Defines Response to H1N1 Vaccination in HIV-Infected Individuals

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**Background:** H1N1 infection is a major co-morbidity among HIV-infected individuals. While H1N1 vaccines offer protection to healthy individuals, responses in HIV-infected individuals are variable. We hypothesized that HIV infection will result in decreased B-cell clonal expansion following H1N1 immunization.

**Methods:** NextGen sequences for immunoglobulin G heavy chain variable region (IgGHV) were generated [median 10,496 (quartile range: 8,600 to 14,405) quality sequences] from mRNA of peripheral blood mononuclear cells and used to quantify IgH biodiversity before and after two doses of H1N1 vaccine administered 4 weeks apart to ten HIV-infected children and adolescents enrolled in IMPAACT P1088. Five individuals demonstrated a ≥4-fold rise from baseline in hemagglutination inhibition titers after the 2<sup>nd</sup> dose, while 5 age-matched individuals failed to respond. Sequences from study participants and reference H1N1 neutralizing antibodies (NAb) from GenBank were clustered at 10% genetic distance to identify putative H1N1-specific sequences. Consensus sequences of clusters were queried to IMGT for variable (V) and joining (J) alleles, and length and somatic hypermutation (SHM) in complementary determining regions (CDRs). T test compared two groups with p < 0.05 as significant.

**Results:** Following vaccination, IgGHV biodiversity was reduced by 12% in responders, but not in the nonresponders. Base line sequences from each group clustered with each of 26 unique reference H1N1 NABs with the same V and J alleles and number of SHM in CDR3 as correspondent H1N1 NABs. Post vaccination, increases in sequence numbers within multiple clusters was observed in all responders, but in only 2 of 5 nonresponders (p = 0.03).

**Conclusions:** HIV-infected children and adolescents harbor H1N1-specific B-cells that fail to expand in vaccine nonresponders, indicating a failure in oligoclonal expansion among memory B-cells. Reduction in biodiversity and increase in number of sequences clustering with reference H1N1 NAB sequences provide novel and sensitive biomarkers to identify responders to vaccines in general.

**326 CD4/CD8 Ratio and K/T Ratio Predict Yellow Fever Vaccine Response in HIV+ Patients**

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**Background:** Weaker immune responses to Yellow Fever vaccine (YFV) in HIV-infected persons may be influenced by chronic immune activation. Indoleamine 2,3-dioxygenase-1 (IDO) catabolizes tryptophan (T) to kynurenine (K) and other metabolites that may contribute to proliferative lymphocyte defects, microbial translocation and immune activation in treated HIV infection. Low CD4/CD8 ratio is strongly associated with IDO activity and may also be a marker of adaptive immune dysfunction in this population.

**Methods:** We prospectively assessed YFV viremia and YF-specific antibodies (NAb) in HIV-infected (CD4>350) and -uninfected adults on days 0, 3, 5, 7, 14, 28, 56, 84, and 365 after YFV. We assessed CD4/CD8 ratio, GBV-c viremia and IDO activity (as measured by plasma K/T ratio) as potential predictors of NAb in the first year after YFV with linear mixed models.

**Results:** Among 12 HIV-infected and 45 -uninfected participants, median age was 33 and 43 years old, and most were men. Groups had similar percentages of previous YFV. Compared to HIV-uninfected participants, those with HIV had lower median CD4 counts (722 vs 941 cells/mm<sup>3</sup>, P=0.003), lower CD4/CD8 ratios (0.7 vs. 1.6, P<0.0001), a trend toward higher K/T ratio (36 vs. 31 nM/μM, p=0.06), and a higher prevalence of detectable GBV-C viremia (66.7% vs. 26.7%, p=0.016). HIV status was not associated with the occurrence or levels of YFV viremia, or with the occurrence of adverse events after YFV.

HIV status was not associated with difference in NAb titers in the visits up to day 84, but predicted lower NAb titers at 1 year after YFV (fold-change 0.32, 95% CI 0.13 to 0.83, p=0.021), independent of sex, age and previous YFV. Among HIV-infected participants, higher CD4/CD8 ratio predicted higher NAb titers; for each 10% increase in CD4/CD8 ratio, post-baseline NAb titers were 14% higher (95% CI 1 to 26, p<0.03). Similarly, higher K/T ratio predicted lower NAb titers; for each 10% increase in K/T ratio, post-baseline antibody titers were 21% lower (95% CI 5 to 37% lower, p=0.02).

GBV-c viremia was not associated with difference in NAb titers.

**Conclusions:** Despite similar responses in the initial 3 months, NAb to YFV were less durable among HIV-infected persons. CD4<sup>+</sup>/CD8<sup>+</sup> ratio and K/T ratio at vaccination predict NAb among HIV-infected participants, suggesting immune activation and/or dysfunction impairs YFV response in this population.

**327 Steady State and Post-Vaccination TFH Dynamics in HIV Patients Treated With cART**

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**Background:** HIV infection dramatically changes the lymph node (LN) tissue architecture and the dynamics of tissue resident immune cells, potentially affecting the ability of HIV infected individuals to respond to subsequent antigenic challenge. The dynamics of circulating and LN-resident CD4 T cell and B cell populations in cART HIV patients after trivalent seasonal influenza immunization were assessed.

**Methods:** LN-derived cells and PBMCs from healthy controls and virologically suppressed HIV+ patients were obtained pre- and post- vaccination and analyzed using multiparametric flow cytometry assays and multiplexed confocal imaging. Fluidigm gene expression analysis was performed to assess the effect of vaccination on the transcriptional profile of follicular CD4 T cells. Actively transcribed virus in sorted LN-cells was analysed by PCR assays.

**Results:** Pre-vaccination frequencies of follicular CD4 T helper cell (T<sub>FH</sub>) between healthy donors and cART HIV+ patients at steady state were collectively similar but were highest in those with the greatest increase in antibody titers post-vaccination. This profile was also reflected at tissue level when relevant populations were investigated with imaging assays. There was a trend for lower T<sub>FH</sub> frequencies post-vaccination in HIV+ compared to HIV- individuals while B cell frequencies remained the same post-vaccination both in LN and PBMC. The transcriptional profile of T<sub>FH</sub> revealed differentially expressed genes (DEGs) between HIV- and cART treated donors. Furthermore, our transcriptional analysis revealed that vaccination had a greater impact on T<sub>FH</sub> compared to non-T<sub>FH</sub> CD4 T cells. In line with the lower frequencies of T<sub>FH</sub>, actively transcribed virus in the T<sub>FH</sub> compartment was reduced after vaccination.

**Conclusions:** Vaccination predominantly alters the frequency and phenotype of follicular CD4 T-cells in the draining LN of HIV infected patients on cART in a manner consistent with the development of IgG antibody titers. The underlying mechanism of follicular CD4 T cell mobilization warrants further investigation as it could bear implications for the rational design of HIV vaccines.

**328 WITHDRAWN****329 Impaired Responses to Vaccine in HCV Infection Are Related to Baseline Inflammation**

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**Background:** Chronic HCV and HIV infection are associated with impaired responses to HAV/HBV vaccines. HAV and HBV infections augment hepatic damage in HCV-infected individuals and HIV disease progression is associated with HBV and HCV coinfection. Understanding why these populations fail to respond effectively to neo-antigen vaccines is necessary to guide strategies to improve these prophylactic vaccine responses.

**Methods:** This study was designed to examine HAV/HBV vaccine responsiveness in untreated HCV (n=15), untreated HIV (n=24), and uninfected (n=10) participants. Baseline levels of soluble factors of inflammation were measured by ELISA and HAV and HBV antibody titers were measured at wk1, 3, 8, and 24 after vaccination with the HAV/HBV vaccine, Twinrix.

**Results:** The proportion of HIV-infected participants who failed to respond to vaccine with an antibody response within 8 weeks post-vaccination to HAV (20%) and HBV (62%) was greater than the proportion of HCV-infected participants (HAV 0%, HBV 21%) or uninfected controls (HAV 0%, HBV 20%). We found that baseline plasma levels of IP10 and IL-6 were elevated in HCV (p=<0.001) and HIV-infected participants compared to uninfected controls, and in HCV-infected participants plasma levels of IP10 inversely correlated with week 8 antibody responses to HAV (r=-0.762 p=0.037). Plasma levels of IL-6 inversely correlated with week 8 HBV antibody responses (r=-0.728 p=0.004). Despite elevated plasma levels of IP10 and IL-6 in HIV-infected participants, levels did not correlate with vaccine antibody response, likely because of the low proportion of participants responding to the vaccine. In HCV-infected participants plasma levels of soluble CD163 (sCD163) were elevated at baseline compared to plasma levels in uninfected controls and untreated HIV-infected participants. Soluble CD163 correlated inversely with week 8 HBV antibody levels in HCV-infected participants (r=-0.643 p=0.015).

**Conclusions:** Elevated baseline levels of soluble factors of inflammation are predictive of poor vaccine response to HAV/HBV neo-antigen vaccine during chronic HCV infection.

**330 Persistence of HIV-Infected Alveolar Macrophages After Suppressive ART**

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**Background:** Although HIV-1 infection in macrophages is documented among viremic individuals, there is no conclusive evidence for long-term HIV persistence in these cells during suppressive ART. Here, we analyzed highly-purified alveolar macrophages (AM) for evidence of HIV-1 infection and transcriptional activity *in vivo*.

**Methods:** Two participants with HIV-1 viremia (plasma HIV-1 RNA >1,000 copies/ml) and 7 consecutive participants on suppressive ART were enrolled from the Pitt Lung HIV cohort and underwent bronchoscopy. Bronchoalveolar lavage (BAL) fluid was separated into cellular and cell-free fractions by centrifugation. AM were purified from BAL cells by plastic adherence with extensive washing. Plasma and peripheral blood mononuclear cells (PBMC) were also collected. HIV-1 RNA in the plasma and BAL supernatant were tested by qPCR targeting a highly-conserved region in HIV-1 *pol*. Cell-associated (CA) HIV-1 DNA and RNA in AM, total BAL cells, and PBMCs were assayed by qPCR targeting the same region of *pol*. To detect contaminating or ingested CD4+ T cells, T-cell receptor (TCR) RNA was tested by PCR.

**Results:** Most subjects (89%) were male. The median age was 50 yrs and CD4 count was 735. In viremic individuals, CA HIV-1 DNA and RNA were detected in AM (median 1.5 and 2.5 log<sub>10</sub> copies/10<sup>6</sup> cells, respectively) and in PBMC (median 2.4 and 1.5, respectively). HIV-1 RNA in BAL supernatant was low compared to plasma (median 0.9 and 4.4 log<sub>10</sub> copies/ml). Among subjects on ART, the median duration of viremia suppression was 3.6 yrs (range: 2.4, 12.3; n=7). CA HIV-1 DNA was detected in AM from 6 of 7 participants (median 1.7 log<sub>10</sub> copies/10<sup>6</sup> cells) and CA HIV-1 RNA in AM from 4 of 6 with detectable HIV-1 DNA (median 1.3). AM extracts were negative for TCR in 6 of 7 participants with samples available. By comparison, CA HIV-1 DNA was detected in PBMC from 6 of 6 individuals tested (median 2.5 log<sub>10</sub> copies/10<sup>6</sup> cells) and CA HIV-1 RNA in 2 of 6 tested (median 1.9). HIV-1 RNA in BAL supernatant was not detectable (< 0.3 copies/ml) despite low-level plasma HIV-1 RNA in 6 of 7 virally-suppressed participants (median 7.7 copies/ml).

**Conclusions:** These results establish the persistence of HIV-infected AM in individuals on suppressive ART, which cannot be explained by contaminating or ingested CD4+T-cells. Studies are in progress to more fully characterize the proviruses in alveolar macrophages and their inducibility.

### 331 Evidence of HIV Evolution in Lymphatic and Cancer Tissues in ART+ Patients

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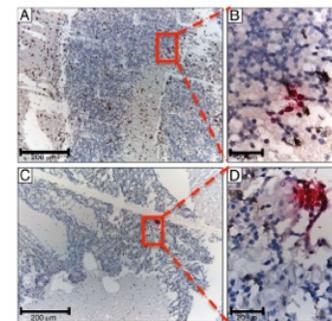
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**Background:** Anti-retroviral therapy (ART) is effective in reducing plasma HIV load to undetectable levels and restoring partial immunity. However, viral populations rapidly rebound once therapy is removed. Furthermore, HIV-associated co-morbidities, particularly certain cancers, remain a significant co-morbidity even with effective ART. We hypothesized that HIV-infected macrophages in tumors may both protect HIV from ART and contribute to metastasis.

**Methods:** The AIDS and Cancer Specimen Resource (ACSR) provided 26 post mortem tissues from three HIV+/ART+ patients with no detectable viral load. Two died from metastatic lymphoma (Pt02, Pt04), and one from lung cancer (Pt05). Tissues were assessed for HIV using digital drop PCR. Single genome amplification was used to generate *env* and *nef* sequences. We inferred maximum-likelihood phylogenies and performed multiple statistical tests to investigate viral evolutionary patterns and compartmentalization. We used an HIV signal detection technique (RNAScope) combined with histological staining to visualize the cellular location of HIV RNA in tissues from Pt02.

**Results:** ddPCR was positive for all tissues. HIV *env-nef* DNA sequences were generated from 8/11, 3/6 and 9/9 tissues from each of the three patients, respectively. HIV RNA was also isolated from three tissues in Pt02 (lymph node tumor, cerebellum and aorta) and two tissues from Pt05 (lymph node and spleen). Maximum-likelihood phylogenies and statistical testing showed little evidence of viral compartmentalization among tissues for RNA and DNA. Considering that the patients had been on ART, some clades showed surprisingly high diversity and evidence of on-going evolution, while others were consistent with a pattern of clonal expansion. RNAScope for Pt02 showed that RNA expression of HIV *gag-pol* was clustered and co-localized with CD163+/CD68+ macrophages in tumor lymph node and the cerebellum, with lymphoma involvement due to meningeal metastasis. Infiltrating macrophages surrounded HIV infected cells (Figure).

**Conclusions:** Our results suggest that lymphatic and cancer tissues may offer a privileged environment for persistent HIV replication within macrophages during ART and may promote tumor growth and metastasis. Two distinct patterns of tissue virus evolution suggest that different modes of replication/spread underlie persistence: ART-resistant HIV infected macrophages with persistent evolution/spread and clonal expansion of HIV infected cell populations.



Cerebellum with HIV RNA amplification (fuchsia) and CD68/CD163 staining of macrophages (brown). Scale bars: 200 μm (A, C) and 20 μm (B, D)

### 332 Role of Transitional-Memory T Cells in Productive HIV Reservoir in ULTRASTOP Patients

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**Background:** The Ultrastop clinical study aims at evaluating frequency and parameters of HIV-remission after treatment interruption (TI) in early-chronic treated patients with an ultra-low HIV reservoir. The success of drug free remission was 10%. Half patients carried protective HLA-B\*27 or B\*57 and CD8-T-cell responses were weak at baseline in all patients including the post treatment controller (PTC). Here, we analysed the reservoir dynamic and HIV-reactivation upon TI in the ULTRASTOP Cohort.

**Methods:** Prospective study of treatment-interruption (TI) followed by treatment-resumption (RxR) in case of immuno-virological failure (pVL>400 cp/mL or CD4<400/mm<sup>3</sup>) in ten early-chronic treated patients enrolled with ultralow HIV-DNA (<66 cp/10<sup>6</sup> PBMC). Monitoring of immune-virological parameters was performed from D0 until W48 off-ART and at W4/W12/W24 after RxR. Dynamics of HIV reservoirs were quantified in PBMCs and sorted resting naïve, central-, transitional- and effector-memory CD4 T cell subsets at D0, RxR and W24 post RxR using ultrasensitive RT-PCR. Reactivation of HIV-1 from CD4 T-cell was performed by anti CD3/CD28, IL2 and IL7 stimulation

**Results:** One HLA-B\*27+ CCR5<sup>wt/wt</sup> patient controlled viremia up to W56 off-ART and was defined as PTC. 9/10 patients, 4 being HLA-B\*27 or B\*57+, had prompt plasma viral rebound (W2-12) accompanied by an increase in cell-associated HIV-DNA which returned to baseline levels at W24 post RxR. At baseline, HIV-DNA distribution did not differ between resting naïve and memory subsets in all patients, and was below the threshold in the PTC's TTM and TEM compartments. HIV-1 was reactivated in-vitro upon total CD4 T-cells stimulation in only 3 patients that displayed the highest HIV-DNA levels at baseline, but not in the PTC. At RxR increase in HIV-DNA levels involved the TCM and TTM subsets with a preferential contribution of TTM to the CD4 compartment. HIV became inducible in-vitro in total CD4 T-cells from all non-controllers as well as in PTC's CD4 T cells. RxR levels of HIV-RNA reactivation correlated with both peak of pVL (p 0.006) and HIV-DNA levels in PBMC (p 0.03) and in TTM (p 0.01).

**Conclusions:** In a highly selected population of early-chronic treated patients with ultra-low HIV reservoir, a success rate of 10% after TI was observed. The relatively short-lived transitional-memory T cells compartment appears to play a key role in contribution to virus production both in vitro and in vivo. these findings open new perspectives in targeting the productive HIV reservoir

### 333 Drug Activity in PrEP Breakthroughs Has a Transient Effect on SHIV DNA Reservoirs

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**Background:** Infection of macaques with simian HIV (SHIV) during concurrent pre-exposure prophylaxis (PrEP) with emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF) is associated with reduced acute plasma viremias and limited virus diversity, thus providing a unique model to assess long-term effects of acute antiretroviral treatment. Here, we investigated the effect of PrEP on acute SHIV DNA dynamics in peripheral blood mononuclear cells (PBMCs), and on the size of the persistent virus reservoir in lymphoid tissues.

**Methods:** Cell-associated SHIV DNA levels were measured in PBMCs during acute infection in 8 macaques infected during concurrent PrEP with FTC/TDF combination or single-agent tenofovir alafenamide fumarate (TAF). Macaques continued treatment with 1-2 weekly drug doses to model suboptimal drug exposure during undiagnosed HIV infection in humans. Peak SHIV DNA and area under the curve values (AUC) over 5 or 20 weeks, as well as RNA levels, were compared to the values seen in untreated SHIV infections ( $n = 10$ ). SHIV DNA levels were also measured in lymphoid tissues collected from FTC/TDF or maraviroc PrEP breakthroughs after 1 year of infection.

**Results:** PrEP breakthrough infections had reduced plasma RNA viremia relative to untreated infections both at peak and during the first 20 weeks of infection ( $p < 0.005$ ). SHIV DNA levels in PBMCs were also reduced in PrEP breakthrough infections both at peak and at week 5 ( $p = 0.022$  and  $p = 0.043$ , respectively), but not after 20 weeks of infection. At 1 year, SHIV DNA reservoirs in lymphoid tissues were similar in size among macaques that received PrEP with FTC/TDF (median = 464 SHIV DNA copies/ $10^6$  cells; range, 40-5,346), PrEP with maraviroc (median = 1088 copies/ $10^6$  cells; range, 554-10,090), or placebo (median = 952 copies/ $10^6$  cells; range, undetectable-24,668) ( $p > 0.05$ ).

**Conclusions:** Antiviral drug activity due to PrEP limits acute SHIV replication but has only a transient effect on cell-associated DNA levels in PBMCs and lymphoid tissues. Our model suggests that suboptimal drug exposure in persons that are taking PrEP and become infected with HIV may not be sufficient to reduce the pool of HIV-infected cells, and that treatment intensification may be needed to sustain the virologic benefit from the PrEP regimen.

### 334 Germinal Centre T and B Cells in Lymph Node Fine-Needle Biopsies During HIV Infection

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**Background:** Measurement of HIV-1 reservoirs is usually studied in peripheral blood (PB), but 98% of CD4 T cells are in lymphoid tissues and other organs. Histology has shown characteristic hyperplastic germinal centres (GC) in lymph nodes, recently associated with increases in the T follicular helper cell (Tfh) subset of CD4 T cells, and there is now recognition that GC and Tfh are an important reservoir of HIV-1. We investigated whether ultrasound-guided fine needle biopsies (FNB) from peripheral lymph nodes (LN) provide a minimally invasive means of longitudinally accessing HIV-1 reservoirs in GC.

**Methods:** FNB of inguinal LN were performed on 10 healthy adult controls (HC), 11 HIV+ ART-naïve and 10 HIV+ on ART subjects, without adverse events. FNB were analyzed for CD3+CD4+CD45RA<sup>neg</sup>PD-1<sup>hi</sup>CXCR5+ICOS+CD127<sup>dim</sup>Tfh cells and CD19+CD20<sup>high</sup>CD38<sup>high</sup>gDnegKi-67+Bcl-6+ GC B cells, using TruCount tubes on a 4-laser LSR II. CD4 T cells, from FNB and PB, respectively, were purified by cell sorting, from 19 patients, and HIV DNA and cell-associated (CA) HIV RNA were quantified by real-time PCR.

**Results:** Overall, the median number of CD4 T cells obtained by FNB from HC was 887,000 (IQR: 206,000-1,187,000), and from all 21 HIV+ subjects (519,000 (IQR: 228,000-1,340,000)). However, the median Tfh cell number in FNB from ART-naïve HIV+ subjects was 46,300, significantly higher than in HC (5,600;  $p = 0.04$ ); HIV+ on ART subjects had a median of 15,800 Tfh cells. The median GC B cell number from ART-naïve HIV+ was 153,493 cells, significantly higher than in HC (median: 1,506;  $p < 0.01$ ). For HIV+ on ART, the median GC B cell number was 30,749 cells, so that 4/10 were above HC normal range. Cell sorting from HIV+ FNB yielded a median of 295,000 CD4+ T-cells (purity >96%). HIV DNA was quantified in 19 out of 19, CA HIV RNA in 13 out of 17, samples. HIV DNA copies per  $10^6$  CD4+ T cells were higher in CD4+ T cells purified from LN FNB compared to PB in both ART-naïve (3.22 vs 2.56,  $p = 0.02$ ) and ART (3.21 vs 3.08,  $p = 0.13$ ) groups. Unspliced HIV RNA copies per  $10^6$  CD4+ T cells were similarly elevated in LN FNB samples from both the ART-naïve (3.97 vs 3.05,  $p < 0.01$ ) and ART (3.66 vs 2.95,  $p = 0.13$ ) groups.

**Conclusions:** Ultrasound guided FNB of unenlarged LN was well-tolerated, and provided sufficient CD4+ T cells and B cells for high-dimensional flow cytometry and cell sorting for PCR, thereby providing access to Tfh and GC B cells for HIV reservoir, pathogenesis, therapy and vaccine studies.

### 335 Tcm CD4 T-Cell Infection Associates With Immune Failure and HIV Persistence on ART

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**Background:** Morbidity and mortality especially from severe non-AIDS conditions are increased for individuals with persistently low CD4 T cell counts and/or persistent immune activation despite virologic suppression on antiretroviral therapy (ART). Current treatments to increase CD4 counts or reduce immune activation have been unable to reduce morbidity or mortality. It is unclear if differential infection of CD4 T cell subsets could further explain divergent CD4 response to ART and maintenance of the latent HIV reservoir.

**Methods:** Thirty HIV-infected subjects were enrolled in a cross-sectional cohort based upon their CD4 response to ART-mediated virologic suppression. Immunologic responders (IR) were defined as having CD4 counts  $>500$  cells/ $\mu\text{L} \leq 2$  years after ART initiation. Immunologic non-responders (INR) were defined as having CD4 counts  $<350$  cells/ $\mu\text{L} \geq 2$  years after ART initiation. The frequency of cells harboring total and integrated HIV DNA were measured in sorted naïve (N), central memory (CM), transitional memory (TM), and effector memory (EM) CD4 T cells for INR and compared to IR. Immunological parameters - including frequencies of CD4 and CD8 T cells and of their N, CM, TM, and EM subsets, their levels of activation/proliferation, and the expression of immune checkpoint molecules - were analyzed by flow cytometry.

**Results:** Median age was 46, 90% were male, 82.8% were African-American. The frequency of cells harboring total and integrated HIV DNA were lowest for N CD4 cells while the other subsets were equivalent. Total and integrated HIV DNA levels were highly correlated ( $r = 0.9240$ ;  $P < 0.0001$ ) and significantly higher for INR than IR in all CD4 T cell subsets ( $P < 0.01$ ). Total/integrated (T/I) HIV DNA ratio was lowest for EM CD4 cells and highest for N CD4 cells, consistent with a higher susceptibility to HIV infection of EM CD4 T cells. Interestingly the T/I ratio was significantly lower in INR than IR only for CM CD4 cells ( $p = 0.0149$ ). Levels of proliferation (Ki-67+) and expression of immune checkpoint molecules previously associated with HIV persistence (PD-1 and TIGIT) were significantly higher in both bulk and CM CD4 T cells of INR.

**Conclusions:** INR have higher levels of HIV DNA in all subsets when compared to IR, thus highlighting a link between the lack of immune reconstitution and viral persistence. Furthermore, INR having a lower T/I ratio only in central memory CD4 cells as compared to IR suggests a higher potential for proviral integration and productive infection of the central memory CD4 cells as an important virologic feature of INR.

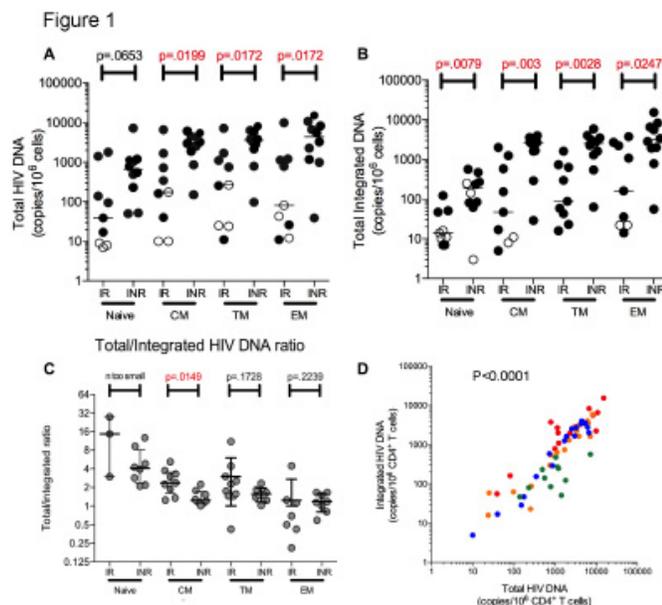


Figure 1. (A) Total HIV DNA, (B) integrated HIV DNA and (C) total/integrated HIV DNA ratio for immunologic responders (IR) and immunologic non-responders (INR) on antiretroviral therapy within naïve, central memory (CM), transitional memory (TM) and effector memory (EM) CD4 T cell subsets. (D) Correlations between Total and Integrated HIV DNA in the different CD4+ subsets. Open symbols depict undetectable HIV DNA. The limit of detection based on cell input is plotted.

**336 Sustained HIV Release by Single Persisting CD4+ T Cells During Latency Disruption**

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**Background:** While the magnitude, timing, persistence, and quality of HIV released from the source CD4+ T cells undergoing latency disruption are unknown, both multi-day sustained HIV release and instantaneous burst stochastic models of HIV replication have been proposed. Which model best represents latency disruption may have important implications for detecting replication-competent virus.

**Methods:** We used HIV *gag* RNA RT-PCR to quantify HIV release from isolated primary CD4+ T cells undergoing latency disruption in limiting dilution cultures. Viral inhibition cultures with efavirenz revealed released virus without new rounds of infection and were compared to respective viral outgrowth cultures. Sustained release and instantaneous burst stochastic models were implemented using the Gillespie algorithm with parameters derived from applying the deterministic model to experimental data.

**Results:** While many outgrowth wells accumulated high amounts of virus, there were also many with declining virus that was nevertheless replication-competent, confirmed by virus transfer from the original wells to new outgrowth wells. Culture wells with >77% probability of being seeded by a single virus-producing cell were analyzed for viral release. Half of cells releasing virus did so in the first 4 days of culture. The accumulated virus for a well varied from less than 120 to 30,000 HIV *gag* RNA copies. Although a low amplitude instantaneous burst dynamic pattern was observed, the vast majority of accumulated HIV RNA was attributed to a higher amplitude virus release pattern sustained for 2-6 days. The intrinsic decay half-life of virus in cultures in which infection was blocked was 3 days. Applying this result to the deterministic model, we estimated an average total sustained release per cell of 5500 HIV RNA copies.

**Conclusions:** Our results are consistent with a multi-day sustained virus release stochastic model that predicts, compared to an instantaneous burst model, higher virus extinction probabilities. Because extinction probabilities are highest at extremely low infected cell numbers, such as single infected cells at limiting dilution, the sustained release stochastic model predicts, and our results show, that replication-competent virus will be present in cultures that are negative for viral outgrowth. Our results further demonstrate that single virus-producing cells can survive and produce virus over several days without succumbing to viral cytopathic effects.

**337 Analysis of HIV Proviruses in Clonally Expanded Cells In Vivo**

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**Background:** Recently, we and others found that clonally expanded populations of HIV infected cells persist during cART. Some cells were highly expanded; a clone with an integrant in the *HORMAD2* gene accounted for c. 50% of all of the infected cells. Integration into specific introns of *BACH2* or *MKL2* increased the expansion and/or survival of infected cells. The proviruses in these clonally expanded cells have not been adequately characterized. One highly expanded provirus was found to be replication-competent (Simonetti et al. CROI 2015). We now report the characterization of other proviruses in highly expanded clones, including those integrated into the *HORMAD2* and *MKL2* genes.

**Methods:** Longitudinal PBMC samples were obtained from two volunteers with chronic HIV infection. Integration sites were determined as previously described (Science 345: 179, 2014). Proviruses integrated in *HORMAD2*, *MKL2*, and an intergenic region of the X chromosome were selectively amplified using specific primers for HIV and the flanking host DNA.

**Results:** The clone carrying the integrant in an intron of the *HORMAD2* was present both pre- and on-cART therapy and accounted for 50-80% of all of the infected cells after 7-8 y on cART, implying that expansion of the clone started early in infection. Proviral sequence analysis revealed a 675 nt single LTR with intact promoter elements. Similarly, analysis of the proviral integrant in an intergenic region of the X chromosome from the second donor showed it was a solo LTR. Seven *MKL2* integrants in introns 4 or 6 of *MKL2* in the same orientation as *MKL2* transcription were also characterized from the second donor. One of the proviruses had a large *pol*-U3 internal deletion; of the remaining, 4 proviruses showed evidence G to A mutation leading to multiple stop codons and 3 were found to lack intact *tat* or *rev*. All seven, however, had intact LTR promoter elements and retained the major splice donor sequence.

**Conclusions:** Proviruses in expanded clones can be intact, hypermutated, partially deleted or consist of only a single LTR. The proviruses that affect the function of the *MKL2* gene to promote cell survival have intact LTR regulatory elements but some lack *tat*, which is normally required for transcription of the HIV genome.

**338 Viral Evolution Analysis for the Distinction of True HIV-1 Elite-Controller Patients**

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**Background:** Elite controllers (EC) are a sub-set of HIV-infected patients able to spontaneously maintain viral loads below the limit of detection without therapy. Some EC remain with undetectable viral load, and they could be considered as the true elite controllers, while other undergoes events of viral replication. *The distinction between both groups of EC is of major importance.* In this work, we propose to study viral evolution as a marker to distinguish true EC.

**Methods:** Serial samples of 15 EC, HIV-1 sero-positive individuals without antiretroviral therapy for at least one year and with 2 viral loads determinations < 50 copies / ml were included. After one year of follow-up, 5 maintained the virological control whereas 10 patients showed an increase in viral load > 50 copies/ml. For virological analysis, we compared samples when viral load was <50 copies/ml (t=0) in both group of patients. Proviral DNA was extracted from PBMC for amplification and sequencing of viral quasispecies by limiting dilution PCR in *env* and *gag* genes. Phylogenetic trees were constructed and calculations of diversity and viral dating (Casado et al., 2000) in both groups were performed. We also analyzed the presence of escape mutations in the *gag* gene.

**Results:** Viral diversity in the *env* C2-V5 region in t=0 was statistically superior ( $p = 0.004$ ) in patients who lost control (mean =  $0.03 \pm 0.01$ ) than in those who maintained control (mean =  $0.0028 \pm 0.003$ ). Viral sequences in patients without loss of control dated close to the time of primary infection, while viral sequences from patients who lost control corresponded to the year of the sample. Overall, no escape mutations in *gag* related to the loss of control were observed. However, when escape mutations (T242N for example) were detected, these mutations pre-existed in the t=0 samples.

**Conclusions:** Viral evolution analysis allowed the distinction of two evolutionary patterns in patients with the same viral control phenotype. EC patients with virological control showed no signs of evolution (evolutionary stasis), suggesting that the virus has not replicated since primo-infection. In the other group of EC patients, we detected viral diversification even with undetectable viral load, indicating viral replication along the infection. Thus, lack of viral evolution could be used as a diagnostic marker for the identification of true EC. These EC patients could be potentially considered as functionally cured.

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**339 Optimization of PCR Amplicons to Predict Clonality of Full-Length HIV-1 Sequences**

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**Background:** Virus-driven clonal expansion of latently infected memory CD4+ T cells may provide a mechanism of HIV-1 persistence in the presence of suppressive antiretroviral therapy. The only conclusive way to prove the presence of a clonally expanded infected cell is to sequence complete, identical viral genomes and integration sites multiple times in independent experiments. Due to the prohibitive difficulty of this measurement, many studies assume clonality when independent viral sequences are identical in short PCR amplicons of ~500-1500bp. In this study, we analyzed previously published full-length HIV-1 sequence alignments from 28 patients to determine which PCR amplicons, if any, are sufficient to demonstrate the clonality of an entire viral genome.

**Methods:** We analyzed full-length HIV-1 sequences from plasma RNA (6 patients), resting CD4<sup>+</sup> T cell proviral DNA (7 patients treated during acute infection, 9 patients treated during chronic infection), and DNA from p24 antigen-negative quantitative viral outgrowth assay culture wells (6 patients). We analyzed between 5 and 121 unique sequences (mean=25, median=16) per patient. We compared the apparent clonality of each patient alignment within previously published PCR amplicons in the gag-pol and envelope V3-V4 regions. We also measured the apparent clonality of each alignment within hypothetical amplicons of various fixed lengths across the genome to identify the optimal genomic region(s) for primer placement and to compare the importance of amplicon location versus amplicon length.

**Results:** Although no short region of the HIV-1 genome is consistently sufficient to distinguish non-clonal full-length viral sequences in all patients, a 1500bp amplicon in the gag-pol region is superior to a 500bp amplicon spanning the envelope V3-V4 region ( $p < 0.001$ ). In sequences from plasma RNA, the sufficiency of a hypothetical amplicon to distinguish non-clonal sequences depends almost entirely on the length of the amplicon. In proviral DNA samples, there is wide variation among patients in terms of which genomic regions work best and in terms of the importance of amplicon length.

**Conclusions:** No short PCR amplicon is sufficient to demonstrate the clonality of HIV-1 genomes within samples from a single patient. Researchers hoping to demonstrate clonality may screen samples with a short PCR but must ultimately sequence full-length viral genomes to demonstrate the clonality of independent samples.

### 340 Clonal Expansion of Replication-Competent Proviruses is Common in Individuals on ART

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**Background:** Clonal expansion of HIV-infected CD4<sup>+</sup>T-cells in patients on long-term ART was recently described by Maldarelli *et al.* and Wagner *et al.* (Science 2014), but the replication-competence of such proviruses is controversial. Cohn *et al.* (Cell 2015) were unable to identify intact, clonally-expanded proviruses *in vivo*. Conversely, Simonetti *et al.* (CROI 2015) described a case of persistent viremia during ART wherein the primary source was a clonally-expanded, replication-competent provirus. Here, we sought additional evidence of clonally-expanded, replication-competent proviruses in individuals on ART.

**Methods:** Viral outgrowth assays (VOAs) were performed on  $\geq 6 \times 10^6$  purified CD4<sup>+</sup> T-cells from 8 consecutive participants who were on suppressive ART for  $\geq 2$  years (median 13.5 years) and who had diverse virus populations consistent with long-term chronic infection prior to therapy. Purified CD4<sup>+</sup> T-cells were seeded in six replicates of 3-fold serial dilutions beginning with  $1 \times 10^6$  cells per well. Single-genome sequencing (SGS) targeting p6-PR-RT was performed on supernatants from p24 positive wells and on HIV cell-associated, unspliced HIV RNA (CAR) and HIV DNA (CAD) in PBMCs from the same individuals. Sequences were examined for matches across VOA wells and to PBMC CAR and CAD by neighbor-joining analyses.

**Results:** In 6 of the 8 individuals studied to date, identical sequence matches have been found across multiple VOA positive wells or between VOA positive wells and CAR or CAD SGS. Replication-competent proviruses constituted a median of 1.3% (range 0.7-7.1%) of all unique viral variants and a median of 9.1% (range 7.7-20%) of all clonally-expanded variants detected by SGS.

**Conclusions:** We detected inducible, clonally-expanded, replication-competent proviruses in more than half of individuals studied on long-term suppressive ART. These expanded clones could serve as a persisting source for rebound viremia after interruption of ART and should be targeted by HIV cure strategies.

### 341 A Novel Assay to Quantify Replication-Competent Latent HIV-1 From rCD4<sup>+</sup>T Cells

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**Background:** The latent HIV-1 reservoir that resides in resting CD4<sup>+</sup> (rCD4<sup>+</sup>) T cells of infected individuals on combination antiretroviral therapy (cART) is a major obstacle to a cure. A primary challenge in defining this reservoir is the lack of a high-throughput, sensitive and robust assay that can quantify the size of the inducible replication-competent pool of latent HIV-1. Currently, the quantitative viral outgrowth assay (Q-VOA) that is widely used is labor intensive, time consuming and expensive, and is not amenable to a high-throughput format. Here we report on the development of a TZM-bl cell based assay (TZA) for the quantification of replication-competent latent HIV-1, which is fast, sensitive, robust and amenable to a high-throughput format.

**Methods:** The strategy for quantifying inducible replication competent HIV-1 in rCD4<sup>+</sup>T cells by TZA involves two steps: (i) induction of latent HIV-1 from rCD4<sup>+</sup>T cells by anti-CD3/CD28 antibodies; and (ii) quantification of induced replication competent HIV-1 in the TZM-bl cell based assay. Using TZA, Q-VOA and a simultaneous ultrasensitive subpopulation staining/hybridization *in situ* (SUSHI) assay we quantified the size of the inducible replication-competent pool of latent HIV-1 in rCD4<sup>+</sup> T cells isolated from 13 aviremic HIV-1 infected individuals on suppressive cART from the Pittsburgh Multicenter AIDS Cohort Study.

**Results:** For the TZA, the amount of replication competent HIV-1 was found to range 1.2-141 (mean 46.4) infectious units per million (IUPM). By contrast, the IUPM values for the Q-VOA ranged 0.28-2.37 (mean 0.70), which is almost 70-fold lower ( $p=0.0029$ ). To confirm the high levels of IUPM obtained from the TZA assay we applied the SUSHI assay in parallel to anti-CD3/CD28 activated rCD4<sup>+</sup> T cells from 10 out of 13 donors. The number of HIV-1 RNA positive cells per million rCD4<sup>+</sup> T cells in the SUSHI assay was similar in magnitude to the TZA assay ranging from 10 to 240 (mean 75). In subsets of HIV-infected subjects, we also evaluated fractional HIV-1 provirus expression (fPVE). For 9 subjects the fPVE as measured by the TZA ranged from 0.12-13.93% (mean 4.03%), while fPVE as measured by the Q-VOA ranged from 0.02- 0.9% (mean 0.14%).

**Conclusions:** Using the novel TZA assay, we show that the size of the inducible latent HIV-1 reservoir in virally suppressed individuals on cART is significantly larger than previous estimates using the Q-VOA, which could pose a challenge for the irradiation of latent virus in rCD4<sup>+</sup> T cells in these patients.

### 342 Cellular HIV-1 RNA/DNA As Biomarkers of Inducible Virion Production

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**Background:** Simple biomarkers of the inducible HIV-1 reservoir have not been identified. We investigated whether the frequency of infected cells in blood and their transcriptional activity is related to the inducible HIV-1 reservoir from resting CD4<sup>+</sup> T cells in persons on virologically-suppressive antiretroviral therapy (ART).

**Methods:** Total blood mononuclear cells (PBMC) were isolated from leukapheresis product obtained from volunteers on suppressive ART for  $\geq 1$  year. Cellular unspliced HIV-1 RNA (CA-RNA) and proviral HIV-1 DNA (CA-DNA) in uncultured PBMC were quantified by qPCR assays targeting 3' integrase. Resting CD4<sup>+</sup> T (rCD4) cells were isolated from PBMC by negative selection, and were then activated with PMA/ionomycin in the presence of 300 nM efavirenz. On day 6 of culture, supernatants were collected from replicate wells, centrifuged (500 x g for 5 min), and stored at -80°C. HIV-1 RNA in supernatants (surrogate for virion production) was quantified by COBAS Roche TaqMan v2.0. Correlations between virion production and cellular HIV-1 RNA/DNA were assessed using Spearman's correlation coefficient.

**Results:** A total of 22 donors were evaluated. Levels of HIV-1 RNA produced after 6 days of treatment with PMA/ionomycin varied  $>1000$  fold among the donors; the median was 4406 copies/mL of culture supernatant, ranging from 38 to 42756 copies/mL. The median level of CA-RNA was 38 (range:  $<1$  to 355) copies per  $10^6$  PBMC, and the median level of CA-DNA was 286 (range: 7 to 2972) copies per  $10^6$  PBMC. CA HIV-1 RNA and DNA levels were strongly correlated with each other ( $\rho=0.80$ ,  $p < 0.001$ ), and both were strongly correlated (see figure) with inducible virion production ( $\rho=0.76$ ,  $p < 0.001$  for CA-RNA;  $\rho=0.75$ ,  $p < 0.001$  for CA-DNA).

**Conclusions:** Inducible virion production from resting CD4+ T cells *ex vivo* is strongly associated with the number of HIV-infected cells and the basal level of HIV-1 RNA transcription in isolated PBMC. These data indicate that simple, high-throughput and relatively inexpensive measures of cellular HIV-1 RNA and DNA can be used to estimate the size of the inducible reservoir in patients on suppressive ART. These approaches could also be useful in assessing the effectiveness of interventions targeting the latent HIV reservoir.

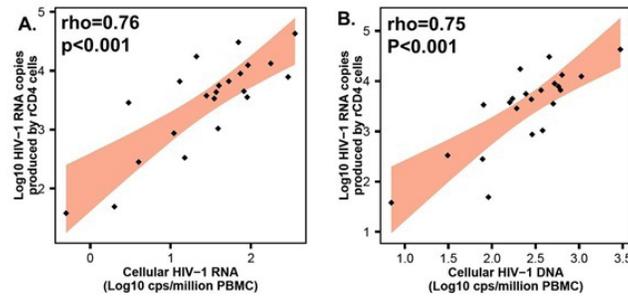


Figure 1. Statistically significant correlations were found between unspliced cellular HIV-1 RNA and total virions produced from resting CD4+ T (rCD4) cells (A), and cellular HIV-1 proviral DNA and total virions produced from rCD4 cells (B). Spearman's correlations were calculated, and 95% confidence intervals for correlations are shown in red.

### 343 FDG PET/CT Imaging of Lymph Nodes As a Measure of the HIV Reservoir in Humans

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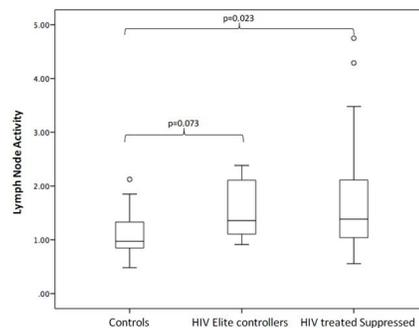
**Background:** The HIV reservoir persists even in the setting of effectively treated and suppressed HIV infection. Preclinical models strongly implicate lymphoid structures as a major reservoir. 18F-fluorodeoxyglucose (FDG) PET/CT is an imaging modality that quantifies inflammation in these tissues. We assessed the association between lymph node inflammation and the size of the reservoir.

**Methods:** A total of 64 participants, ages 40 and older, were recruited from the SCOPE cohort. There were 45 HIV-infected participants and 19 controls that were matched by age, gender, Framingham Score, and race. Thirty-four of those with HIV were treated and suppressed and 7 were elite controllers. Participants underwent 18FDG-PET/CT imaging after an overnight fast and FDG uptake in the axillary lymph nodes (LN) was assessed as a mean standardized uptake value (SUV). A target to background ratio (TBR) for LN was generated by dividing LN SUV by blood background SUV. Viral persistence was measured using frequency of cells harboring integrated HIV DNA in CD4+ T cells.

**Results:** Lymph node inflammation was lower in HIV-uninfected controls (TBR: 0.97 (0.84-1.37)) compared to treated and suppressed individuals (1.39 (1.01-2.23)  $p=0.02$ ) and elite controllers (1.36 (0.92-2.19),  $p=0.07$ ) as shown in Figure 1. Higher inflammation in the lymph nodes was associated with higher HIV RNA viral load ( $r=0.53$ ,  $p<0.001$ ), even after correcting for antiretroviral therapy, CD4 count, or history of opportunistic infections ( $p=0.006$ ). Additionally, higher lymph node inflammation was associated with markers of viral persistence among the elite controllers (Integrated HIV DNA,  $r=0.78$ ,  $p=0.039$ ), but not the treated suppressed ( $r=0.051$ ,  $p=0.794$ ). Among those on effective ART, lymph node inflammation was associated with higher markers of CD4+ T-cell activation (HLADR+CD38+ cells:  $r=0.53$ ,  $p=0.003$ ) and higher levels of IL-6 ( $r=0.43$ ,  $p=0.02$ ).

**Conclusions:** Lymph node activity as assessed by FDG-PET/CT was higher among HIV-infected individuals compared to controls and was associated with markers of viral persistence and inflammation. Our findings support the hypothesis that among elite controllers, HIV serves as the main driver of inflammation as opposed to those on ART. FDG PET/CT imaging of the axillary lymph nodes may provide a method to non-invasively measure reservoirs of viral persistence in HIV and assess response to different antiretroviral therapies or anti-inflammatory interventions.

Figure 1: Lymph Node Activity in Treated Suppressed HIV, Elite Controllers, and HIV-negative Controls



### 344 Activation of Nasopharynx-Associated Lymphoid Tissue in HIV Infection

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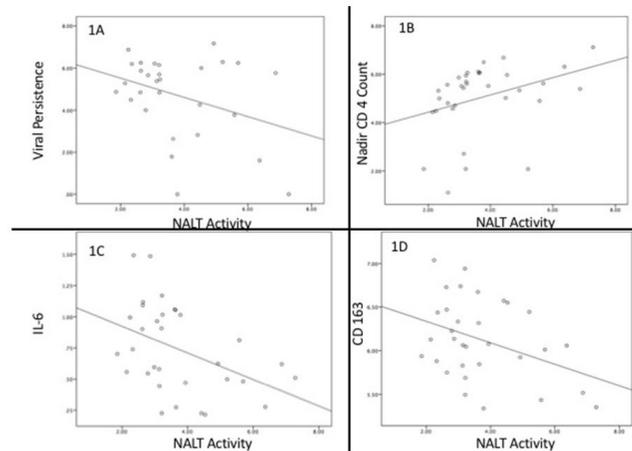
**Background:** Nasopharynx-associated lymphoid tissue (NALT) plays an important role in stimulating immune responses against alimentary and airborne antigens. Tissue-resident inflammatory cells are metabolically active and can be quantified using an infusion of 18F-fluorodeoxyglucose (FDG) followed by a PET scan to identify heightened inflammation. We compared NALT in HIV-infected individuals and matched HIV-negative controls and assessed the association between NALT and inflammatory markers, markers of immune activation, and size of the HIV reservoir.

**Methods:** A total of 64 participants, ages 40 and older, were recruited from the SCOPE cohort. There were 45 HIV-infected participants and 19 HIV-negative controls (who were matched by age, gender, and Framingham risk score to HIV patients). Thirty-four (76%) of the HIV-infected individuals were treated and virally suppressed, 7 (16%) were elite controllers, and 1 (2%) was treated and unsuppressed. NALT activation (NALT-A) was measured as average of maximum standardized uptake value (SUVmax) of FDG uptake within nasopharyngeal tissue. Immunophenotyping of T-cells and monocytes was performed by multiparameter flow cytometry on cryopreserved PBMCs. HIV reservoir was ascertained by measuring the frequency of CD4+ T-cells harboring integrated HIV DNA.

**Results:** Average maximum standard uptake value of NALT was higher in HIV-infected individuals on ART vs matched HIV-negative controls (5.18 +/- 2.19 vs 3.40 +/- 1.04,  $p=0.023$ ). SUVmax of NALT was positively correlated with nadir CD4 ( $r=0.507$ ,  $p=0.002$ ) and was inversely associated to markers of inflammation (IL-6:  $r=-0.429$ ,  $p=0.014$ ; sCD163:  $r=-0.380$ ,  $p=0.032$ ). A trend for an inverse association between NALT and the HIV reservoir was observed ( $r=-0.350$ ,  $p=0.0563$ ). However, among the elite controllers only, this finding was highly significant (NALT-A:  $N=5$ ,  $r=-0.98$ ,  $p=0.005$ ).

**Conclusions:** HIV-infected individuals had higher levels of NALT-A compared to matched HIV-negative controls. In HIV infection, activation of NALT tissue was associated with lower indices of inflammation, higher nadir CD4 count, and lower levels of the HIV reservoir. These novel observations suggest that an intact and active NALT may play a role in generating a response in the setting of HIV.

Figure 1: Scatterplots with regression line in HIV-infected subjects



### 345 HIV DNA Identified in Most Tissues of a Plasma-Negative HIV Autopsy Cohort

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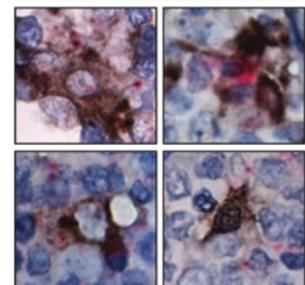
**Background:** While combined antiretroviral therapy (ART) can reduce plasma viral loads to undetectable levels, the degree to which virus is eliminated from other anatomical sites remains unclear. The high frequency of comorbidities in ART<sup>+</sup>HIV<sup>+</sup> patients suggests that persistence of virus may contribute to tissue pathologies. The goal of this study was to identify subjects with undetectable plasma and CSF viral load at death, assay a panel of their autopsy tissues for HIV, and assess tissue histopathology to discover the extent of residual anatomical HIV levels during cART and the potential relationship to tissue injury.

**Methods:** The National Neurological AIDS Bank (NNAB) and AIDS and Cancer Specimen Resource (ACSR) autopsy cohort was screened to identify 20 HIV<sup>+</sup>/ART-treated participants who had undetectable plasma and CSF viral loads at autopsy. Extensive medical histories were compiled for each participant. Detailed histopathological findings were noted in multisite autopsy specimens ( $n=212$ , including up to 6 brain and 6 lymphoid tissues per subject). All tissues were assayed for the presence of HIV DNA using quantitative and digital drop PCR. A subset of tissues was evaluated for HIV RNA using RNAscope *in situ* hybridization assay.

**Results:** The median patient age and length of HIV infection was 46.5 years and 12 years, respectively. Fifteen of the 20 patients developed cancer. Abnormal histological findings in the spleen, lung, lymph node and liver were identified in 90% of the participants. Aorta and kidney were abnormal in 50 and 60% of the participants, respectively. 75% of participants demonstrated a degree of atherosclerosis and all brain tissues analyzed demonstrated slight to severe pathology. Overall, 66% of the tissues studied contained HIV DNA copies >200/million cell equivalents. Selected RNAscope studies localized HIV to macrophages within cancer tissues (See Figure). In HIV RNA positive brain tissue, infiltrating macrophages surrounded HIV<sup>+</sup> cells.

**Conclusions:** This study confirms the presence of HIV within diverse anatomical tissues in virally suppressed ART<sup>+</sup> patients. Persistent virus replication in tissues could promote inflammatory diseases, including cancer, atherosclerosis and other organ-associated diseases. These ACSR/NNAB cohorts, along with others of their kind, are highly valuable resources for future studies of HIV reservoirs and persistence.

#### HIV-infected macrophages



#### HIV RNA/CD163/CD68/Nuclei

HIV infection of macrophages in B-cell lymphoma tissues was evident by the presence of viral RNA in the nucleus and cytoplasm of CD163/CD68-positive cells

### 346 Relationship Among Viral Load Outcomes in HIV Treatment Interruption Trials

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**Background:** Analytic treatment interruption (ATI) trials are crucial for evaluating potential HIV curative strategies, but interpretation of these trials is complicated by the use of varying virologic outcome measures and an incomplete understanding of relationships between potential virologic endpoints. While viral load (VL) set point has historically been the most common virologic outcome of ATI studies, there is increasing use of time to viral rebound as an alternative endpoint. In addition, other studies have presented lower ATI VL set point compared to pre-ART VL as evidence of therapeutic efficacy in clinical trials. Using a pooled dataset of AIDS Clinical Trials Group (ACTG) ATI studies, we completed an in-depth characterization of ATI VL endpoints.

**Methods:** We performed a pooled analysis of 6 ACTG ATI studies with 235 total participants who were virologically suppressed prior to ATI. We evaluated the relationship between two common VL outcomes—timing of confirmed VL rebound  $\geq 200$  HIV RNA copies/mL and VL set point (mean  $\log_{10}$  VL during ATI weeks 12-16). Associations between set point and timing of viral rebound were analyzed using Spearman correlation, Kruskal-Wallis, Mann-Whitney, and Chi-square tests.

**Results:** For participants who had HIV rebound at or before week 12, there was no significant association between timing of viral rebound and the VL set point. However, participants with viral rebound after 12 weeks also had significantly lower set points (rebound  $\leq 12$  [N=176] vs.  $>12$  weeks [N=14]: median 4.1 vs. 1.9  $\log_{10}$  HIV RNA copies/mL,  $P < 0.001$ , Figure 1). Participants treated during chronic infection had higher ATI VL set points than those treated during early infection (chronic [N=141] vs. early [N=50]: median 4.2 vs. 3.4  $\log_{10}$  HIV RNA copies/mL,  $P < 0.001$ ). Pre-ART VL was correlated with ATI VL set point (Spearman  $r = 0.38$ ,  $p < 0.001$ ), but not rebound timing. In 68% of participants, ATI VL set point was lower than pre-ART VL with a median 2-fold decrease, but there was significant variation in the fold-change between ATI VL set point and pre-ART VL (Q1, Q3: 5-fold decrease, 1.7-fold increase).

**Conclusions:** Our results reveal a complex relationship between several key virologic factors in HIV ATI trials, including pre-ART VL, viral rebound timing, and ATI VL set point. The association of delayed viral rebound with lower ATI VL set point suggests the presence of virologic and/or immunologic factors mediating both outcomes.

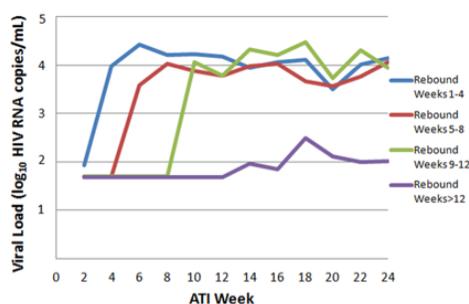


Figure 1. Biweekly median viral loads for participants stratified by timing of viral rebound.

### 347 Viral and Immune Characteristics of HIV Post-Treatment Controllers in ACTG Studies

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**Background:** HIV post-treatment controllers (PTCs) are individuals who can maintain low levels of viremia after ART discontinuation. They have primarily been identified from patients treated during acute infection and little is known about PTCs who were treated during chronic infection. Understanding the mechanisms of HIV control in PTCs has implications for the design of novel strategies for HIV remission.

**Methods:** We evaluated 497 participants from 8 ACTG analytic treatment interruption (ATI) studies to identify PTCs who generally maintained viral loads  $\leq 400$  HIV RNA copies/mL for  $\geq 24$  weeks. Non-PTC control participants were matched by study arm and selected randomly. Total HIV DNA and CA-usRNA were measured in PBMCs at 3 time points: pre-ATI, early and late post-ATI. T cell intracellular cytokine staining (ICS) was performed on PBMCs stimulated with HIV Gag peptide pool and NK cell ICS was performed with PBMCs stimulated with K562 cells. Soluble markers of inflammation were measured with ELISA-based assays.

**Results:** 16 PTCs were identified (3.2% of participants), including 6 treated during early infection (6.6% of this group) and 10 treated during chronic infection (2.5% of this group). Median duration of documented virologic control was 96 weeks. There were no significant differences in pre-ATI CD4+ count, duration of ART, or frequency of protective HLA alleles between PTCs and non-PTCs. Pre-ATI HIV DNA and CA-RNA were detectable in 14% and 29% of PTCs, respectively. HIV DNA and CA-RNA levels did not significantly increase in PTCs after ATI (median DNA and CA-RNA  $< 50$  copies/ $10^6$  PBMCs for all time points). For individuals treated during chronic infection, non-PTCs had higher HIV DNA levels pre-ATI and higher DNA and CA-RNA levels early post-ATI. No significant differences were detected in HIV-specific T or NK cell activity between PTCs and non-PTCs. While there were no significant pre-ATI differences in soluble and T cell markers of inflammation, non-PTCs had increased CD8+ cell activation as well as IP10 and sCD163 levels post-ATI.

**Conclusions:** While rare, PTCs can be identified from individuals who were not treated during acute HIV infection. PTCs maintained a small HIV reservoir even after ART discontinuation without a significant increase in immune activation or HIV-specific T or NK cell activity. The detection of pre-ATI HIV DNA and CA-RNA did not preclude the possibility of post-treatment control, suggesting inefficient viral replication or antiviral immune activity may mediate this control.

### 348 HIV-1 in the Blood and Intestine Contributes to Viremia During Treatment Interruption

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**Background:** Transcriptionally silent HIV-1 proviral genomes contribute to HIV-1 persistence in long-lived CD4+ T cells. Administration of panobinostat to HIV-1 infected individuals on long-term antiretroviral therapy (ART) activates HIV-1 RNA transcription in these latently infected cells. The extent to which the induced HIV-1 RNA transcripts and related HIV-1 DNA sequences represent virus able to contribute to viremia upon treatment discontinuation is unknown. In this study, we compared HIV-1 sequences in CD4+ T-cells from the peripheral blood and intestinal lamina propria mononuclear cells (LPMCs) during experimental therapy with panobinostat to sequences that were collected from the plasma during a post-panobinostat analytical treatment interruption (ATI).

**Methods:** CD4+ T cells were obtained before, during, and after panobinostat administration (n=15). For nine of the participants, LPMCs were collected during and after panobinostat treatment. Additionally, plasma samples were collected during an ATI for nine of the trial participants. Five participants participated in both the ATI and collection of LPMCs. We used single-proviral/genome sequencing to determine the genetic composition HIV-1 DNA and RNA. Phylogenetic analyses were conducted using MEGA 6.0.

**Results:** We identified an expansion of clonal cell-associated HIV-1 DNA in the peripheral blood, which is indicative of previous cellular proliferation, that matched viral RNA sequences from the ATI. Cell-associated HIV-1 DNA sequences from CD4+ T cells from both blood and LPMCs were closely related to HIV-1 RNA sequences detected in plasma during the ATI (>99% similarity; blood n=38 sequences, LPMC n=12 sequences). Furthermore, we identified cell-associated HIV-1 RNA sequences in CD4+ T cells from blood and LPMCs collected during panobinostat treatment that were closely related to plasma sequences from the ATI (>99% similarity; blood n=6 sequences LPMC: n=1 sequence).

**Conclusions:** Clonally expanded HIV-1 is capable of contributing to viremia, indicating that replication-competent virus is maintained in proliferating cells. Importantly, we found that cell-associated HIV-1 DNA in the peripheral blood CD4+ T cells and LPMCs both contribute to plasma viremia following discontinuation of ART. Furthermore, HIV-1 RNA from virus that later emerged during ATI was expressed in the blood and LPMCs during panobinostat treatment. Thus, both of these important compartments of latent virus should be prioritised in remission and curative strategies.

### 349 HIV DNA Set Point Remains Elevated in Untreated vs Treated Acutely Infected Thais

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**Background:** The dynamics of HIV reservoir seeding in early acute HIV infection (AHI) and the longitudinal comparison of reservoir markers in antiretroviral therapy (ART) untreated and treated AHI is poorly understood.

**Methods:** Total and integrated HIV DNA were measured in the peripheral blood mononuclear cells (PBMCs) from two prospective AHI protocols of untreated (RV217, n=12) and treated (RV254, n=71) Thais. HIV DNA levels were log<sub>10</sub> transformed prior to analysis. Differences between groups were assessed using two-tailed Student's t test.

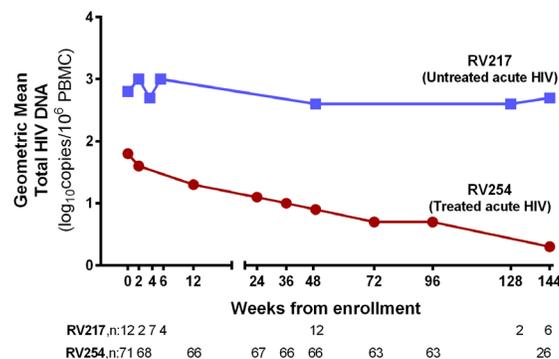
**Results:** The untreated cohort was younger (median age 23 vs. 29 years in the treated cohort). The distribution of Fiebig stages were similar: untreated: 7 (58%) FI/II and 5 (42%) FIII/IV; treated: 33 (46%) FI/II and 38 (54%) FIII/IV. Baseline HIV RNA was higher in those untreated (6.4 vs. 5.7 log<sub>10</sub> copies/ml in treated, p=0.04). Mean time to ART initiation was 2 days from enrollment in the treated cohort. Geometric mean total HIV DNA over time is shown in the figure.

Mean baseline values were higher in the untreated vs. treated cohorts for total DNA (2.8 log vs. 1.8 log<sub>10</sub> copies/10<sup>6</sup> PBMC, p=0.02) and integrated DNA (1.6 vs. 0.7 log<sub>10</sub> copies/10<sup>6</sup> PBMC, p=0.006). After ART, the treated cohort had a marked reduction in total and integrated HIV DNA levels whereas these changed little in those untreated. The mean values at week 48 for total HIV DNA were 2.6 vs. 0.9 log<sub>10</sub> copies/10<sup>6</sup> PBMC and integrated HIV DNA were 1.5 vs. 0.1 log<sub>10</sub> copies/10<sup>6</sup> PBMC for the untreated and treated cohorts, respectively.

The mean differences of total HIV DNA between the untreated vs. treated cohorts increased from 1 log<sub>10</sub> copies/10<sup>6</sup> PBMC at baseline to 1.7 log<sub>10</sub> copies/10<sup>6</sup> PBMC at week 48 (p<0.0001) and 2.3 log<sub>10</sub> copies/10<sup>6</sup> PBMC at week 144 (p<0.0001).

Similarly, the mean differences of integrated HIV DNA between the untreated vs. treated cohorts increased from 0.9 log<sub>10</sub> copies/10<sup>6</sup> PBMC at baseline to 1.4 log<sub>10</sub> copies/10<sup>6</sup> PBMC at week 48 (p<0.0001) and 1.9 log<sub>10</sub> copies/10<sup>6</sup> PBMC at week 144 (p<0.0001). The total and integrated HIV DNA differences between cohorts remained after adjusting for baseline HIV DNA values.

**Conclusions:** Levels of HIV DNA "set point" appears to be established early during Fiebig I-IV AHI and determines the reservoir size in chronic infection. Over three years without ART, persons with AHI have HIV DNA that is 2 logs higher than those on ART. The window of opportunity to significantly alter HIV DNA levels with ART is possibly very early.



### 350 Impact of Long-term Antiretroviral Therapy (ART) on Cellular HIV and Inflammation

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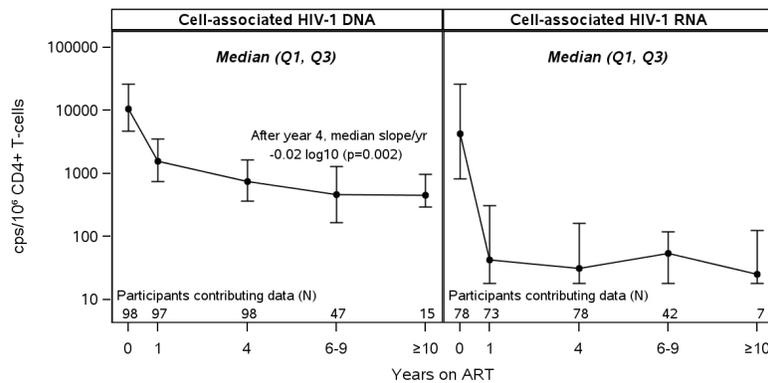
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**Background:** The longitudinal impact of ART on markers of HIV persistence and inflammation is incompletely understood.

**Methods:** Participants with chronic HIV who initiated ART in ACTG trials had pre- and on-ART samples tested for cell-associated HIV RNA and DNA (CA-RNA, CA-DNA), plasma HIV RNA, IL-6, hsCRP, sCD14, sCD163.

**Results:** 101 participants with median duration of virologic suppression on ART of 7 yrs (range 4-15) were analyzed. Prior to ART, higher CA-DNA correlated with higher CA-RNA (r=0.67, p<0.001) and lower CD4 count (r=-0.51, p<0.001). After 4 yrs of ART, CA-DNA fell 15-fold whereas CA-RNA dropped 525-fold (Figure). Slope of CA-DNA decay was greatest during the first 4 yrs but DNA continued to decline slowly thereafter (5%/yr decline, p=0.002; 69% had negative slope). By contrast, after yr 1 there was no further decline in CA-RNA. Despite suppression of plasma viremia, participants with high CA-DNA before ART continued to have high DNA while on ART (r≥0.61, p<0.001). Other factors associated with high on-ART CA-DNA included low pre-ART CD4 count and low on-ART CD4:CD8 ratio (eg, at yr 1 of ART, r=-0.44, p<0.001). The correlation between CA-DNA and CA-RNA persisted at all time points (r=0.51-0.57, all p<0.001) but only DNA correlated with plasma RNA by single-copy assay (r=0.32, p=0.013; assessed at yr 4). IL-6 and sCD163 dropped significantly during the first yr of ART and then stabilized; sCD14 and hsCRP did not decline after ART. No consistent correlations were found between CA-DNA or CA-RNA and inflammatory biomarkers at pre- or on-ART time points.

**Conclusions:** Following initiation of ART, CA-RNA declines more rapidly and to a greater extent than CA-DNA, indicating that a subset of cells expressing RNA has a shorter half-life than infected cells that do not express RNA. At 4 yrs of ART, CA-DNA remains correlated with plasma RNA and continues to decline slowly thereafter (similar to rate shown for plasma RNA (Riddler JID 2015)). CA-RNA does not appear to decline after yr 1, suggesting persistence of a cell population expressing defective or partial HIV genomes that do not kill infected cells at a rate higher than their proliferation. ART reduces some inflammatory markers but there was no evidence for a link between inflammation and HIV persistence. Low CD4:CD8 is associated with high CA-DNA, supporting early ART initiation to reduce the infected cell pool. These data underscore the need for interventions to accelerate the slow decay on ART of infected cells capable of virus production.



**351 Impact of cART & Systemic inflammation on Semen HIV-1 Reservoir in Primary Infection**

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**Background:** The risk of sexual transmission is conditioned by the presence of HIV-1 infected cells and viral particles in genital secretions. Infected cells in semen represent an increased risk of HIV-1 cell to cell transmission Primary HIV Infection (PHI) is brief but very efficient in terms of viral transmission. We studied the impact of early-cART in PHI and the influence of the systemic activation on HIV-semen and blood reservoir dynamic.

**Methods:** Patients from the ANRS-147-OPTIPRIM randomized trial received two years of early-cART. Blood and seminal samples were collected at inclusion and month 24. Total cell-associated-HIV-DNA and HIV-RNA were quantified in blood, semen cells and seminal plasma (Biocentric, Bandol, France). Interferon-γ-inducible interferon 10 (IP-10) and interleukin-6 (IL6) were quantified by ELISA in blood plasma. Spearman correlation tests were performed.

**Results:** Twenty-one patients participated to this substudy (median age: 36 years, time from estimated date of infection: 33 days), 20 were symptomatic and 8 presented acute infection (WB ≤1 Ab). At enrolment, median HIV-RNA was significantly higher in blood (5.39 log<sub>10</sub> cp/mL) than in semen (4.22) (p< 0.0001). Median blood and seminal HIV-DNA level was 3.59 and 0.31 log<sub>10</sub> cp/10<sup>6</sup>PBMC, respectively. Semen HIV-RNA was correlated with CD4 count (r=-0.54, p=0.018) and CD8 count (r=-0.54 p=0.018). Furthermore, IP-10 positively correlated with blood HIV-RNA (r=0.46 p=0.046), blood and semen HIV-DNA (r=0.53 p=0.018; r=0.51 p=0.026), IL-6 (r=0.68 p=0.003) and negatively with CD4/CD8 ratio (r=-0.59 p=0.006) (Figure1). Among the 8 patients with acute infection, semen-HIV-RNA correlated with blood-HIV-RNA (r=0.81, p=0.015), CD4 count (r=-0.98, p<0.0001), CD4/CD8 ratio (r=-0.85, p=0.0075). Two years effective cART induced an important and significant decrease in blood and semen HIV-RNA levels

**Conclusions:** This is the first evidence of HIV-reservoir cells in semen of patients with PHI, showing that levels are linked with the immunosuppression severity and plasma IP-10 level. Early treatment allows purging viral particles and also infected cells, which reduces the high risk of HIV transmission during PHI.

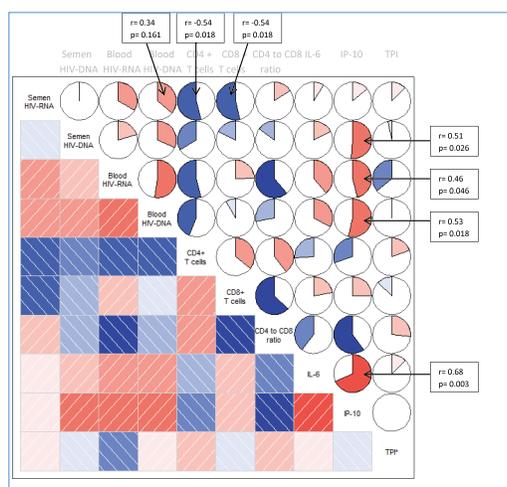


Figure 1: Correlogram of baseline virological and immunological markers.  
 \*TPI: Time between estimated date of infection and enrolment  
 Heatmaps and pie-charts indicate associations between the variables. Red color displays a positive correlation and blue a negative correlation. Their intensity and size represent the strength of the association.

### 352 Predictors of Viral Control After ART Interruption in SIV-Infected Rhesus Macaques

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<sup>1</sup>Emory Univ, Atlanta, GA, USA; <sup>2</sup>Yerkes Natl Primate Rsr Cntr, Emory Univ, Atlanta, GA, USA; <sup>3</sup>Univ of Montreal, Montreal, QC, Canada; <sup>4</sup>Case Western Reserve Univ, Cleveland, OH, USA; <sup>5</sup>Univ de Montréal, Montreal, QC, Canada; <sup>6</sup>Frederick Natl Lab, Frederick, MD, USA

**Background:** Antiretroviral therapy (ART) does not eradicate HIV and the virus rebounds upon ART interruption. Recently, a sustained control of HIV replication in the absence of ART has been achieved in a subset of subjects starting ART early after infection (post-treatment controllers; PTC). However, the virologic and immunologic determinants of post-ART control of HIV replication are still unclear, particularly in tissues. Understanding the mechanisms behind durably containing HIV replication may guide new strategies towards HIV remission. Here, we used the well-established model of SIV-infected rhesus macaques (RMs) to investigate the features associated with post-ART SIV control.

**Methods:** We identified five SIV<sub>mac239</sub>-infected RMs (B\*08 and B\*17) that, after seven months of ART initiated at two-months p.i., controlled viral rebound (<200 copies/mL) after structured treatment interruption (STI). Blood (PB), rectum (RB) and lymph node samples were longitudinally collected from these animals as well as from RMs that, under the same experimental conditions, experienced a robust SIV rebound after STI (NC). Total SIV-DNA and RNA was measured on purified PB CD4 T cells and mucosal tissues by qPCR; immunological parameters were determined by flow cytometry. Predictors associated with PTC status were evaluated by odds ratio analyses.

**Results:** Before ART initiation, PTC had markedly reduced SIV-RNA levels in plasma and RB, lower SIV-DNA content in PB CD4 T cells and RB, reduced T-cell activation, and higher CD4 counts as compared to NC (P<0.01 for all measures). Levels of intestinal CD4 T cells were similar, but PTC had higher frequencies of Th17 and Th22 cells. On ART, PTC had significantly lower levels of residual plasma viremia (<3 copies/mL) and SIV-DNA content in PB CD4 T cells. The bulk and SIV-specific CD8 T cell compartment was comparable between the two groups, thus suggesting that PTC can achieve partial viral control without CD8 antiviral responses superior to those of NC. Remarkably, PB and RB SIV-RNA and DNA contents rapidly increased in NC after ART interruption, while remain stable or progressively decreased in PTC. Finally, partial control of SIV rebound in PTC resulted in higher CD4 levels and reduced inflammation in PB, RB and LN during the off-ART period.

**Conclusions:** Lower pre-ART viremia, cell-associated SIV-DNA, and T cell activation with concomitant preservation of Th17 cell homeostasis are highly associated with prolonged viral control post ART interruption in SIV-infected RMs.

### 353 Antiretroviral Therapy in SIV Natural Hosts: Implications for AIDS Pathogenesis

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<sup>1</sup>Emory Univ, Atlanta, GA, USA; <sup>2</sup>Emory Univ Sch of Med, Atlanta, GA, USA; <sup>3</sup>Frederick Natl Lab, Frederick, MD, USA; <sup>4</sup>Yerkes Natl Primate Rsr Cntr, Emory Univ, Atlanta, GA, USA

**Background:** The major obstacle to Human Immunodeficiency Virus (HIV) eradication is the presence of a small pool of long-lived latently infected cells that are not affected by antiretroviral therapy (ART), particularly, central memory (T<sub>CM</sub>) and memory stem (T<sub>SCM</sub>) CD4+ T cells. We recently showed that in sooty mangabeys (SMs) these subsets are relatively resistant to SIV infection when compared with rhesus macaques (RMs), while effector memory CD4+ T cells were infected at similar levels between the two species. Based on these data, we hypothesized that SMs may experience a significant decay of the viral reservoir during ART and, possibly, a slower rebound of viremia upon treatment interruption.

**Methods:** Twelve experimental chronically SIV-infected SMs with viral load of 10<sup>3</sup>-10<sup>5</sup> vRNA copies/ml were treated with antiretroviral therapy (ART) regimen consisting of PMPA (20 mg/kg), FTC (30 mg/kg), Raltegravir (300 mg/day) and Darunavir (800 mg/day). The cohort of SMs was divided in four-treatment interruption groups, each of three animals, receiving ART for to 2, 6, 9 and 12 months. Virological and immunological parameters were monitored prior to, during and after ART administration.

**Results:** All animals receiving ART experienced a rapid and significant decline of the plasma viral load with eleven out of twelve SMs showing viremia suppression below the limit of detection (60 copies/ml). Analysis of total circulating CD4+ T cells showed minor changes in terms of frequency and absolute number. Interestingly, the depletion of intestinal memory CD4+ T cells, which is associated with SIV infection of SMs, was reverted when viral replication was controlled by ART and higher levels of intestinal CD4+ T cells were maintained even after therapy interruption. Moreover, ART was associated with decreased frequency of CD8+ HLADR+ T cells in blood and mucosal tissues. While ART induced variable decrease in the level of cell-associated SIV-DNA in blood, therapy interruption resulted in viral rebounds in all treated SMs.

**Conclusions:** This is the first study in which a potent and prolonged four-drug ART regimen was administered to SIV-infected SMs. The treatment was safe and effective in suppressing viral replication, with ART inducing a remarkable level of immunologic reconstitution in the gut. However, ART interruption resulted in rapid viral rebound in all animals, thus indicating that the virus reservoir persists for at least a year despite low levels of infection in CD4+ T<sub>CM</sub> and T<sub>SCM</sub>.

### 354 Bryostatin-1 for Latent-Virus Reactivation: A Phase I, Double-Blind Clinical Trial

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**Background:** The protein kinase C (PKC) agonist bryostatin-1 has been found to be highly potent in inducing latent viral expression through NF-κB signaling. It may also have synergistic effects when combined with other latency reversing agents (LRA). However, no clinical trials with the drug have been performed in HIV-infected patients.

**Methods:** In this pilot, double blind phase I clinical trial (NCT 02269605), we included aviremic HIV-1 infected patients on suppressive antiretroviral therapy (ART) to evaluate the effect of two different single doses of bryostatin-1 (10 µg/m<sup>2</sup> or 20 µg/m<sup>2</sup>) compared with placebo. The primary outcomes were safety and the change from baseline in the cell associated unspliced (CA-US) HIV-1 RNA. Secondary endpoints were PKC activation, plasma HIV-1 RNA, and markers of early immune activation. Bryostatin-1 plasma levels were assessed at baseline, and at 15 min, 30 min, 60 min, and at 2, 4, 8, 12, and 24 hours post-infusion.

**Results:** Twelve patients were included, four in each Study Group. Bryostatin-1 was well tolerated in all the patients regardless the dose of the drug they received. No abnormalities were detected in blood cell counts or blood chemistry. No detectable increases of intracellular HIV-1 mRNA was induced by the single dose infused, without differences between the bryostatin-1 and placebo groups (p=0.44). Using a transcription mediated amplification (TMA) assay, the proportion of positive and negative HIV-1 RNA plasma samples were similar in the three groups, without correlation with the CA-US HIV-1 RNA levels (p=0.676) (Figure 1). We also failed to detect any effect on PKC activity with any of the two doses of bryostatin-1 (p=0.23), as well as in biomarkers activation (CD25 and CD69). After the single dose of bryostatin-1, plasma levels were undetectable in all the patients that received 10 µg/m<sup>2</sup>, and below 50 pg/mL in those receiving 20 µg/m<sup>2</sup> (Figure 2).

**Conclusions:** In this first clinical trial in HIV-infected patients, bryostatin-1 was safe at the doses administered. However, the drug did not show any effect on PKC activity or on the transcription of latent HIV, probably due to the low plasma levels. Given the safety profile of the drug, clinical trials with multiple doses of bryostatin-1 or with a combination of bryostatin-1 and other drugs shown to be synergistic in *ex vivo* studies are warranted.

Figure 1  
Effects of bryostatin on cell-associated unspliced HIV RNA

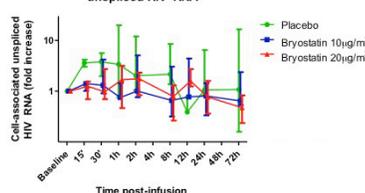
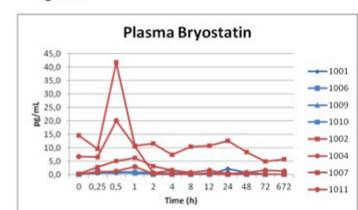


Figure 2



355 **Clinical Administration of Vorinostat Increases NK Cell Capacity to Produce IFN-g**

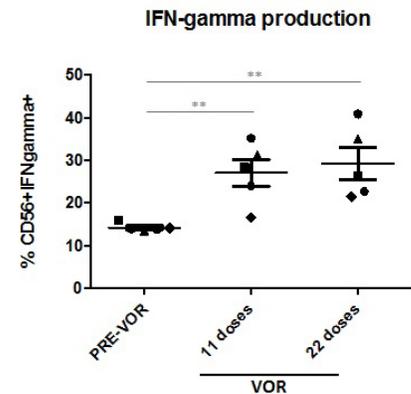
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**Background:** Vorinostat (VOR) can upregulate HIV RNA expression in latently infected resting CD4<sup>+</sup>T cells from treated aviremic HIV-infected patients. However, to achieve viral eradication, latency reactivation must be followed by immune clearance, and thus it is essential to investigate whether reactivation therapies have an impact on immune function. While VOR effect on T cell function has been studied, here we evaluate the impact of *in vivo* administration of VOR on Natural Killer (NK) cell function and phenotype.

**Methods:** Five participants received 22 doses of VOR over 8 weeks (Archin JID, 2014). Cryopreserved PBMC from these patients were used to assess the impact of VOR on CD4, CD8, NK and NKT cell frequency, proliferation and activation. In addition, NK cells were isolated to analyze NK phenotype, including the expression of activating receptors CD16, NKG2D, NKp30, NKp46 and DNAM-1. NK degranulation (CD107a expression) and IFN-g production after culture with the target cell line K562 was measured. Cells were studied before VOR administration, after 11 doses and after 22 doses, and differences assessed using the Mann Whitney test.

**Results:** The frequency of CD3<sup>+</sup>CD4<sup>+</sup>, CD3<sup>+</sup>CD8<sup>+</sup> or CD3<sup>+</sup>CD56<sup>+</sup> cells were unaltered by VOR. However, a modest increase in NK cell (CD3<sup>+</sup>CD56<sup>+</sup>) frequency was observed after VOR treatment (p=0.059 and p<0.05 after 11 and 22 doses). Interestingly, we observed a significant decrease of Ki67 expression in all cell subpopulations after 11 VOR doses, which persisted after 22 doses (p<0.01). Expression of the activation marker CD69 remained stable during VOR treatment. Phenotypic analysis on isolated NK cells showed that CD16, DNAM-1 and NKp30 expression did not change upon VOR exposure, while NKG2D and NKp46 expression increased (p<0.05). Regarding NK cell function, we observed a trend towards an increase in degranulation after VOR exposure (p=NS), and remarkably, a significant increase in IFN-g production after VOR administration (p<0.01).

**Conclusions:** NK cell capacity to produce IFN-g was improved after *in vivo* treatment with VOR. In addition, activating receptors NKG2D and NKp46 were upregulated. Interestingly, Ki67 expression was significantly reduced after 11 and 22 doses of *in vivo* VOR in all cell subpopulations. These results suggest *in vivo* VOR dosing is not reducing NK cell function, and could even provide some enhancement. We are currently testing this hypothesis by evaluating the potential of NK cells exposed to VOR to reduce viral production in latency clearance assays.

356 **CCR5 Gene-Edited Cells Undergo Positive Selection in SHIV-Infected Nonhuman Primates**

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**Background:** Nuclease-mediated gene editing holds great promise in the cure of HIV infection, but the efficacy of this approach in patients is unclear. We have targeted the CCR5 locus in hematopoietic stem cells (HSCs) and assessed engraftment following autologous transplant in the pigtailed macaque. CCR5 disruption in this model should directly protect against infection with simian/human immunodeficiency virus (SHIV). We are evaluating the extent to which CCR5-disrupted cell progeny impact SHIV infection in naïve animals, and viral reservoirs in previously infected, combination antiretroviral therapy (cART)-suppressed animals.

**Methods:** Animals are challenged with CCR5-tropic, HIV enveloped SHIV, and suppressed by three-drug cART following viral set point. Zinc Finger Nucleases (ZFNs) are used to disrupt CCR5 in autologous HSCs; these stem cells and their progeny are subsequently measured *ex vivo* and *in vivo*. In previously infected and suppressed animals, assays are conducted to measure the size of the latent SHIV reservoir before and after transplant.

**Results:** In SHIV-naïve animals, we observe approximately 5% steady state bulk disruption of CCR5 *in vivo*, which persists following SHIV challenge. This approach is equally feasible in SHIV-infected, cART-suppressed animals. Virus-dependent selection for CCR5-disrupted HSC progeny is observed, namely in memory T-cell subsets. Viral reservoir assays demonstrate that sites of viral persistence are present in cART-suppressed animals, and are impacted by the transplant procedure.

**Conclusions:** We have built on our unprecedented demonstration of successful long-term engraftment of CCR5 gene-edited HSCs *in vivo* by showing that these cells can undergo virus-dependent positive selection. We are currently developing gene-editing approaches to enrich for CCR5-edited cells without the need for ongoing viral replication, using adeno-associated virus (AAV) to knock in a chemoselection marker at the disrupted CCR5 locus. This should allow for significant enrichment of infection-resistant cells *in vivo* under the cover of ongoing cART. These results have important implications for cART-independent control of viremia in HIV<sup>+</sup> patients, and should be a promising component of combinatorial HIV cure strategies.

357 **Predicting Determinants of Long-Term HIV Control With Gene Therapy Strategies**

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**Background:** Gene therapy to render lymphocytes resistant to HIV infection is a proposed strategy to achieve long-term antiretroviral-free remission (e.g. CCR5 modification or delivery of antiviral gene products). Preliminary *in vivo* studies of gene therapy, however, have had limited success, and there is a need to elucidate the conditions under which modified cells have sufficient selective advantages to reduce target cell density below a critical level required to maintain infection.

**Methods:** We designed a mathematical model of the competition between wildtype and genetically-modified CD4<sup>+</sup>T cells *in vivo* and the accompanying dynamics of HIV infection. The model was parameterized using data on lymphocyte kinetics, HIV viral dynamics, and the effects of modification. The dynamics were analyzed to determine the conditions under which gene therapy strategies will be successful, and to better understand factors that could be manipulated to improve outcomes.

**Results:** In summary, complete control of HIV with these strategies is difficult. Under a range of model structures and parameters, the most likely outcome is that modified cells co-exist with wild-type cells below the level required to prevent HIV persistence. To obtain viral control off ART, edited CD4<sup>+</sup>T cells must have a higher proliferation rate or a longer lifespan even in the absence of virus, or, edited hematopoietic stem cells must be included. The enrichment level of edited cells is highly dependent on the strength of competition between cells for homeostatic proliferation signals. Interestingly, lower thymic contribution to wild-type T cell levels makes invasion easier and engraftment higher, promoting viral control. Higher viral fitness in wildtype cells can make it easier for edited cells to expand initially but harder to control infection. The potential benefit can be boosted if edited cells are also resistant to causes of bystander cell death that are not viral-load-dependent, or if they provide enhanced immune effector function. Using this model to interpret recent clinical data, observed outcomes may be more consistent with transfusion-mediated changes in cell dynamics than due to resistance of edited cells.

**Conclusions:** A mathematical model of gene therapy strategies for HIV demonstrates that viral control is possible only under a narrow range of conditions, and that further measurement and manipulation of immunological dynamics during engraftment may be necessary to improve outcomes.

**358LB T-Cell Homeostasis and CD8 Responses Predict Viral Control Post SB-728-T Treatment**

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**Background:** Infusion of ZFN driven CCR5 modified T-cells (SB-728-T) in HIV infected subjects was previously shown to durably improve CD4 counts. Long-term CD4 reconstitution and decay of the HIV reservoir were previously correlated with a novel memory stem cell (TSCM) CD4 subset that expands post-infusion and is enriched in CCR5-modified cells. This current study aims at identifying correlates and mechanisms that lead to control of viremia post treatment interruption (TI).

**Methods:** 18 ART treated subjects with CD4 counts above 500 were preconditioned with 0.1-2.0 g/m<sup>2</sup> of Cytoxin prior to infusion of SB-728-T. Subjects initiated TI at 6 weeks post infusion. T cell count and viral load (VL) were monitored regularly for 12 months following treatment. Immunologic and viral analyses (immuno-phenotyping, reservoir analyses, anti-HIVgag effector functions, etc) were performed at selected time points.

**Results:** Of the 9 subjects pre-conditioned with Cytoxin doses of 1.0 and 1.5 g/m<sup>2</sup>, 6 subjects demonstrated durable control of viremia (VL<10,000) in extended TI (duration=14-26 months), with 2 subjects showing consistent ongoing VL measurements <1000 (duration=17 & 20 months). Using a univariate linear regression model, greater levels of CCR5 modified cells before TI (p=0.03) and frequencies of CD4 TSCM during TI (p=0.01) correlated with lower VL, suggesting that greater levels of the HIV resistant T-cell compartment could be critical in conferring post-treatment control possibly by restoring immune homeostasis and providing help to HIV specific CD8 T cells. Multivariate analyses were used to determine parameters that further predict viremia control during TI. Results indicate that higher CD4 TSCM levels, along with greater polyfunctional anti-HIV gag CD8 response during TI (p=0.04) were associated with reduced viral load. Further, HIV reservoir size prior to TI showed a significant interaction with CD8 response in this model (p=0.03), suggesting that greater HIV reservoir were associated to HIV specific CD8 responses that failed to control virus upon TI.

**Conclusions:** Control of viremia during treatment interruption was demonstrated in 6 subjects treated with CCR5 modified T-cells. In a multivariate model, data from immunologic and reservoir analyses suggest that the best predictors for post treatment viral control are restored T cell homeostasis as suggested from greater levels of SB-728-T engraftment, polyfunctional antiviral CD8 responses during TI and lower HIV reservoir levels prior to TI.

**359 Integration Analysis of Latently Infected Cell Lines: Evidence of Ongoing Replication**

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**Background:** HIV cure is limited by persistence of long lived latently infected CD4<sup>+</sup> T cells. Latently infected cell lines are widely used *in vitro* to study HIV latency. We identified and tested the stability of HIV integration sites in latently infected cell lines, obtained from NIH AIDS reagent program, using a newly developed high throughput method.

**Methods:** **Method:** To determine assay sensitivity/efficiency, high molecular genomic DNA of seven latent HIV cell lines were isolated and DNA equivalent of 20 cells each were mixed with genomic DNA from a million HIV negative PBMCs. Additionally, the latently infected cell lines ACH-2, U1 and J1.1 that contain 1 or 2 integrated copies of replication competent HIV and J-Lat 8.4, 9.2, 10.6 and 15.4 cell lines that contain a single replication deficient HIV were passaged 8 times. Genomic DNA isolated after passage 0, 2, 4, 6 and 8 was enzymatically cut to random sized fragments. The fragments were end-repaired, a-tailed and a linker was ligated to the fragments which enables nested amplification. The fragments were subjected to LTR based nested PCR with barcoded nested primers and prepared for Miseq sequencing. Chromosomal alignment was determined using the Blat-UCSC Genome Browser (GRCH38/hg38).

**Results:** The procedure was optimized for robotic processing to increase sample throughput and decrease risk of contamination. The efficiency was 35% and detected 1 HIV integration site in 50,000 uninfected PBMCs. Latently infected cell lines infected with a deficient virus demonstrated a single integration site. In contrast, cell lines harbouring a replication competent virus demonstrated multiple distinct HIV integration sites per 150,000 cells (74 in ACH2, 42 in U1 and 93 in J1.1). J1.1, which is reported to have a single integrated copy per cell, demonstrated two major integration sites in equal frequency. Moreover, ACH-2 cells, when passaged, demonstrated a 2-fold increase in unique HIV integration sites found across the human genome.

**Conclusions:** **Conclusion:** Cell lines latently infected with replication competent HIV demonstrated multiple unique HIV integration sites indicating these cell lines are not clonal. Furthermore, the increase and change in sites of HIV integration observed in ACH-2 cells over time is suggestive of low level virus replication. These findings have implications for the use of latently infected cell lines as models of HIV latency and as standards for quantification of HIV integration.

**360 HIV Integration Sites in Cellular Models of Viral Persistence**

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**Background:** The location of HIV integration sites is thought to play a critical role in the clonal expansion of infected cells and HIV persistence. Cell culture models are often used to study and assess HIV latency and latency-reversing agents, but it is unclear how these models reflect HIV integration *in vivo*. We have developed a high-throughput sequence-capture assay to evaluate the integration site characteristics of 3 cellular models.

**Methods:** Integration site analysis was performed in three cellular models of HIV persistence: ACH-2 cells, Bcl-2 transduced primary CD4<sup>+</sup> cells, and cells from a cultured T<sub>CM</sub> model. A clonal population of ACH-2 cells was also included after limiting dilution and expansion with ART. Sequence enrichment was performed with a diverse biotinylated probe library composed of 52 consensus probe sequences designed using the LANL database. Libraries were sequenced on the MiSeq platform (Illumina) with 300 bp paired-end reads. Sites were annotated with UCSC canonical gene annotations and gene ontology analysis was performed using the Gene Set Analysis Toolkit. Genes identified from the cellular models were also compared to highly represented genes from two previously published patient datasets.

**Results:** While ACH-2 cells are historically thought to have a single site of integration, we identified two main integration sites. In addition, we found that 47% of reads were outside of the two major integration sites. Integration site analysis of the clonal ACH-2 population showed near elimination of single integration sites (ACH-2 bulk culture vs. ACH-2 clone: 39.4% vs. 0.2%, Fisher's P <0.01), suggesting that this assay has an extremely low false-positive rate and confirming the diversity of integration sites within the ACH-2 population. Across the 3 cellular models, there were significant differences in integration site characteristics, including the proportion of clonally-expanded sites, sites within genic regions, exons, and the orientation of the integrated HIV relative to the host gene. Gene ontology analysis of highly represented genes from the patient samples found little overlap with HIV-containing genes from the cell lines.

**Conclusions:** Integration site differences exist amongst the commonly used cellular models of HIV persistence and in comparison to integration sites found in patient samples. These results may contribute to differences seen in the response to latency-reversing agents amongst cellular models and patient-derived cells.

**361 Clonal Integration Site Frequency and Replication-Competent Virus in Patients on ART**

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**Background:** The latent HIV-1 reservoir remains one of the major obstacles to cure HIV. To monitor HIV cure strategies, a validated biomarker is needed that can evaluate the reservoir over time *in vivo*. Consequently, numerous assays are now being investigated to estimate the size of the replication competent provirus.

**Methods:** A comprehensive study was designed to evaluate and compare potential HIV-1 reservoir biomarkers. A cohort of 25 ART treated patients was sampled in which plasma viral load (<50 cp/ml) was suppressed for median of 7 years (5-11). Total, integrated HIV-1 DNA and unspliced (us-) HIV-1 RNA were quantified in peripheral blood. A quantitative

viral outgrowth assay (qVOA) was performed. Subsequently, a nested substudy of 10 patients was set-up to analyse HIV-1 integration sites and tat/rev induced limiting dilutions assay (TILDA). The selected patients had low level of total HIV-1 DNA (<250 cp/10<sup>6</sup>PBMCs) or high level of qVOA (> 2 IU/PM). The percentage of integration site clonality was estimated based on results from the integration site analysis, and the frequency of cells with inducible HIV RNA transcription were estimated using maximum likelihood method. **Results:** Integrated and total HIV-1 DNA were detected in all patients, and both measures correlated well ( $p=0.002$ ,  $R^2=0.85$ ). Replication-competent virus was detected in 80% of patients by the qVOA and this correlated with integrated and total HIV-1 DNA ( $p=0.05$ ,  $R^2=0.44$ ;  $p=0.019$ ,  $R^2=0.54$ ; respectively). In total 317 integration sites were analysed. A wide range of percentage of clonality (25.0-91.4%) was observed between patients. The majority of patients had an average of 30% clonality, which on average comprised of 3.4 predominant clonal integration sites per patient. Patients with higher clonal diversity ( $\geq 6$  clones) had higher estimate for TILDA (frequency of cells with inducible msRNA > 200 cells /10<sup>6</sup> PBMCs) and higher CD4 nadir count ( $p=0.048$ ,  $R^2=0.46$ ). Interestingly, one patient had an extremely high clonality (>90%), which was represented by a single clone. This patient also had no inducible virus in both VOA and TILDA, suggesting that the clonal provirus in this patient is replication incompetent. The single predominant clone was associated with the extremely CD4 nadir count in this patient (3 cells/ul). **Conclusions:** We observed a good correlation between VOA and integrated and total HIV-1 DNA. Integration site sequencing revealed that most of the ART treated patients in this study had an HIV DNA reservoir consisting of 30% clonally expanded cells. These levels of clonal provirus did not affect the amount of inducible virus, except for the one patient with >90% clonality.

### 362 Measurement of Integrated HIV DNA by Pulse-Field Gel Electrophoresis and ddPCR

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**Background:** Assays that measure the size of the latent reservoir with high sensitivity, precision and throughput are needed for clinical studies directed toward eradication. Droplet digital PCR (ddPCR) provides an efficient, standard-independent method to measure HIV DNA, but distinguishing between integrated and unintegrated molecular forms remains a challenge. Previous attempts to remove contaminating episomal and linear unintegrated DNA have been limited by low sensitivity or assay throughput. We sought to develop an assay specific for integrated HIV DNA by initial isolation of high-molecular weight DNA via automated pulse-field gel electrophoresis (PFGE) with the BluePippin system followed by ddPCR.

**Methods:** 8E5 cells were grown in culture, while CD4 cells were enriched from PBMCs and infected with NL43 HIV for 7 days. DNA was extracted by Qiagen QIAamp DNA Blood Mini Kit. 8E5 DNA was loaded at 1000ng or 100ng while CD4 DNA was loaded at 500ng or 50ng per well into Pippin Gel 0.75% DF Cassettes, and separated using a PFGE protocol. After collection, DNA samples were assayed by ddPCR to measure HIV 2-LTR junction, HIV gag, and cellular RPP30. Recovery efficiencies were calculated by assuming that measured copy numbers were Poisson-distributed.

**Results:** Preliminary PFGE protocols resulted in poor recovery of high molecular weight DNA (3-10%). Further optimization, including a custom high-pass PFGE protocol greatly improved recovery of high molecular weight DNA; 29-38% for 8E5 DNA, and 24-61% for CD4 DNA. Efficient recovery of high molecular weight DNA increased with the amount of loaded DNA. Contaminating episomal 2-LTR HIV DNA in infected CD4s was reduced by 98% for 500ng DNA (CI: 97-99%) and 97% for 50ng DNA (CI: 93-100%) as measured by ddPCR. In 8E5, which lack low molecular weight HIV DNA, HIV gag and RPP30 were recovered with similar efficiency. The coefficient of variation of the normalized HIV gag copy number per cell for the combined PFGE-ddPCR protocol was 14%.

**Conclusions:** Automated PFGE to remove episomal and linear species of HIV DNA permits the recovery of chromosomal HIV DNA with high purity and efficient recovery. This technology provides a sensitive and precise approach to the measurement of integrated HIV DNA with sufficient throughput for translational research studies.

### 363 Inhibition of the IN-LEDGF/p75 Interplay by LEDGINS Reduces Reactivation From Latency

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**Background:** Despite the success of cART, HIV-1 persists in a latent state in patients. Persistence is a consequence of proviral integration. LEDGF/p75 is the pivotal chromatin tethering factor targeting HIV integration into active transcription units through its interaction with HIV-1 integrase. We investigated the role of integration site selection in the establishment of HIV persistence employing LEDGF/p75 knockout cells and LEDGINS interfering with the LEDGF/p75-IN interplay.

**Methods:** To evaluate whether the reactivation potential of the quiescent proviral reservoir is dependent on integration site selection during reservoir establishment, LEDGF/p75 WT and KD/KO cell lines were infected with HIV<sub>NL4.3-tCD34</sub> or a double reporter virus that allows direct visualization of the quiescent pool. Formation of the quiescent pool in primary CD4<sup>+</sup> T-cells was studied in the presence and absence of LEDGINS during primary infection. Cells were reseeded 2 weeks post infection and reactivation by different latency reversing agents was monitored by FACS analysis. Integration sites were amplified using LM-PCR.

**Results:** LEDGF/p75 depletion hampers HIV-1 reactivation by latency reversing agents in cell culture. LEDGIN-mediated inhibition of the LEDGF/p75-IN interaction shifts integration out of transcription units and relocalizes the 3D nuclear location of the provirus away from the nuclear rim, comparable with previous observations for LEDGF/p75 depletion. Interestingly, LEDGIN treatment increases the relative fraction of quiescent provirus and blocks HIV reactivation from latency, both in a dose-dependent manner ( $IC_{50}$  CX014442  $\approx 7.24 \mu M$ ). This approach may represent a viable alternative to the shock-and-kill strategies for functional eradication of HIV in patients as LEDGIN treatment apparently succeeds in rendering almost 100% of the virus into a quiescent state refractory to reactivation.

**Conclusions:** LEDGF/p75 determines the integration site pattern of HIV. The presented data in cell lines and in primary cells support our hypothesis that LEDGINS, small molecules that inhibit the interaction between integrase and LEDGF/p75, retarget integration away from the nuclear rim and in genomic regions that are less sensitive to reactivation. If further corroborated in clinical trials, this strategy may pave the way towards a functional cure. Pushing the provirus into quiescence could drive the basic reproduction number of HIV below a threshold required for sustained infection even after treatment interruption.

### 364 Treatment of HIV and AML by Allogeneic CCR5-d32 Blood Stem-Cell Transplantation

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**Background:** The Berlin patient is presumed to be the only person cured from HIV-infection by hematopoietic stem cell transplantation (HSCT) from a homozygous CCR5-d32 unrelated donor. Attempts to reproduce cure by HSCT have failed because of either viral rebound or death due to the underlying malignancy. We here report a patient alive, well and negative for proviral DNA 900 days after allogeneic CCR5-d32 HSCT.

**Methods:** A 41y old HIV-infected male patient was diagnosed acute myeloid leukemia (AML, inv16, CBF-MYH11) in 01/2011. Since the diagnosis of HIV-infection in 10/2010 he had been treated with TDF/FTC+ DRV (01/2011 VL 44 cop/mL; CD4<sup>+</sup> 474 cells/ $\mu$ l). To avoid interactions with chemotherapy DRV was switched to RAL in 03/2011. He achieved CR of the AML after 1 induction course (ICE) and received a 2<sup>nd</sup> induction and 3 consolidation courses according to AML-SG 07/04. In 09/2012 AML relapsed and he was treated with A-HAM and a 2<sup>nd</sup> cycle high-dose cytarabine. While in 2<sup>nd</sup> CR he received 8.74x10E6/kg unmodified peripheral blood stem cells from a female 10/10 CCR5-d32 DKMS-donor after conditioning with fludarabine and treosulfan in 02/2013. Before transplant HIV resistance analysis was performed for PR, RT, IN and viral tropism was determined.

**Results:** There were no significant resistance mutations and the coreceptor-usage was predicted as R5-tropic (Sanger sequencing: FPR 44.5%; NGS: 0.14% X4 at 3.5% FPR; geno2pheno). The proviral DNA load was 29400 cop/mL and in the western blot all anticipated bands could be detected. During transplant and until today the patient remained on

ART (since 06/2014 ABC/3TC/DTG) and the viral load remained undetectable in plasma and liquor. He had a 2<sup>nd</sup> relapse of AML in 06/2013 but re-entered molecular remission after a total of 8 courses of 5-azacytidine and 4 donor lymphocyte infusions. Concerning HIV, all collected samples were negative for proviral DNA by conventional and digital droplet PCR\* in two different labs, namely PBMCs (06/2014, 01/2015\* and 02/2015), rectal biopsy (04/2015) and bone marrow (08/2015\*). Western blots from 06/2014 and 02/2015 showed incomplete patterns with fading bands.

**Conclusions:** Like in the Berlin patient, all tests from the Duesseldorf patient so far suggest that HIV may have been eradicated and that he may be the second individual cured from HIV by allogeneic CCR5-d32 HSCT. Further investigations will be performed before considering the discontinuation of ART.

### 365 HIV-1 Viral Rebound Following Allogeneic Hematopoietic Stem-Cell Transplantation

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**Background:** Allogeneic stem cell transplantation (SCT) has been shown to lead to significant changes to the HIV-1 reservoir and the HIV-1 immune response. However, these changes may not result in viral control upon cessation of antiretroviral therapy (ART).

**Methods:** We studied one patient who had undergone allogeneic SCT with reduced intensity conditioning (RIC) for treatment of non-Hodgkin's lymphoma (NHL). HIV-1 antigens and antibodies (Ag/Ab) were measured by 4<sup>th</sup> generation chemiluminescence microparticle immunoassay (CMIA), Western blot (WB) and HIV-1 Ab avidity assays. HIV-1 specific CD4+ T cell responses were measured by CD25/CD134 upregulation. HIV-1 RNA levels in plasma were measured by two separate real-time PCR assays with 20 and single copy/ml sensitivity; HIV-1 DNA levels were assessed in peripheral blood mononuclear cells (PBMCs) and in isolated CD4+ T cells by PCR using three different primer sets. Viral genotypic sequence analysis was performed by sequencing of the pol RT, PR and INT regions. The presence of the CCR5Δ32 mutation was tested by PCR.

**Results:** The patient received an HLA matched, allogeneic SCT in 2010 for NHL. ART was continued throughout and following the procedure. Post-transplant he experienced systemic grade 2 graft-versus-host disease (GVHD). He was heterozygous for the CCR5Δ32 mutation post-transplant, had no detectable HIV-1 RNA in plasma by either real-time PCR assay, and no detectable HIV-1 DNA by PCR in PBMCs or CD4+ T cells. CD4+ T cell responses to HIV-1 antigen were absent. Ag/Abs to HIV-1 were detectable at very low levels by CMIA, WB and Ab avidity assays. However, the patient experienced viral rebound 4 ½ years following the SCT with high levels of HIV-1 pVL (>10,000,000 copies), seroconversion by CMIA and WB and increasing HIV-1 Ab avidity. HIV-1 DNA levels were readily detectable by real-time PCR following the viral rebound. The patient reported strict treatment adherence but ART drug levels were profoundly reduced at the time of viral rebound, possibly attributable to poor drug absorption. There was no new ART drug-resistance or superinfection based on genotypic sequence analyses.

**Conclusions:** This case describes HIV-1 viral rebound in a patient with profoundly reduced HIV-1 Ag/Ab and DNA levels following SCT, underlining the poor predictive value for viral rebound of these markers. This is highly relevant for the interpretation of these measures in the context of interventions targeting the HIV reservoir.

### 366 A Tale of Two Stem-Cell Transplantations in HIV+ Patients: Clues to Eradicate HIV

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**Background:** To date, Berlin patient's case provides the only evidence of long-term HIV-1 control after allogeneic stem cell transplantation (SCT), potentially due to the CCR5Δ32/Δ32 donor genotype. The case of the two Boston patients, without resulting in HIV cure, showed that the SCT procedure itself involves a tremendous reduction of the viral reservoir. The reason why this happened is unraveled yet. Herein we analyzed two patients from the European cohort EpiStem receiving different types of SCT

**Methods:** Patient 1 (Pt1) and Patient 3 (Pt3) were transplanted in 2012 and 2013 in Hospital Gregorio Marañón due to a Burkitt NHL and NK-NHL respectively. Analysis of the HIV-1 reservoir was performed 29 (Pt1) and 20 (Pt3) months post-transplant using qVOA, ddPCR, and semiquantitative PCR in CD4 T cells from peripheral blood and ileum, and CD3+ cells from bone marrow (BM). SCA was performed in plasma and CSF. Chimerism was performed by short tandem repeat PCR (STR-PCR). Antibody titers were determined by ELISA. Activation markers (CD38 and HLA-DR) were determined in CD4 and CD8 T-cells

**Results:** Pt 1 received a myeloablative single cord blood SCT, supported with third party HLA-mismatched CD34+ cells (haplo-cord SCT). Pt 3 underwent reduced intensity conditioning SCT with peripheral blood progenitor cells (PBPC) of an HLA-matched sibling. Both patients were kept on cART. Donors were wt for CCR5. Pt 3 reached full chimera 1 month after SCT, while Pt 1 still harbors 0.2% of host cell in BM and 0.1% in PB. Pt 3 developed chronic GvH disease but not Pt 1. CD4 T cells from both patients were susceptible to *in vitro* R5 and X4-tropic HIV infection. IUPM in Pt 1 were 0.034 but undetectable (IUPM < 0.006) in Pt3. Total DNA in peripheral CD4 were 25 copies/10<sup>6</sup> cells in Pt1, with 5 HIV RNA copies/ml in plasma. On the contrary, we were unable to detect virus in peripheral CD4, ileum CD4, BM, plasma or CSF in Pt3. Although both patients had a reduction in HIV-specific antibody titers, Pt 3 had lower levels. CD4 and CD8 activation levels were lower in Pt 3 than Pt 1

**Conclusions:** Within the EpiStem cohort, we compared two different SCT procedures, with different outcome regarding the viral reservoir. Allogeneic SCT with PBPC of an HLA-matched sibling donor, with a short engraftment time and a chronic GvH disease resulted in a more drastic reduction of the latent reservoir down to undetectable levels. We hypothesize that the "graft versus HIV-1 reservoir effect" contributes to facilitate the clearance of the viral reservoir

### 367 Ex Vivo Determination of Stem-Cell Transplantation Graft-Versus-HIV Reservoir Effects

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**Background:** Allogeneic hematopoietic stem cell transplantation (HSCT) is one of the few strategies that leads to substantial decreases in HIV-1 reservoir size. Graft-versus-host (GVH) responses are likely responsible for ongoing clearance of residual recipient cells capable of harboring HIV. Beneficial GVH responses, such as those that permit donor cells to clear tumor or residual host hematopoietic cells, may be mediated largely by the innate immune system. To investigate the role of NK cells and other lymphocytes in reactivating and eliminating latent HIV following HSCT, we designed a novel *ex vivo* assay to determine the activity of HLA-matched, post-HSCT donor effector cells on latently infected, pre-HSCT host CD4 T cells.

**Methods:** We adapted a latency model to enable infection of high numbers of CD4 T-cells from individuals with hematopoietic malignancies prior to HSCT with an iGFP-gag HIV viral strain. The infected pre-HSCT CD4 T cells were then co-incubated with PBMC obtained from the same individuals 9-12 months after HSCT. The PBMC were obtained following engraftment and development of full donor cell chimerism (*i.e.* post-HSCT cells are of donor origin despite being recovered from the transplant recipient). We then determined lymphocyte activation, proliferation, viral reactivation and death over a two week period using flow cytometric analyses.

**Results:** We included samples from a total of 30 HIV-negative individuals who received either full myeloablative or reduced intensity conditioning HSCT. Up to 95% pre-HSCT CD4+ T cells were infected with iGFP-HIV-1, with subsequent resting resulting in large numbers of latently infected cells. Flow cytometry was performed 0-13 days following lymphocyte mixing and co-culture. Of note, higher levels of non-proliferating HIV reactivated cells were found in the autogeneic setting compared to that of the allogeneic samples. Conversely, higher levels of proliferating HIV infected cells were seen in the allogeneic samples, peaking at day 7. While expression of activation markers increased on NK,

NKT and CD8 T cells, there were no differences found between the autogeneic and allogeneic groups. However, CD8 T cell activation was strongly correlated with HIV production ( $R^2 = 0.975$ ).

**Conclusions:** Our findings suggest that lymphocytes, including NK and NKT cells, may play an important role in surveillance and clearance of residual HIV-infected cells following HSCT.

368 WITHDRAWN

### 369 Differential Effect of Selenium on HIV Replication and Reactivation in Primary Cells

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**Background:** Sodium Selenite ( $\text{Na}_2\text{SeO}_3$ ) can modulate cellular dynamics that may alter viral replication, and has been correlated with CD4<sup>+</sup> T cell recovery in HIV-infected persons *in vivo*, however cellular mechanisms responsible for these findings are poorly understood. Understanding these factors could elucidate key viral/host cell events controlling viral replication, persistence/reactivation that can be explored as a therapeutic intervention.

**Methods:** Human macrophages (M $\Phi$ ) and peripheral blood mononuclear (PBM) cells were treated with various concentrations of  $\text{Na}_2\text{SeO}_3$  for 2hr prior to infection (HIV-1<sub>bal</sub>). Cells were maintained for 6 days before viral quantification (p24-ELISA and FACS). M $\Phi$  or PBM cells were treated with various concentrations of drug for 6 days and stained with Zombie-Violet live/dead dye and quantified by FACS (M $\Phi$ ) or MTT (PBM cells). Resting memory CD4-T cells were infected with 89.6 prior to IL-7/TGF- $\beta$ -induced latency and reversal was quantified (p24<sup>+</sup> cells, FACS). Latent M $\Phi$  were reactivated with various concentrations of drug with or without PMA+TNF- $\alpha$  and extracellular virus was quantified (p24-ELISA).

**Results:**  $\text{Na}_2\text{SeO}_3$  selectively inhibited HIV-1 replication in M $\Phi$  ( $\text{EC}_{50/90}$  3.9 $\pm$ 1.7 / 19.2 $\pm$ 4.1 $\mu\text{M}$ ), without apparent cytotoxicity ( $\text{IC}_{50} > 100\mu\text{M}$ ), but did not inhibit HIV in lymphocytes ( $\text{EC}_{50} > 40\mu\text{M}$ ).  $\text{Na}_2\text{SeO}_3$  significantly reduced ( $p < 0.01$ ) levels of reactivation from *in vitro* latently infected CD4-T cells *versus* untreated controls at 5 $\mu\text{M}$ , resulting in reactivation levels similar to non-activated controls, without apparent toxicity ( $\text{IC}_{50} > 100\mu\text{M}$  in uninfected lymphocytes).  $\text{Na}_2\text{SeO}_3$  induced reactivation of latent HIV-1 in M $\Phi$  ( $\text{EC}_{50}$  3.9 $\mu\text{M}$ ) without apparent toxicity ( $\text{IC}_{50} > 100\mu\text{M}$ ) and did not inhibit PMA+TNF- $\alpha$  induced reactivation.

**Conclusions:**  $\text{Na}_2\text{SeO}_3$  inhibited HIV-1 replication in M $\Phi$ , but not lymphocytes, and inhibited reactivation of latent HIV-1 from resting CD4-T cells, but surprisingly induced reactivation from latent M $\Phi$ . These findings suggest the effects of  $\text{Na}_2\text{SeO}_3$  on viral replication and reactivation are distinct across CD4-T cells *versus* M $\Phi$ , and that its antiviral potency/reactivation profile could be determined by cell-type specific milieu. These data suggest that  $\text{Na}_2\text{SeO}_3$  could represent an add-on therapy to existing ARV to 1)reactivate latent HIV-1 in M $\Phi$ , 2)specifically inhibit viral replication in M $\Phi$ , and 3)inhibit reactivation in CD4 T cells, which could also prevent viral reseeding and reduce the viral reservoir.

### 370 Kinetics of HIV-1 Latency Reversal Measured by a New Flow-Based Technique

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**Background:** During HIV-1 infection, the virus early establishes a pool of latently infected cells. While current antiretroviral treatment can suppress viral replication, HIV-1 persists in cellular reservoirs. New therapeutic approaches aim to diminish the reservoir by reactivation of latently infected cells. However, the reactivation kinetics of viral mRNA and viral proteins production remain largely unknown. We designed a methodology to assess the dynamics of HIV-1 latency reactivation on the single cell level by flow cytometry to differentiate cells in which only viral mRNA is being produced from cells in which viral proteins or novel HIV-1 viruses are being produced.

**Methods:** J89 cells, a Jurkat cell line containing a single full copy of HIV-1, were used as an HIV-1 latency model. J89 cells were stimulated at several time points (ranging from 0 hr to 48 hrs) with different concentrations of hTNF $\alpha$  cytokine and Romidepsin. Using a new flow cytometry technique that allows for synchronized detection of mRNA targets and intracellular proteins, as well as cell surface markers, combined intracellular staining for p24 Gag protein and gag mRNA was performed.

**Results:** After stimulation of J89 cells with 10 ng/mL of hTNF $\alpha$  for 3h, moderate HIV-1 gag mRNA expression with no intracellular Gag protein was detected. Longer incubation times with hTNF $\alpha$  lead to an increased expression of HIV-1 gag mRNA as well as intracellular Gag protein synthesis. Three distinct populations were identifiable by flow cytometry: only HIV-1 gag mRNA positive cells, HIV-1 gag mRNA and Gag protein double-positive cells, and double negative cells. Romidepsin stimulation (5nM) induced gag mRNA production only after 12h. However, the overall stimulation of J89 with RMD induced a faster gag mRNA and Gag protein production at 18h and 24h compared to hTNF $\alpha$ . Using both treatments CD4 receptor was downregulated on the cell surface following HIV-1 reactivation.

**Conclusions:** We here describe a novel method allowing for the first time to quantify the kinetics of HIV-1 mRNA and protein synthesis after latency reactivation on the single cells level using flow cytometry. This new technique will enable the phenotypic characterization of latently infected cells at different stages of latency reversal, and the identification of specific surface markers from these cells as targets for innate and adaptive immune responses.

### 371 Reactivation of Latent HIV-1 in J-Lat T Cells by Type I Interferon Agonists

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**Background:** We are investigating innate antiviral immune responses to target and eradicate latent HIV reservoirs. Type I interferon pathways are triggered when cellular receptors bind and recognize pathogen-associated molecular patterns (PAMP) in viral RNA, activating transcription factors NF- $\kappa\text{B}$  and IRF3/7 to induce antiviral interferon-

stimulated genes (ISG) that restrict virus replication. As NF-kB also stimulates transcription of the HIV-1 LTR promoter, we hypothesized that viral RNA PAMP might induce a transcriptional cascade that enhances HIV expression in latently infected cells. We are studying how novel host factors, including RNA-binding proteins and chromatin-modifying complexes, modulate HIV reactivation by viral RNA PAMP.

**Methods:** To test this hypothesis, we transfected J-Lat (H2) CD4+ reporter T cell lines containing an integrated HIV-1 sequence, 5' LTR promoter and a GFP reporter gene, with viral RNA PAMP: short chain poly-I:C, long chain poly-I:C (PIC-H), and Sendai virus RNA, in comparison to latency-reactivating drugs SAHA (a chromatin modifier) and prostratin (an NF-kB and PKC activator). Transcription factors and novel host proteins thought to be involved were targeted by RNA interference (mRNA depletion) using siRNA.

**Results:** In J-Lat cells, HIV-1 transcription was activated by viral RNA PAMP, with PIC-H exhibiting a 10-fold increase. PIC-H concurrently induces IFN-beta. Expression of cell stress marker MICB was also induced, raising the possibility of detection of newly-reactivated HIV by natural killer (NK) cells. Interestingly, latency-reversing drugs prostratin and SAHA not only reactivate latent HIV, but significantly induced IFN-alpha/beta, and ISG56, MXA, and antiretroviral gene MXB. We are also studying novel negative regulators of latent HIV reactivation. Targeting RNA binding proteins PKR, NF90, hnRNP A1, and RNase H2 with siRNA resulted in a 2- to 5-fold increase in HIV-1 transcription in cells transfected with PIC-H or Sendai virus RNA. Knockdown of PKR or NF90 also enhanced HIV-1 reporter expression by prostratin more than 2-fold.

**Conclusions:** Viral RNA PAMP can induce latent HIV-1 reactivation in a T cell reporter model, a process regulated by RNA-binding host factors. We are currently exploring the molecular mechanisms involved, and seeking to identify RNA species bound to host factors using next-generation sequencing. These innate antiviral immune pathways might be leveraged in new antiviral therapies to target and eradicate latent HIV reservoirs.

### 372 CSF1R Antagonists Sensitize HIV-Infected Macrophages to Death

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**Background:** HIV can persist in the face of antiviral suppression and there is a need to develop new strategies to eradicate the viral reservoir in infected individuals. HIV activates the colony-stimulating factor 1 receptor (CSF1R or MCSF-R) by phosphorylation to turn monocyte-derived macrophages (MDM) resistant to apoptotic pathways driven by TNF-related apoptosis-inducing ligand (TRAIL). In this study we assessed if CSF1R antagonists sensitize HIV-infected MDM to cell death.

**Methods:** The ability to block the phosphorylation of CSF1R in MDM by CSF1R antagonists was quantified by ELISA after priming the cells with soluble CSF1 in the presence of active or inactive compounds. HIV-infected MDM were treated with CSF1R antagonists in the presence of soluble TRAIL, stained for intracellular gag p24 and analyzed by flow cytometry to assess the amount of infected cells at the end of the cultures. Reporter GFP-HIV was also used to infect MDM and monitor the infection rates by GFP expression. Quantitative PCR and reverse transcriptase assays were performed from extracted cell-associated DNA and cell-free supernatants, respectively, to quantify the effect of the compounds on the absolute cell loss and the viral output. Treatment effects with active compounds were compared to inactive compounds or DMSO by two-way ANOVA.

**Results:** The active CSF1R antagonists PLX3397 and PLX5622 avoid the activation of CSF1R by inhibiting its phosphorylation in human MDM. Inactivation of CSF1R in cultures of infected cells correlated with a 33-38% reduction of the viral output and 45-60% myeloid cell loss, which was unaffected when treated with a non-nucleoside reverse transcriptase inhibitor, nevirapine, or an inactive CSF1R antagonist. Intracellular gag<sup>p24+</sup> cells were specifically sensitized to apoptosis after 4 days of treatment, showing a 25-38% infection decrease. Infections with reporter HIV showed also a 50% decrease in the amount of productively infected GFP<sup>+</sup>-cells after a 5-day treatment.

**Conclusions:** The CSF1R antagonists PLX3397 and PLX5622 reduce the percentage of HIV-infected MDM and the overall viral production *in vitro*. The promising results suggest that compounds targeting CSF1R could potentially purge highly resistant HIV-infected myeloid cells by restoring their apoptotic sensitivity to TRAIL and impact the size of the macrophage viral reservoir in HIV-infected individuals.

### 373 Effect of HIV-1 Nef on MHC-I After Latency Reversal in Primary CD4 T Cells

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**Background:** The main obstacle to clearing HIV-1 infection is the elimination of latently infected cells. This dormant reservoir remains unaffected by ART and is capable of producing replication-competent virus upon reactivation. The accessory HIV-1 protein Nef is expressed early during that viral replication cycle and is known to down-regulate MHC-I from the cell surface during acute infection. Our study aim is to demonstrate that latency reversal in a primary cell model using drugs of potential clinical relevance results in Nef-mediated modulation of MHC class-I and that this is associated with resistance to killing by CTL.

**Methods:** We established a modified version of the Bosque and Planelles primary CD4 T cell latency model using replication-competent viruses and the drugs tenofovir and raltegravir to stop spreading infection in the cultures. We examined Nef-function upon viral reactivation with clinically relevant latency reversing agents (LRAs), specifically romidepsin, bryostatin-1 and prostratin. To analyze infected cells, we used an NL4-3-based clone in which the murine HSA protein (CD24), a cell surface marker, is expressed within the *vpr* ORF. To study the effect of Nef on MHC-I, we created a Nef-M20A mutant, which is selectively defective in the down-regulation of MHC-I. Additionally, we investigated the ability of a CTL clone to eliminate infected primary cells by flow cytometric assays.

**Results:** Our results indicate that Nef down-regulates MHC-I from the surface of infected cells after latency reversal in a primary T cell-based *in vitro* model. Unexpectedly, the data also suggest that certain LRAs might up-regulate MHC-I expression on CD4 T cells, whereas others might enhance the activity of Nef. We now plan to demonstrate that CTL specific for the gag p17 epitope SL9 are able to kill these cells after latency reversal more efficiently when the downregulation of MHC-I by Nef is genetically absent (i.e., in case of the Nef-M20A mutant).

**Conclusions:** Nef is functional with respect to the down-regulation of MHC class-I after latency reversal by drugs of potential clinical relevance. Unexpectedly, certain LRAs may affect the level of MHC-I directly as well as in concert with Nef. If we can now show that the lack of this Nef-activity sensitizes cells in which latency is reversed pharmacologically to killing by CTL, then we expect to invigorate drug discovery targeting Nef as an adjunct to latency reversal in viral eradication strategies.

### 374 Effect of Primary CD8 T Cells on Romidepsin and Bryostatin-Treated CD4 T Cells

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**Background:** Latently infected CD4 T cells represent a major barrier to HIV-1 cure efforts. The current "shock and kill" strategy relies upon the use of latency reactivating agents (LRAs) to selectively induce production of HIV-1 mRNA and proteins in resting CD4 T cells to make them visible to the adaptive immune system. Previous work has shown that treating primary latently infected cells with combinations of LRAs is more effective than single drug treatments. One such combination is bryostatin and romidepsin, the latter of which has further been shown to induce reactivation *in vivo*. However, romidepsin and other HDAC inhibitors have been associated with impaired CTL responses. Thus, we sought to examine the stimulated, primary effector CD8 T cell response of cART-treated HIV+ individuals to latently-infected CD4 T cells cultured with the LRAs bryostatin and romidepsin (B/R).

**Methods:** Resting, CD4 T cells from 6 cART-treated HIV+ chronic progressors were treated with the combination B/R to reactivate latently infected cells. To determine the ability of the CD8 effector response to identify and kill these cells, primary CD8 T cells that had been stimulated with overlapping Gag and Nef peptides and IL-2 for seven days were added to culture at 6 hours post-stimulation. Cell-associated and supernatant HIV mRNA from 24 hours post-stimulation was measured via qPCR to determine the efficacy of the CD8 effector response.

**Results:** B/R treatment of primary, resting CD4 T cells resulted in a  $12.3 \pm 3.0$  fold increase in cell-associated HIV mRNA and a  $30.4 \pm 10.7$  fold increase in supernatant HIV mRNA. Despite the stimulation of the primary CD8 T cells, there was no significant difference in either cell-associated or supernatant HIV mRNA when B/R-treated CD4 T cells were incubated with CTL in 3 patients. However a 2-fold reduction in cell-associated mRNA was seen in the other 3 patients.

**Conclusions:** While the combined treatment of latently infected CD4 T cells with romidepsin and bryostatin induces upregulation of HIV mRNA, primary CD8 T cells stimulated with Nef and Gag peptides in the presence of high levels of IL-2 were unable to eliminate the reactivated cells in the majority of chronic progressors on ART. However effective responses were seen in several patients. The results suggest that in some cases, effective priming of the cytotoxic T lymphocyte responses may lead to elimination of reactivated latently infected CD4+ T cells.

### 375 Dendritic Cell T-Cell Culture Facilitates HIV Latency in Proliferating CD4+ T Cells

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**Background:** Latent infection of resting CD4+ T-cells is a major barrier to HIV eradication. We have previously shown that latency occurs in non-proliferating CD4+ T-cells following co-culture with myeloid dendritic cells (mDC) and monocytes, and have now examined whether latency can be established in proliferating CD4+ T-cells under similar conditions.

**Methods:** Resting CD4+ T-cells stained with the proliferation dye eFluor670 were cultured alone or with syngeneic mDC, plasmacytoid DC (pDC) or monocytes (CD14+) in the presence of staphylococcal enterotoxin B (SEB). After 24hrs, cultures were infected with CCR5-tropic enhanced green fluorescent protein (EGFP)-reporter HIV. On day-5 post-infection, non-productively-infected, non-proliferating and proliferating T-cells were sorted and further cultured until day 12 with IL-7, T-20 (fusion inhibitor) and L8 (integrase inhibitor). On day 5 and 12 post-infection EGFP was quantified following activation with  $\alpha$ CD3/ $\alpha$ CD28+L8 as a marker of inducible latent infection. At day 5, expression of markers of activation (CD69, CD25), proliferation (Ki67), immune checkpoints (PD-1, Tim-3) and T cell subsets (CCR7, CD27) was determined. T-cell receptor V $\beta$  chains that were SEB specific (17, 3) and non-specific (13.1) were measured on T-cells.

**Results:** Inducible latent infection was detected in non-productively infected, proliferating T-cells co-cultured with mDC, pDC and monocytes following activation with  $\alpha$ CD3/ $\alpha$ CD28+L8 (median(interquartile range), 403(53-1470), 40(1-213), 177(1-980) EGFP+ cells/10,000 viable cells respectively; n=15) and only in mDC and monocyte cultures using ALU-LTR nested PCR (1000 copies per 10<sup>6</sup> cells each; n=7). At day 12, inducible latent infection was observed in proliferating T-cells co-cultured with mDC and monocyte, but not pDC where cell viability was less than 20% (n=4). Proliferating CD4+ T-cells from mDC, pDC and monocyte co-cultures expressed high levels of CD25 (97, 86, 99% respectively, n=6), CD69 (39, 32, 46%; n=5), Tim-3 (23, 67, 48; n=4), lower levels of PD-1 (0.1, 1.9, 3%; n=4) and Ki67 (7, 18, 11%; n=7). The cells remained CCR7+CD27+ until day 12. SEB non-specific V $\beta$  was not enriched in either non-proliferating or proliferating T-cells, indicating that these cells do not differ in MHC-II interaction.

**Conclusions:** Proliferating latently infected cells may be an important mechanism for HIV persistence and these cells should be included in studies of HIV persistence in HIV-infected individuals on ART.

### 376 Role of Dendritic Cell Polarization in the Induction of HIV-1 Latency Reversal

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**Background:** Dendritic cell (DC)-based strategies have been used to induce antigen specific CTL immunity in HIV during combination ART (cART), with reduction in viral load after cART interruption. Recent findings suggest DC also play a role in HIV-1 latency reversal. We have demonstrated that a DC-based therapeutic HIV vaccine increases residual viremia in cART-suppressed individuals after analytic treatment interruption. We therefore assessed the in vitro capacity of differentially polarized DC to induce HIV-1 latency reversal during cART.

**Methods:** Monocyte derived immature DC (iDC) were generated with IL-4 and GM-CSF for 5 days. The iDC were then exposed for 2 days to a combination of IFN- $\gamma$ , TNF- $\alpha$ , IL-1 $\beta$ , IFN- $\alpha$  and Poly(I:C) to generate high IL-12p70 producing, type-1 polarized DC (DC1), or TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and PGE<sub>2</sub> to generate IL-12p70 deficient DC (DC2). Two models of HIV-1 latency reversal were tested: 1) Total resting CD4+ (rCD4) T cells from HIV-1 uninfected donors were treated with CCL19, infected with HIV-1<sub>XLAR</sub>, exposed to low-dose IL-2 with Efavirenz to establish latency, and subsequently stimulated with DC in the presence or absence of superantigen staphylococcal enterotoxin B (SEB). 2) DC derived from HIV-1 infected individuals on cART (viral RNA <20 copies/mL plasma) were co-cultured with autologous rCD4 T cells, in the absence or presence of Efavirenz and/or SEB. Cellular and culture supernatant viral RNA were quantified by qRT-PCR.

**Results:** All antigen expressing DC resulted in the propagation of replication competent virus from rCD4 T cells from HIV-1 infected participants on cART. However, DC1 consistently induced significantly greater levels of replication of HIV-1 RNA in rCD4 T cells than either iDC or DC2 in both models of HIV-1 latency reversal. Importantly, the greatest amounts of latency reversal were mediated by antigen expressing DC1.

**Conclusions:** While they are known to have a strong capacity to produce IL-12p70 and drive antigen specific CTL responses in vitro, this study demonstrates that DC1 are also superior to iDC and DC2 as a potential HIV-1 latency reversing tool. Although the mechanisms of this effect have yet to be elucidated, the enhanced ability of DC1 to drive HIV-1 latency reversal was antigen dependent. Information gathered from these studies will be used in the development of novel DC1-based vaccine strategies to facilitate both the "kick" and "kill" of the HIV-1 reservoir.

### 377 Naïve CD4+ T Cells Harbor a Large Inducible Reservoir of Latent HIV-1

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**Background:** The latent viral reservoir in resting CD4+ T cells represents a major barrier to eradicating HIV-1 infection. Central memory (T<sub>CM</sub>) and transitional memory CD4+ T cells are thought to constitute the major reservoirs of latent HIV-1 because the frequency of infected cells, as assessed by total HIV-1 DNA, is greater compared to the other resting CD4+ T cell subsets. By contrast, HIV-1 DNA is detected less frequently in naïve (T<sub>N</sub>) CD4+ T cells, and consequently there has been little emphasis on studying the establishment and reversal of viral latency in these cells. Here, we compared ex vivo production of HIV-1 from T<sub>N</sub> and T<sub>CM</sub> cells after exposure to latency reversing agents (LRAs).

**Methods:** Resting CD4+ T<sub>N</sub> and T<sub>CM</sub> cells were purified using antibody-coated beads from PBMCs of HIV-1-infected individuals obtained via leukapheresis following written consent who had been on suppressive ART for  $\geq 5$  years. Total HIV-1 DNA was measured by quantitative real-time PCR. Reversal of latency was assessed by measuring virion-associated HIV-1 RNA, using a single copy assay, following treatment with a panel of LRAs (anti-CD3/CD28 antibodies, PHA + IL-2, PMA + ionomycin, prostratin, panobinostat or romidepsin). Infectious virus production was quantified by the quantitative viral outgrowth assay (Q-VOA).

**Results:** Levels of total HIV-1 DNA were higher in CD4+ T<sub>CM</sub> cells (mean: 2,215 copies/10<sup>6</sup> cells; range: 723-4,533) compared to T<sub>N</sub> cells (mean: 735 copies/10<sup>6</sup> cells; range: 181-1,023), although this difference was not significant (p=0.09). Following exposure to anti-CD3/CD28 antibodies, virion-associated HIV-1 RNA levels were similar between T<sub>CM</sub> cells (mean: 19,299 copies/mL; range: 2,260-71,350) and T<sub>N</sub> cells (mean: 22,866 copies/mL; range: 0-57,663). In cells from 3 of the 5 donors, the virion-associated HIV-1 RNA levels produced were higher for T<sub>N</sub> compared to T<sub>CM</sub> cells, independent of the LRA used. Replication-competent virus was recovered from both T<sub>N</sub> and T<sub>CM</sub> CD4+ T cells by Q-VOA.

**Conclusions:** Although the frequency of HIV-1 infection is lower in CD4+ T<sub>N</sub> compared to T<sub>CM</sub> cells purified from individuals on long-term suppressive ART, as much, if not more, virus is produced from T<sub>N</sub> cells after exposure to LRAs. This finding shows that quantifying HIV-1 DNA alone may not be predictive of the size of the inducible latent reservoir in different CD4+ T cell subsets and that greater attention should be given to the reservoir of HIV-1 in T<sub>N</sub> cells.

**378 Follicular Tregs Reduce HIV Replication and Reactivation From Latency Ex Vivo**

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**Background:** Most HIV replication occurs within B cell follicles in follicular T helper cells ( $T_{FH}$ ), which likely contribute substantially to the HIV reservoir in virologically suppressed individuals. Follicular Tregs ( $T_{FR}$ ), also reside in B cell follicles, yet little is known about the effects of  $T_{FR}$  regulation on HIV replication and HIV latency within  $T_{FH}$ . We hypothesized that  $T_{FR}$  are able to suppress both HIV replication in  $T_{FH}$  and reactivation of HIV from latent infection of CD4 T cells during infection *ex vivo*.

**Methods:** Tonsil cells were spinoculated with X4- or R5- tropic GFP reporter viruses, or mock spinoculated, and  $T_{FH}$  (CD3+CD8-CXCR5+CD25lo/-) were analyzed by flow cytometry after 2 days. In select experiments, CD25 cells were depleted prior to spinoculation. For latency experiments, isolated CD4 T cells were spinoculated with X4 HIV and cultured with CXCR4 antagonist (bicyclam) to prevent *de novo* infection in culture, sorted to remove GFP+ cells after 2 days, and cultured an additional 4 days to confirm stable latency in culture. During removal of GFP+ cells,  $T_{FR}$  (CD3+CD8-CXCR5+CD25hi) were also isolated and added back to CD4 T cells at a 1:1 ratio. To stimulate reactivation of cells, cultures were treated with 500 nM SAHA for 24 hours at day 4. Cell phenotypes and GFP expression were determined by flow cytometry. Statistical analyses were performed by Wilcoxon matched-pair tests using GraphPad Prism 6.

**Results:** Removal of CD25+ regulatory cells led to an increase in %GFP+  $T_{FH}$  (median 1.7 fold in X4,  $p=0.002$ , 2 fold in R5,  $p=0.004$ ;  $n=10$ ) and an increase of GFP intensity in  $T_{FH}$  (median 1.2 fold in X4,  $p=0.002$  1.5 fold in R5,  $p=0.002$ ;  $n=10$ ) compared to undepleted cultures. A natural and stable HIV reservoir is generated by spinoculation of unstimulated tonsillar CD4 T cells and removing productively infected (GFP+) cells during culture. Latently infected tonsil CD4 T cells are reactivated during SAHA treatment (median 2.38%  $p24+$ GFP+;  $n=3$ ), but not in the presence of  $T_{FR}$  (median 0.1%  $p24+$ GFP+;  $n=3$ ).

**Conclusions:** Regulatory T cells inhibit HIV replication in  $T_{FH}$  and prevent HIV reactivation from latency in CD4 T cells *ex vivo*. They likely contribute to viral persistence in virologically suppressed individuals *in vivo*. Targeting the suppressive mechanisms of  $T_{FR}$  that prevent HIV reactivation in latently infected cells may be an important therapeutic approach to efficiently activate and purge the latent reservoir *in vivo*.

**379 A Subset of Infectious Proviruses Persist and Expand Following Activation Ex Vivo**

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**Background:** The most effective latency reversing agents for HIV-1 are also potent T-cell activators (Cillo, PNAS 2014). Recent studies show that virus producing cells can persist and expand *in vivo* (Maldarelli, Science 2014). We hypothesized that activation of HIV-infected CD4+ T-cells could lead to clonal expansion of proviruses rather than their elimination.

**Methods:** We established an *ex vivo* cell culture system involving stimulation of patient-derived CD4+ T cells with PMA/ionomycin (day 1-7), followed by rest (day 7-21), and then restimulation (day 21-28). Raltegravir (300 nM) and efavirenz (300 nM) were added to cultures to block virus spread throughout the experiments. Cell-associated HIV-1 DNA (CAD) and virion RNA in the supernatant were quantified by qPCR at weekly intervals. Single genome sequencing (SGS) was performed to characterize proviruses and virion RNA. The replication-competence of proviruses in cultured cells was determined by the viral outgrowth assay (VOA) at multiple time points.

**Results:** Experiments were performed with purified CD4+ T-cells from five consecutive donors who had been suppressed on ART for  $\geq 2$  years (median = 13.4 years). In all experiments, HIV-1 RNA levels in supernatant increased following initial stimulation, decreased or remained stable during the rest period, and increased again with restimulation. Cell-associated HIV-1 DNA levels did not show a consistent pattern of change. SGS revealed several different outcomes of proviral populations: 1) virus production following the first but not the second stimulation; 2) virus production following only the second stimulation; 3) virus production following both stimulations; 4) no virus production after either stimulation, 5) cellular expansion of non-induced proviruses; and importantly 6) persistence and likely expansion of induced proviruses, including replication-competent proviruses. This latter outcome was observed in 3 of 3 donors tested, as evidenced by identical sequence matches between  $p24$  positive VOA wells and virion RNA sequences at multiple time points.

**Conclusions:** These results indicate that reversal of HIV-1 latency with CD4+ T cell activation results in multiple outcomes of proviral populations, including persistence and expansion of replication-competent proviruses. These findings underscore the complexity of eliminating HIV reservoirs with latency-reversal agents and highlight the need for new strategies to kill HIV-infected cells before they can clonally expand.

**380 Transcription of Novel HIV-1 RNA Species in the Setting of "Undetectable" Virus**

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**Background:** Proviruses in circulating PBMCs have been characterized as "defective" and are thought to represent a "graveyard" of viral sequences with little contribution to HIV-1 pathogenesis. We have previously demonstrated the ability of such defective provirus to transcribe viral RNA and its long-term persistence *in vivo*. In the present study, we demonstrate that the presence of transcription-competent "defective" proviruses is a consistent finding in patients with HIV-1 infection and examine the importance of their persistence in the context of the persistent immune activation seen in patients with prolonged viral suppression.

**Methods:** Four HIV-infected patients (pts) with  $pVL \geq 40$  copies/ml (range 26,758-225,658) and four on suppressive cART with  $pVL < 40$  copies/ml for  $> 6$  yrs (range 6.1-11.9) were studied. Nuclear and cytoplasmic fractions of the same population of CD4+ T cells served as sources for HIV-DNA and RNA, respectively. Single genome amplicons generated via a U5-U5/R PCR were used to sequence full-length HIV-1 genomes. Western blots were performed using the Cambridge Biotech HIV-1 Western Blot kit.

**Results:** Single-genome sequencing of 175 HIV-1 proviruses determined that 53% (48/90) in  $pVL \geq 40$  pts and 99% (84/85) in  $pVL < 40$  pts were defective. The majority of defective proviruses could be characterized as truncated forms with gross internal deletions (87% in  $pVL \geq 40$ ; and 93% in  $pVL < 40$ ). The remainder, while full-length, contained multiple lethal mutations (13% in  $pVL \geq 40$ ; and 7% in  $pVL < 40$ ). Truncated proviruses were also found following *in vitro* infection of primary CD4+ T cells with the DH12 clone of HIV-1 as early as 24 h post infection with a frequency of 20% (4/20). A number of novel RNAs and corresponding truncated proviral DNAs (including 2 cases with evidence of clonal expansion) were identified in  $pVL < 40$  pts, suggesting ongoing transcription of these defective proviruses. These novel RNA sequences contained open reading frames not corresponding to known mRNA species. Western blots obtained from the sera of these patients showed persistence of viral bands in a variety of patterns suggesting an asynchronous decay in viral proteins *in vivo*.

**Conclusions:** Cells harboring defective proviral DNA capable of transcribing novel RNAs with open reading frames can be seen following *in vitro* and *in vivo* infection. The proteins encoded by these "zombie" proviruses may explain the persistence of antibodies to HIV-1 and the persistent immune activation seen even in patients with "undetectable" virus.

### 381 Advanced MRI Predict Cognition at 1-Year Follow-up in Mildly Impaired HIV+ Patients

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**Background:** Multi-contrast quantitative MRI has shown to be highly sensitive to detect brain damage in virally suppressed HIV patients. In this study we assessed the value of quantitative MRI in (i) monitoring the longitudinal evolution over 1 year of brain structural abnormalities in aviremic patients with and without minor cognitive impairment (MND+ and MND-) and in (ii) predicting cognitive outcome at one year in HIV+ MND+ patients.

**Methods:** We performed T1/T2\* relaxometry and Magnetization Transfer Imaging at 3T MRI in 36 aviremic HIV+ patients, at three time points (tp): study entry (tp0), 6 months (tp1) and 1 year follow-up (tp2). All patients underwent extensive cognitive and psychiatric examination at all time points. We applied three generalized linear models to fit each cognitive score at tp1 and tp2 as a function of (i) the information obtained by structural MRI data in each region of interest (T1 or T2\* or magnetization transfer ratio-MTR) at tp0 and (ii) covariates (age and gender). Multiple comparison correction was done using Bonferroni and cross-validation was performed with a leave-one-out-test. Multivariate Analysis of Variance was used to study the influence of independent variables (age, gender, patients group, MRI sequence and time-points) on three dependent variables (regional mean T1 relaxation time (rt), T2\* rt and magnetization transfer ratio) at the 3 time points.

**Results:** At 6 months, cognitive performances were mainly predicted by T1/T2\* in basal ganglia and thalamus (table 1); at 1 year, they were also predicted by T1/T2\* rt in global white and grey matter (WM and GM, table 1). No significant evolution of microstructural brain properties was observed in both MND+ and MND- over 1 year. These results suggest that in HIV+ patients (i) the integrity of the cortico-striatal-thalamic loop highly influence executive function, working memory and reaction time and that (ii) WM integrity plays a major role in processing speed.

**Conclusions:** Multi-contrast MRI metrics appeared to be predictors of cognitive function in well-treated HIV patients. Predicting the evolution of cognitive deficits in HIV patients is important as it might help selecting patients benefitting of more aggressive antiretroviral therapy or of targeted cognitive intervention. Future studies should aim at developing tools based on quantitative metrics to assess microstructural brain alterations on a single-patient basis.

SIX MONTHS F.U.	PREDICTIVE MODELS BASED ON SINGLE MRI CONTRASTS
TMTZB	T1 rt in WM, GM, caudate and pallidum (p-corr = 0.007, Adjusted R <sup>2</sup> = 0.97, R(cv-corr) = 0.5)
ECIZ	T1 rt in thalamus and pallidum (p-corr = 0.02, Adjusted R <sup>2</sup> = 0.98, R(cv-corr) = 0.5) T2* rt in thalamus and pallidum (p = 0.003, Adjusted R <sup>2</sup> = 0.98, R(cv-corr) = 0.5)
SWMEZ	T2* rt in caudate and pallidum (p = 0.0006, Adjusted R <sup>2</sup> = 0.96, R(cv-corr) = 0.5)
ONE YEAR F.U.	
SOCPZ	T1 rt in WM, GM, caudate, pallidum and putamen (p = 0.006, Adjusted R <sup>2</sup> = 0.99, R(cv-corr) = 0.8)
DSBZ	T1 rt in WM, GM and pallidum (p = 0.0002, Adjusted R <sup>2</sup> = 0.99, R(cv-corr) = 0.8)
RTIZ	T2* rt in WM (corrected p-value of the model: 0.02, Adjusted R <sup>2</sup> = 0.95, R(cv-corr) = 0.5)
SWMEZ	T2* rt in WM, caudate, pallidum and putamen (p = 0.03, Adjusted R <sup>2</sup> = 0.98, R(cv-corr) = 0.3)

**Table 1:** GLM models predicting cognitive performances in HIV+ patients with mild cognitive impairment at 6 months and 1 year follow-up (f.u.). TMTZ-B: Trail making test B z-score; DSBZ: Digit Span Backward; SWMEZ: Spatial Working Memory errors z-score; SOCPZ: Stockings of Cambridge completed problems z-score; RTIZ: reaction time z-score; rt, relaxation times; GM/WM: grey and white matter; p-corr and cv-corr: p-value and cross-validation R after Bonferroni correction.

### 382 Topographies of Cortical and Subcortical Volume Loss in HIV and Aging in the cART Era

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**Background:** Studies of HIV-associated brain atrophy often focus on *a priori* brain regions of interest, which can introduce bias. We used a minimally-biased multivariate approach to analyze changes in brain volumetrics associated with HIV and its relationship to aging, viral factors, and combination antiretroviral therapy (cART).

**Methods:** A cross-sectional, comparative study of 51 HIV-negative (HIV-) and 146 HIV-infected (HIV+) participants was conducted. Structural MRI was acquired and analyzed from participants using principal component analysis (PCA) to reduce dimensionality and determine topographies of volumetric changes. Neuropsychological (NP) assessment was examined using global and domain-specific scores. The effects of HIV-disease factors (e.g. viral load, CD4, duration infection, etc.) on brain volumes and cognitive function were investigated using least absolute shrinkage and selection operator (LASSO) regression.

**Results:** Two components of interest were visualized using PCA. An aging effect predominated both components. The first component, a cortically-weighted topography, accounted for a majority of variance across subjects (43.5% of variance) and showed an independent HIV effect. A secondary, subcortically-weighted topography (4.6%) showed HIV-status accentuated age-related atrophy. In HIV patients, the cortical topography correlated with global NP scores and nadir CD4, while subcortical volume loss correlated with recent viral load.

**Conclusions:** Cortical regions showed the most prominent volumetric changes due to aging and HIV. Within HIV+ participants, cortical volumes were associated with immune history while subcortical changes were associated with current immune function. Cognitive function was primarily associated with cortical volume changes. Observed changes in chronically infected patients may reflect both past history of infection and current viral status. Future longitudinal studies should follow HIV patients soon after seroconversion.

### 383 Meta-Analysis of Large HIV Cohort Reveals CD4 Effects on Longitudinal Brain Atrophy

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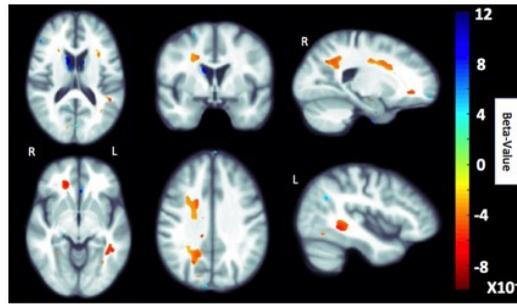
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**Background:** While advances in ART have dramatically improved life expectancies of HIV+ people, chronic infection is still associated with neurological deficits and brain injury. By pooling datasets across independent studies of HIV, we can boost statistical power to map brain disease progression and generalize findings to the world-wide HIV epidemic. In a pilot tensor based morphometry (TBM) study for the HIVNC consortium, we examined the effects of baseline CD4 count on longitudinal maps of local volumetric changes in 169 chronically HIV+ individuals on stable cART from 7 independent sites.

**Methods:** For each site, anatomical T1-weighted image pre-processing included removal of extra-cerebral tissue, N3 bias field correction, ONLM denoising, and linear registration to a target brain template. For each subject, follow up T1-weighted scans were linearly and elastically registered to their respective baseline scans and 3D Jacobian expansion factor maps were created that characterized voxel-wise percent annual rate of volumetric changes. Longitudinal Jacobian maps were then spatially normalized to a common baseline study-specific template. For each site, voxel-wise regression models, adjusting for effects of sex and age, were used to test for effects of baseline CD4 count on structural variations in Jacobian maps. Across sites, an inverse variance weighted fixed-effects meta-analysis was used to combine results from independent measurements. The standard FDR method was used to correct for multiple comparisons ( $q=0.05$ ).

**Results:** While no single site yielded significant findings, the meta-analysis took advantage of the consistent associations across sites and revealed that HIV+ individuals (mean baseline CD4: 381 +/- 235, Age: 48 +/- 8.6, HIV duration: 12 yrs +/- 6.7) with a lower baseline CD4 count displayed significantly higher rates of atrophy (expanded ventricles and white matter atrophy) than healthier subjects (critical  $p=0.0003675$ ).

**Conclusions:** Lower CD4 count at baseline was associated with increased longitudinal rates of atrophy. This analysis represents the first large-scale, multi-site, longitudinal study of brain change in HIV and demonstrates the power of meta-analysis. HIV findings are often complicated by a host of factors including ART adherence/efficacy, substance abuse, comorbidities, and chronic inflammation. Pooling additional independent, heterogeneous study samples will further increase power to determine how various predictors of brain change generalize to the HIV epidemic.



### 384 Brain Volumetric Changes After 2 Years of ART Initiated During Acute HIV Infection

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**Background:** Brain alterations (e.g., neuroinflammation, cortical atrophy, enlargement of brainstem) in the first year of HIV infection have been reported. However, little is known about whether changes in brain volumes may be detected over time in individuals on combination antiretroviral therapy (cART) initiated during acute HIV.

**Methods:** We prospectively enrolled individuals in Bangkok who had acute HIV infection (Fiebig stages I-IV), on average 15 days after the estimated date of exposure. Participants underwent brain magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) at 1.5T and immediately thereafter initiated cART. MRI/MRS was repeated at regular intervals. Global and regional gray matter (GM) and white matter (WM) volumes at baseline and 24 months post-cART were obtained from T1-weighted MRI data by the FreeSurfer longitudinal processing stream. Wilcoxon signed-rank tests for related samples assessed changes from baseline to 24 months. For brain regions showing significant change, relationships between baseline metabolite concentration ratios over creatine (Cr) and 24-month volumes were examined by linear regression controlling for baseline volumes and patient age.

**Results:** We evaluated 38 individuals (33 male) whose median (range) age was 29.0 (18-48) years, CD4 count 386 (132-1127) cells/mm<sup>3</sup> and plasma HIV RNA 5.54 (2.78-7.57) log<sub>10</sub> copies/mL at baseline prior to cART. Over the first 24 months of cART, volumetric decreases of 2% were found in caudate (p=0.002), putamen (p=0.00008) and pallidum (p=0.034), with a 1% decrease in total subcortical GM (p=0.002). Cerebellar GM and brainstem volumes increased by 5% (p=0.00003) and 1% (p=0.026), respectively. Clinical variables at baseline did not correlate with volumes at 24 months. Baseline N-acetylaspartate (NAA)/Cr in frontal GM predicted 24-month volumes of caudate ( $\beta=0.11$ , p=0.030) and putamen ( $\beta=0.15$ , p=0.016) independently of age and baseline volume. Pallidum volumes at 24 months were associated with baseline choline/Cr ( $\beta=0.19$ , p=0.018) in frontal WM.

**Conclusions:** HIV patients demonstrated regional brain volumetric changes despite very early initiation of cART. Longitudinal studies of HIV-uninfected Thai individuals are needed to determine whether our observed atrophy rates are higher than those in healthy controls. Frontal neuronal injury during acute infection may predict basal ganglia atrophy. The cause and effect of cerebellar cortical enlargement are yet to be understood.

### 385 Regionally Specific Cortical Thinning in HIV+ Patients in the cART Era

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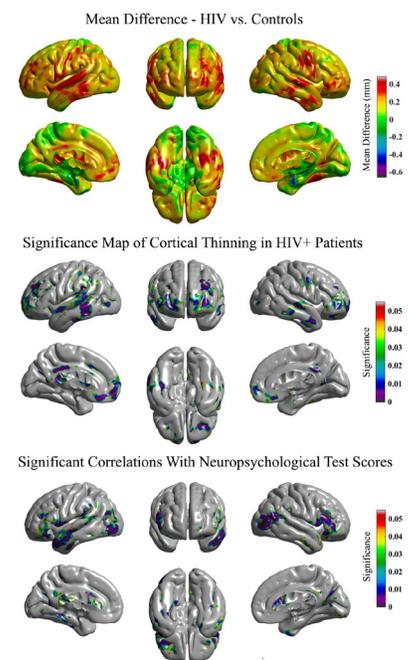
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**Background:** HIV infection and its treatment affect the brain directly and indirectly, and can lead to cognitive impairment. Existing neuroimaging work has found evidence of brain volume loss in HIV+ patients, mainly in subcortical structures. However, the methods used to date may miss subtle cortical changes. Cortical extraction tools provide a sensitive measure of cortical thickness. In this cross-sectional study, we compared cortical thickness in HIV+ patients against healthy controls, and correlated it with measures of current and past viral suppression, and neuropsychological scores (NPZ-4).

**Methods:** 133 HIV+ participants were recruited from a US Infectious Disease clinic, and 58 healthy controls (CTL) were recruited from the local community. All underwent T1-weighted brain magnetic resonance imaging and neuropsychological testing (Hopkins Learning Test-Revised, Digit-Symbol Modalities Test and Trail-Making Tests A&B). Cortical extraction captured the cortical surface and measured its thickness. Cortical thickness estimates were regressed on a vertex-wise basis against HIV status, nadir CD4+, current CD4+, viral load and NPZ-4.

**Results:** The two groups were demographically similar (HIV+ age [mean  $\pm$  SD]: 48  $\pm$  12, nadir CD4+: 216  $\pm$  200, current CD4+: 529  $\pm$  314, 72% virologically suppressed; CTL age: 43  $\pm$  12). HIV+ patients performed worse than controls on neuropsychological testing (NPZ-4: HIV+ -0.85  $\pm$  1.27; CTL -0.14  $\pm$  0.91, p<0.001). Significant cortical thinning was observed in specific brain regions in HIV+ patients compared to controls. The cortex was 0.2–0.4 mm thinner in the HIV+ group bilaterally in the lateral temporal and frontal lobes, as well as posterior cingulate and orbitofrontal cortex. Lower NPZ-4 scores were associated with thinner cortex in the left lateral temporal pole, left inferior occipital, right lateral occipital and right inferior lateral frontal cortices. There was no significant relationship between cortical thinning and measures of current or past viral suppression.

**Conclusions:** Regionally specific cortical thinning was observed in this large sample of HIV+ patients, despite good current viral suppression in the majority. Cortical thinning was related to NPZ-4 scores, but was unrelated to systemic indicators of the severity of HIV infection. These findings show that even well-controlled infection has an impact on cortical thickness, and neuropsychological performance, perhaps related to central nervous system viral reservoirs, accelerated aging, or cART neurotoxicity.



386 **NeuroHIV: A Novel High-Resolution Subcortical Shape Analysis**

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**Background:** Over 50% of HIV+ individuals experience neurocognitive impairment with brain injury having been documented in those on stable antiretroviral therapy. We applied a novel high-resolution subcortical shape analysis technique to better understand the pattern of brain change in a large cohort of HIV+ adults. We hypothesized that shape analysis would detect subcortical morphometric variations associated with clinical and neurocognitive metrics with higher sensitivity compared to traditional volumetric analysis.

**Methods:** Cross-sectional T1-weighted brain MRI scans from 253 chronic HIV+ patients on stable cART were collected through the HIV Neuroimaging Consortium and HIV-associated Brain Dysfunction Study (Age: 47.1± 8.5yrs; M/F: 197/56; Infection Duration: 12± 15yrs). Two shape metrics of local thickness (radial distance) and surface area (Jacobian determinant) were derived from structural MRI across thousands of homologous surface points for bilateral nucleus accumbens, amygdala, caudate, hippocampus, putamen, pallidum, and thalamus shape models. A linear mixed effects model was fit separately for gross subcortical volume (a single value) and shape analysis metrics (thousands of thickness and surface area points) to evaluate associations with current and nadir CD4 counts, and neurocognitive domain-specific T-scores (processing speed (PS); learning (LT); motor function (MF)). Analyses were adjusted for age, sex, ethnicity, intracranial volume, HIV duration, antiretroviral treatment and history of drug use as fixed effects, and scan site as a random effect. All results were corrected for multiple comparisons and a standard FDR correction was applied ( $q=0.05$ ).

**Results:** There were no significant associations between single value gross volumes and CD4 measures or neurocognitive scores. However, subcortical shape modeling revealed clusters of largely lower local thickness and surface area metrics associated with lower nadir and current CD4 values. Furthermore, lower thickness and surface area metrics were associated with more impaired PS, LT and MF neurocognitive performance scores (**Table 1**).

**Conclusions:** To our knowledge, this study represents the largest subcortical shape analysis of HIV. Our high-resolution subcortical shape analysis technique was more sensitive to associations between brain volume and CD4 counts as well as neurocognitive scores over traditional whole volume subcortical analyses and represents a powerful new technique to track HIV-associated brain change.

	Current CD4 (N=253)	Nadir CD4 (N=253)	Processing Speed (N=78)	Learning (N=78)	Motor function (N=78)
<b>L Accumbens</b>			J (+)		
<b>L Amygdala</b>	T (+)				
<b>R Amygdala</b>		T (+/-)			
<b>L Hippocampus</b>			J (+)		
<b>R Hippocampus</b>		T (+)	J (+)		
<b>R Pallidum</b>	J (+)				T (+)
<b>R Putamen</b>		J (+); T (+/-)			
<b>L Thalamus</b>				J (+)	
<b>R Thalamus</b>			J (+); T (+)		

**Table 1:** Significant associations detected by shape analysis after correction for multiple comparisons (N=sample size given available data). J: Jacobian determinant or surface area expansions/contractions; T: Thickness or local increases/decreases in radial distance; (Sign of the beta value in regions passing FDR correction).

For example, T(+) for Current CD4 and L Amygdala indicates that higher thickness values in the left amygdala were associated with higher current CD4 count. Some associations (+/-) indicate clusters of both positive and negative beta values for a particular model, though it is important to note that the majority of significant clusters had positive beta values.

387 **Cigarette Pack-Years May Affect Cingulate and Frontal Cortex in Chronic HIV Infection**

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**Background:** Worldwide, smoking is twice as prevalent in the HIV-infected community as in the general population. Smoking exposure is linked to cortical gray matter loss in HIV+ and healthy individuals. However, enhanced frontal and executive functions have been associated with smoking in HIV patients. Chronic nicotine treatment improves memory in HIV transgenic but not in healthy rats.

**Methods:** Cortical volume and neuropsychological (NP) data, obtained in HIV+ subjects on antiretroviral therapy, were cross-sectionally examined for associations with cigarette pack-years. Subjects provided smoking data by self-report, underwent T1-weighted magnetic resonance imaging (MRI) at 3.0 Tesla, and completed an NP test battery that yielded global and domain-specific (psychomotor; executive function; learning and memory [NPZ\_lm]; working memory) z-scores. Non-smokers (defined as never-smokers) had zero pack-years. Analysis of covariance compared volumes of frontal and cingulate cortex (CCx) between smokers and non-smokers, adjusting for age and intracranial volume (ICV). Effects of pack-years on volumes were assessed by linear regression controlling for age and ICV.

**Results:** We evaluated 53 subjects [45 male; age 51 ± 7 years; median (IQR) current and nadir CD4 count 466 (299-592) and 125 (39-250) cells/mm<sup>3</sup>; 44 with plasma HIV RNA < 50 copies/mL; 19 non-smokers; 34 smokers (19 ± 12 pack-years)]. Smokers and non-smokers did not differ in demographics, clinical variables, cortical volumes or NP z-scores ( $p>0.05$ ). Pack-years were associated with volumes of isthmus ( $\beta = -0.34, p=0.006$ ), caudal anterior ( $\beta = -0.27, p=0.04$ ) and total ( $\beta = -0.34, p=0.002$ ) CCx; lateral orbitofrontal cortex ( $\beta = -0.30, p=0.02$ ); rostral anterior ( $\beta = -0.21, p=0.05$ ) and posterior ( $\beta = -0.23, p=0.08$ ) CCx; and total cortex ( $\beta = -0.19, p=0.07$ ). Higher NPZ\_lm correlated with smaller caudal anterior ( $r = -0.34, p=0.02$ ) and total ( $r = -0.26, p=0.07$ ) CCx volume. Pack-years did not relate to NP z-scores.

**Conclusions:** Cingulate cortex may play a crucial role in nicotinic effects on cognitive performance in chronic HIV disease. HIV+ individuals demonstrated negative associations between cigarette pack-years and volumes of cingulate and orbitofrontal cortex. Paradoxically, we found that reduced cingulate cortical volume correlated with better learning and memory. Future research (e.g., with functional connectivity MRI) may elucidate the underlying mechanism of this association, possibly leading to effective treatment for HIV-related neurocognitive impairment.

### 388 Cardiovascular Risk Factors and the Rate of Change in Brain Structure in HIV Disease

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**Background:** Since the introduction of cART, factors not directly associated with HIV disease are now having a significant impact on brain structure and function. In particular, cardiovascular disease (CVD) is known to affect brain structure and performance on cognitive tests. The purpose of this analysis was to identify associations between CVD risk factors and change in brain structure over time.

**Methods:** 122 men (56% HIV-infected) participating in the Multicenter AIDS Cohort Study (age:  $55.8 \pm 4.9$ ) underwent two brain MRI scans 3.2 years apart (T1 weighted magnetization-prepared rapid gradient-echo sequence). Gray matter, white matter, and CSF volumes (expressed as percent of total intracranial volume) were measured using FMRIB's Automated Segmentation Tool, and an annualized rate of change was calculated. Correlation analyses identified hypertension, diabetes, urine protein/creatinine ratio (as an index of small vessel disease) and age as having significant associations with the outcome variables. Lipid levels and illicit drug use were not linked to rate of change. Linear regression models evaluated the associations between change in the three tissue class volumes and the significant predictor variables (from the unadjusted analysis), as well as CD4+ cell counts and plasma HIV RNA viral load (among infected men only).

**Results:** After adjusting for age, race, and cohort of entry, HIV serostatus was unrelated to rate of change in any of the three brain tissue compartments. The rate of expansion of the CSF compartment was significantly faster among individuals with diabetes; no other CVD-related variable predicted brain volume change. Among the HIV-infected men only, nadir CD4+ cell count was associated with changes in gray and white matter volumes. CSF expansion was significantly greater among individuals in the most recent enrollment cohort, and among individuals with diabetes.

**Conclusions:** In the cART era, CVD risk factors are at least as important as HIV-associated factors in predicting rate of change of brain tissue compartments. Finally, among HIV-infected individuals, factors associated with HIV treatment history are likely critical for predicting subsequent brain health.

### 389 New Method Measuring Intracranial Vessel Caliber Reveals Arterial Remodeling In HAND

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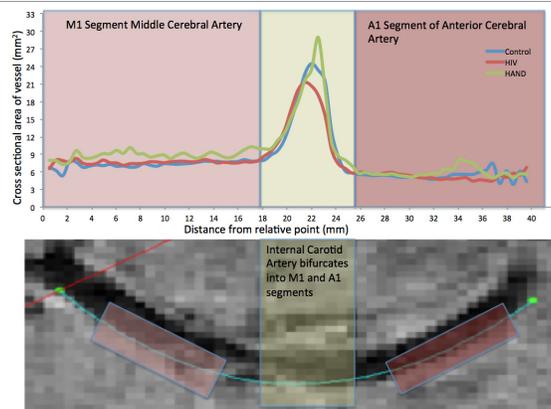
**Background:** In vivo MRI measurements of cerebrovascular caliber could offer insight into the pathology and spread of HIV in the CNS. Previous postmortem studies have shown a decrease in blood-vessel wall thickness in HIV infected individuals, indicating possible arterial remodeling.<sup>1</sup> Here, we measured the vessel caliber in HIV patients in vivo using high-resolution T2\* weighted MRI and compared it to that from matched control subjects.

**Methods:** T2\* weighted MRI was acquired at 0.55 mm isotropic resolution on a Philips 3T scanner with 8-channel head coil in 13 subjects with HIV-associated neurocognitive disorders (HAND, mean and standard deviation of age  $49 \pm 9$  years, 62% male), 43 HIV without HAND (HIV,  $51 \pm 8$  years, 56% male) and 23 socioeconomically matched controls without HIV (Control,  $44 \pm 11$  years, 57% male). Cross-sectional areas (averaged values from right and left arteries) of the A1 segment of anterior cerebral arteries and M1 branch of middle cerebral arteries were measured using a MATLAB program developed in house. One-way ANOVAs and two-tailed t-tests were used to assess group differences, and results are reported as mean  $\pm$  standard deviation.

**Results:** HAND group had significantly higher M1 cross-sectional areas ( $8.8 \pm 2.2$  mm<sup>2</sup>) than either the Control ( $7.7 \pm 1.6$  mm<sup>2</sup>) or HIV ( $7.5 \pm 1.5$  mm<sup>2</sup>; ANOVA  $p < 0.01$ ) groups. However, such differences were not seen in A1 segment among the three cohorts (ANOVA  $p = 0.9$ ) (Figure). The technique also detected a difference between the M1 and A1 segment areas ( $7.8 \pm 1.7$  mm<sup>2</sup> and  $6.9 \pm 2.2$  mm<sup>2</sup> respectively,  $p < 0.01$ , paired t-test). Men had consistently larger M1 areas than women in Control ( $8.1 \pm 1.6$  mm<sup>2</sup> and  $7.0 \pm 1.2$  mm<sup>2</sup> respectively,  $p < 0.05$ ) and in HIV ( $8.0 \pm 1.6$  mm<sup>2</sup> and  $7.0 \pm 1.0$  mm<sup>2</sup>,  $p < 0.01$ ) groups. There were no observed correlations between any of the cross-sectional areas and age.

**Conclusions:** We found that HIV patients with HAND had larger MCA cross-sectional areas than did HIV subjects without HAND or control subjects, which could be consistent with the reports of significantly thinner vessel walls seen in HIV patients post-mortem. The differences in vessel size between men and women and between the M1 and A1 branches show the ability of this method to detect subtle differences in arterial caliber. Another strength of this method is its ability to characterize vascular caliber in-vivo, which should allow for multiple longitudinal measurements over time.

Reference: <sup>1</sup>Gutierrez et al. *Neuropathology* 2013; 33, 256–263



**Figure 1.** Average M1 and A1 cross-sectional areas along the length of the vessel.

### 390 Isolated Cingulate Correlates With Reduced Executive Function in HIV

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**Background:** Executive dysfunction is a common neurocognitive deficit among persons living with HIV (HIV+), and has been attributed to damage in fronto-striatal circuits. The specific neural mechanisms underlying executive dysfunction, however, remain to be elucidated. A recent study with a task-switching paradigm revealed that HIV+ older adults adapt less quickly to changing task demands than HIV- controls, and the behavioral impairments correlated with disrupted brain activation in the anterior cingulate cortex (ACC)

(Magnus et al., CROI 2014), suggesting ACC dysfunction might underlie executive dysfunction in HIV. Here, we further investigated the brain dysfunction in the cingulate cortex using resting-state functional connectivity MRI (rs-fcMRI).

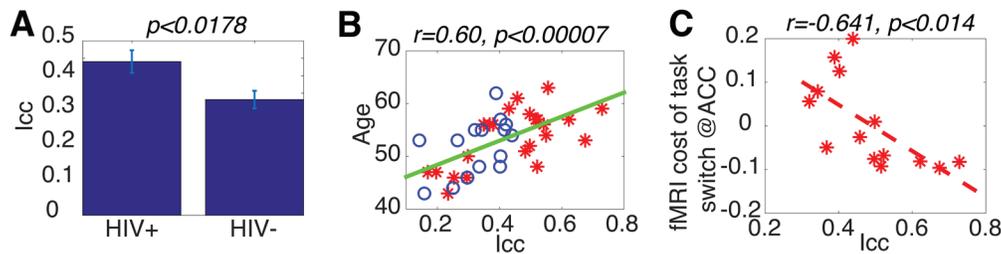
**Methods:** Thirty-eight adults (43–63 years old, 23 HIV+ on antiretroviral therapy) without major psychiatric disorders and other confounding health problems participated in this study. HIV+ and HIV- subjects did not differ significantly on a battery of standard neuropsychological tests. One run of rs-fcMRI data was collected. After controlling for confounding parameters, we extracted the time series from the posterior, middle, and anterior regions of cingulate, then obtained the two sets of voxel-wise correlations,  $R_{wi}$ , within each region, and  $R_{btw}$  between two different regions. The disruption to cingulate connectivity ( $I_{cc}$ ) was defined as

$$I_{cc} = (R_{wi} - R_{btw}) / (R_{wi} + R_{btw})$$

with higher  $I_{cc}$  represents more reduced inter-regional connection.

**Results:** Results are shown in Figure 1. (A) Two-sample t-test revealed an increase in  $I_{cc}$  due to HIV. (B) Pearson's correlation analysis revealed a significant correlation between age and  $I_{cc}$ . (C) For a subset of subjects from the Magnus et al. (2014) study, there was a significant correlation between  $I_{cc}$  and the cost of task switch (the difference between the task-switch and non-switch trial) in fMRI at ACC, suggesting the disconnection between cingulate regions might contribute to the disrupted neural activity in ACC and executive dysfunction in HIV.

**Conclusions:** The rs-fcMRI technique revealed that both aging and HIV disrupt the connection between different cingulate regions. The disrupted connections correlate with the disrupted brain activations in the ACC. These results suggest that the cingulate might be one of brain regions affected early on during the evolution of HIV brain disease, and the additive impairments due to aging and HIV might underlie the increased risk of executive deficits in HIV+ older adults.



### 391 Multifactorial Neurochemical Alterations Are Slowly Evolving Despite Viral Control

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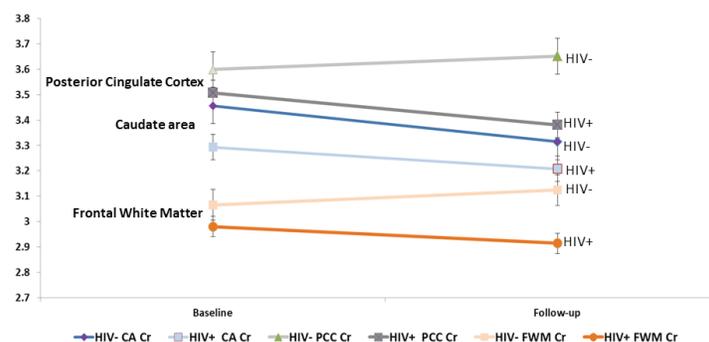
**Background:** Chronic HIV infection in the context of long-term viral suppression and aging represents a new context for HIV neuropathogenesis research. While HIV-related brain injury can be detected using in vivo proton Magnetic Resonance Spectroscopy (<sup>1</sup>H-MRS) in HIV+ persons, the evolution of such brain damage is unclear.

**Methods:** The study sample included 108 men (mean age=55±6), 73 were HIV+ (71% with historical AIDS, nadir CD4 ≤350, all on antiretroviral treatment; baseline plasma and CSF viral suppression>97%; follow-up plasma suppression>94%); and 35 demographically comparable HIV-uninfected controls. All underwent <sup>1</sup>H-MRS of the frontal white matter (FWM), the posterior cingulate cortex (PCC) and the caudate area (CA) at baseline and 18-months later. Neurocognitive performance was also examined at the same time points. Major brain metabolites: Creatine (Cr), N-Acetyl Aspartate (NAA), Choline (Cho), Glutamate (Glu) and Myo-Inositol (MI) were fitted in jMRUI in reference to H<sub>2</sub>O. Change in brain metabolites was determined using linear regression with a time effect, a group effect and a time\*group interaction effect. Age, HIV disease and treatment and cardiovascular markers, having a history of HAND, baseline neurocognitive performance and neurocognitive change were evaluated as predictors/covariates in voxels where significant HIV status-related neurochemical changes were found.

**Results:** Across the study period, in the HIV+ sample and relative to HIV- controls, FWM Cr (p<.01), PCC Cr (p<.01) CA Cr (p<.05), PCC NAA (p<.01) and CA NAA (p<.05) were reduced; PCC MI (p<.05) was elevated. Chronological age did not predict these between-group differences. When only considering the HIV+ sample, lower baseline neurocognitive performance was associated with lower CA NAA (p<.05) over time. History of HAND was associated with reduced FWM Cr (p=.01), lower PCC NAA (p<.05), and lower PCC Cr over the study period (p<.05). Viral blips (n=6) were associated with reduced PCC NAA over time (p<.05). History of cardiovascular disease was associated with lower PCC NAA and Cr over time (p<.05).

**Conclusions:** Metabolite abnormalities were stable over time and included widespread decreased cellular energy (related to glial activation in a neuroAIDS macaque model), cortical and subcortical neuronal integrity loss and restricted neuroinflammation. Predictor/Covariate findings support slowly evolving multi-dimensional neuropathogenesis emphasizing the need for long-term monitoring of brain functions in chronic HIV infection.

Illustration of Creatine concentrations in the three voxels in the HIV+ sample compared to the HIV- sample over the study period. Creatine is significantly reduced in all instances



### 392 Effect of cART on Functional Connectivity in Cart-Naïve HIV-Infected Individuals

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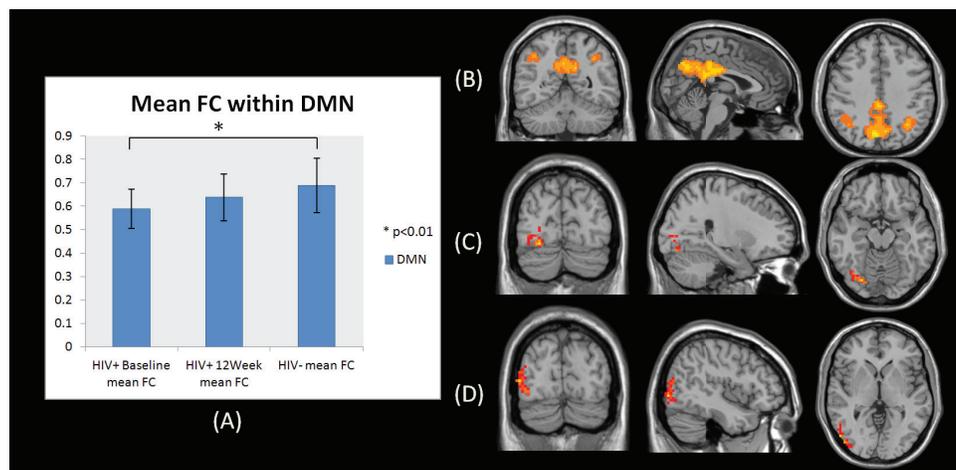
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**Background:** Neuroimaging biomarkers provide an opportunity to investigate HIV-associated CNS injury at the time when clinical changes may be silent. In this study we assessed whether virologic response to combination antiretroviral therapy (cART) in naïve subjects was associated with changes in resting state fMRI.

**Methods:** Subjects with confounding CNS disorders and history of CNS infection, other than HIV, were excluded. All subjects underwent a detailed neurocognitive and functional assessment. HIV infected subjects were scanned before starting cART and 12 weeks after initiation of treatment. **Imaging protocol/analyses:** Resting state fMRI was acquired on a 3T Siemens MAGNETOM Trio MRI scanner. Group ICA was used to identify the resting state networks and to determine differences in functional connectivity (FC) within the Default Mode Network (DMN) (Figure B). The anterior cingulate cortex (ACC) and posterior cingulate cortex (PCC) were used as ROIs to evaluate changes in FC between DMN and other brain areas. Statistical analyses were conducted using two-sample t test or paired t-test (HIV+ baseline vs. HIV+ after 12-week treatment). Multiple comparison correction was performed using values generated by AlphaSim (voxel  $p < 0.01$ , cluster size  $> 64$ , which corresponds to a corrected  $p < 0.05$ ).

**Results:** Cohort: 17 HIV+ male, mean age  $33 \pm 3$  years, 17 HIV- (10 female and 7 male) mean age  $32 \pm 3$  years. At baseline, 10 of the HIV+ subjects had normal cognitive performance, 6 had asymptomatic impairment and 1 had mild neurocognitive disorder. The mean CD4 count and viral load at baseline were  $479 \pm 48 \text{ mm}^3$  and  $121,804 \pm 43586$  copies/ml respectively. After 12 weeks, mean CD4 count and viral load were  $636 \pm 55/\text{mm}^3$  and  $899 \pm 861$  copies/ml. **Imaging results:** FC within the DMN network was significantly lower in HIV+ subjects at baseline as compared to HIV- subjects (Figure A). There were no significant differences in FC within DMN between HIV+ after 12 week treatment and HIV- subjects. There was a significant increase in FC between DMN and occipital lobe in the HIV+ after 12 week treatment as compared to HIV+ subjects at baseline (Figure C: seed in PCC, D: seed in ACC).

**Conclusions:** Our results suggest that in relatively young cART naïve subjects with relatively preserved immune function, there are signs of decreased functional connectivity that tend to improve after 12 weeks of treatment.



### 393 HIV Infection in Astrocytes Via a CD4-Independent, CXCR4-Dependent Mechanism

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**Background:** HIV reservoir in the brain represents a major barrier for curing HIV infection. As the most abundant, long-lived cell type, astrocytes play a critical role in maintaining the reservoir; however the mechanism of infection remains unknown. Here, we determine how viral transmission occurs from HIV-infected lymphocytes to astrocytes.

**Methods:** Human astrocytes were exposed to HIV-infected lymphocytes and monitored by live-imaging, confocal microscopy, transmission and 3-dimensional electron microscopies. A panel of receptor antagonists was used to determine mechanism of viral entry. A transwell culture system was used to test whether the infection of astrocytes could also be established by viral particles that were released from the infected lymphocytes and passed through the pores of transwell membranes.

**Results:** We found that cell-to-cell contact resulted in efficient transmission of X4- or X4R5-using viruses from T lymphocytes to astrocytes. In co-cultures of astrocytes with HIV-infected lymphocytes, the interaction occurred through a dynamic process of attachment and detachment of the two cell types. Infected lymphocytes invaginated into astrocytes or the contacts occurred via filopodial extensions from either cell type, leading to formation of virological synapses. In the synapses, budding of immature or incomplete HIV particles from lymphocytes occurred directly onto the membranes of astrocytes. This cell-to-cell transmission could be almost completely blocked by anti-CXCR4 antibody and its antagonist. Furthermore, we also found that newly produced HIV particles from the infected lymphocytes on the top of transwells could go through the transwell membrane and infect astrocytes via the same mechanism.

**Conclusions:** Cell-to-cell transmission is mediated by a unique mechanism by which immature viral particles initiate a fusion process in a CXCR4-dependent, CD4-independent manner; and newly produced, immature HIV can also infect astrocytes without cell-to-cell contact. These observations have important implications for developing approaches to prevent formation of HIV reservoirs in the brain.

### 394 A Unique In Vitro Neuropathic Phenotype of Clade D Transmitted/Founder Viruses

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**Background:** Expression of the anti-inflammatory, antioxidative phase II detoxifying enzyme heme oxygenase-1 (HO-1) is decreased in the brain prefrontal cortex of HIV+ individuals in association with increased neuroinflammation and neurocognitive impairment. *In vitro* HIV infection of monocyte-derived macrophages (MDM) induces HO-1 deficiency and increases release of neurotoxic levels of glutamate. We recently showed that HO-1 deficiency is consistently seen with HIV MDM infection by a broad panel of macrophage-tropic clade B HIV-1 strains (n=13). This suggests that induction of HO-1 deficiency is a highly conserved macrophage-tropic HIV phenotype that could be particularly relevant for neuropathogenesis driven by infection within the CNS myeloid reservoir.

**Methods:** Human MDM (n=4 donors) were infected with viruses derived from infectious molecular clones of each of 5 clade D transmitted/founder (T/F) strains or the clade B 89.6 strain. Viral replication, glutamate concentration, and neurotoxicity of MDM culture supernatants were determined via reverse transcriptase (RT) activity, glutamate assay, and neuron-based MAP2 ELISA, respectively. Data were analyzed by ANOVA with Holm-Sidak post hoc test or Pearson's correlation.

**Results:** In contrast with MDM infection by 89.6, infection by macrophage-tropic clade D T/F viruses did not significantly decrease HO-1 expression or increase glutamate release, despite similar replication levels as 89.6. Unexpectedly, infection by clade D T/F viruses significantly increased MDM culture supernatant neurotoxicity despite no induction of glutamate release (n=4 donors, p<0.001 for equivalent replication to 89.6). This suggests that macrophage-tropic clade D T/F viruses can activate pathways of neurotoxin production independent of HO-1 and associated effects on glutamate metabolism.

**Conclusions:** Our results suggest that macrophage-tropic clade D T/F viruses differ from clade B viruses in several potentially critical determinants of neurovirulence; differential ability to *i)* reduce expression of MDM HO-1 expression and to *ii)* induce glutamate release from MDM. Nonetheless, macrophage-tropic clade D T/F viruses can induce release of neurotoxins other than glutamate (as yet unidentified) from MDM without altering HO-1 expression, and this suggests potential clade- or T/F virus-specific mechanisms of HIV neurotoxin production. Such differences could contribute to variance in severity of neurological dysfunction among populations infected with different HIV clades.

### 395 Neurotoxicity Screening of Antiretroviral Drugs With Human iPSC-Derived Neurons

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**Background:** While antiretroviral therapy (ART) has become increasingly effective and well-tolerated, there remains a patient subset that experience central nervous system (CNS) side effects, and neurocognitive performance may actually improve following change in specific ART agents. Understanding the risk associated with these drugs will allow informed selection of optimal therapy. Using human induced pluripotent stem cell-derived neurons (hiPSC), we screened 10 ART drugs and generated a neurotoxicity profile based on mitochondrial membrane potential, reactive oxygen species, cell health and neurite growth.

**Methods:** hiPSC cortical neurons (Cellular Dynamics International) were treated in dose response with ABC,ATV,COBI,DRV,DTG,EFV,EVG,RPV,RTV,TDF. Neurons were assayed for mitochondrial function and neurite growth using image based high content, high throughput screening. Statistical significance was determined as Z-score greater than 2 compared with vehicle control mean across replicate wells.

**Results:** The majority of tested drugs demonstrated neurotoxicity: 7 caused mitochondrial toxicity and 3 affected neurite growth. Non-nucleoside reverse transcriptase inhibitor, protease inhibitor (PI) and pharmacoenhancer drugs exhibited the highest degree of mitochondrial toxicity. Only EFV was overtly cytotoxic suggesting mitochondrial dysfunction was a primary target, not a side effect of cell death. After 3 days exposure to ART drugs, morphology and cell health was assessed. DTG and EFV resulted in minor but significant neurite growth. RPV was cytotoxic at high dose but caused neurite inhibition at lower doses. Of note, neurite outgrowth or inhibition could contribute to CNS pathology. At high doses, COBI, EVG, RPV and EFV caused neurite inhibition coincident with cytotoxicity suggesting the morphological effect was secondary to cell death. While PIs influenced mitochondrial function, no effect was found on morphology. In contrast, COBI, EVG, RPV and EFV presented a distinct profile with mitochondrial dysfunction followed by changes in morphology and/or cytotoxicity.

**Conclusions:** We characterized toxicity of 10 ART drugs and linked the majority with impacts either on mitochondrial function, neurite growth, or both. Although immature, hiPSC neurons are human and scalable for drug screening. Further studies are needed to determine whether our *in vitro* assays reflect neurotoxicity *in vivo*, but our results suggest that increased drug concentrations in the CNS could have adverse clinical consequences.

### 396 Chronic Low-Level HIV-1 Tat Expression Promotes a Neurodegenerative Phenotype

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**Background:** Brain volumetric changes occur following HIV-infection and often persist in individuals despite cART. The underlying mechanism(s) for these structural changes are not understood, but it has been suggested that chronic low-level expression of the HIV-1 *trans*-activating protein Tat, may contribute to neural damage over time, but this has not been tested. Here we evaluated the effects of chronic low-level *tat* gene expression on neuropathology.

**Methods:** Tetracycline-inducible GFAP-driven HIV-1 Tat transgenic mice (rtTA-Tat) and mice expressing only the rtTa (rtTA) promoter (3-12 month of age) were used in these studies. Volumetric measures of macrostructural brain regions and cortical thickness were obtained by T2-weighted *in vivo* MRI. Neuronal and synaptic integrity were determined by immunoblot analysis of  $\beta$ III-Tubulin, synaptophysin, and PSD95. Inflammation was assessed by qRT-PCR of proinflammatory cytokines. Brain sphingolipid metabolism was determined by mass spectrometry.

**Results:** We took advantage of a leaky tetracycline-inducible gene system to produce chronic low-level *tat* expression in the absence of gene induction. *Tat* mRNA was undetectable in rtTA mice, detectable in non-induced rtTA-Tat mice, and increased three-fold following a 30-day doxycycline-induced gene induction. All brain volume measures were similar in 3-month old rtTA, non-induced rtTA-Tat and induced-rtTA-Tat mice. In 11-12 month old mice non-induced rtTA-Tat mice exhibited increased total ventricular and dentate gyrus volumes and decreased motor cortical thickness compared to control rtTA mice. Induction of *tat* gene expression with doxycycline did not further alter regional brain volumes. Cortical thinning observed in non-induced rtTA-Tat mice corresponded with decreased  $\beta$ III-Tubulin, synaptophysin, and PSD95 levels. Expression of inflammatory cytokines were similar rtTA and non-induced rtTA-Tat mice, but were increased following induction of *tat* gene expression. Hierarchical clustering analysis of cortical lipid content revealed that brain lipid content of non-induced rtTA-Tat mice closely resembled those of doxycycline-induced rtTA-Tat mice.

**Conclusions:** These findings demonstrate that chronic low-level *tat* expression is associated with changes in brain volume, alterations in bioactive lipid content, and synaptic simplification that were independent of measurable changes in inflammatory gene expression. These data suggest that chronic low level *tat* expression is sufficient to produce structural changes in the brain.

### 397 Glutaminase Regulates Extracellular Vesicles Release in HIV-1-Infected Macrophages

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**Background:** Extracellular vesicles (EVs) are important in the intercellular communication in the central nervous system and their release is increased upon neuroinflammation and neurological disorders. Our previous data demonstrated an increased release of EVs from HIV-1-infected macrophages that have neurotoxic effects. However, the mechanism of elevation of EV release in those HIV-1-infected cells remains unknown. In the current studies, we investigated glutaminase (GLS), which is a mitochondria enzyme critical for glutamine metabolism. GLS is upregulated in HIV-1-infected macrophages and microglia. We propose that HIV-1 infection increases GLS, leading to a metabolic status that favors the EVs generation and release. The new understanding of the metabolic control of EV release in HIV-1-infected cells will shed light on HIV-1 pathobiology and neurological complications.

**Methods:** Human primary microglia and monocyte-derived macrophages culture system and macrophage-tropic HIV-1<sub>ADA</sub> were used to study the regulation of EVs during HIV-1 infection. EVs were isolated through differential centrifugations. A gene overexpression system, delivered via adenovirus vector, was utilized to overexpress GLS in the cell culture to mimic the upregulation of GLS during HIV-1 infection. A brain-specific GLS transgenic mouse line was created to model GLS elevation *in vivo*. BPTES was used to specifically inhibit GLS activity. Transmission electron microscopy and Western blot were used to quantify the EVs released from cells and brain tissues. Glutamate and glutamine levels were determined by reverse phase high performance liquid chromatography.

**Results:** An elevated number of EVs was found in the supernatants of HIV-1-infected macrophages and microglia when compared with controls. Overexpression of GLS in macrophages and microglia cultures leads to increased release of EVs. Conversely, blocking the GLS activity by BPTES significantly reduced EV release and glutamate generation in HIV-1-infected macrophages and microglia, suggesting a critical role of GLS in EV release. Interestingly, we detected an elevated release of EVs in the brain tissues of GLS transgenic mice, suggesting that GLS is also important for EV release *in vivo*.

**Conclusions:** GLS is essential for EVs release in HIV-1-infected macrophages and microglia. Therefore, blocking EV release through GLS inhibitors may serve as a novel therapeutic strategy against the HIV-1 pathobiology and neurological complications.

### 398 HIV-1 Replication Dynamics in the CNS of TB-Coinfected Individuals

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**Background:** HIV-1-associated opportunistic infections of the central nervous system (CNS) are common but how they affect HIV replication dynamics at the site of infection is unknown. The most common opportunistic infection in sub-Saharan Africa is tuberculosis. HIV-1 and *Mycobacterium tuberculosis* co-infection of the central nervous system may alter the local microenvironment leading to independent viral evolution and clonal amplification or alternatively disrupt the blood-brain barrier resulting in loss of compartmentalization and virus influx from the periphery. In this study, we characterized viral load discordance and examined HIV-1 subtype C genetic diversity and compartmentalization between cerebrospinal fluid and plasma in patients with tuberculous meningitis (TBM) versus other meningitides.

**Methods:** One hundred and three matched blood and cerebrospinal fluid (CSF) samples were obtained from patients presenting with suspected meningitis in Durban, South Africa. Patients were categorized as definite TBM (n=16), non-TBM (n=35), probable TBM (n=46) and 6 patients unclassified. The viral RNA for both plasma and CSF were quantified using the COBAS TaqMan HIV-Test. Genetic compartmentalization was assessed by single genome amplification (SGA) and sequencing of the *env* gene on eight of the patients. Phylogenetic analysis was done using sequencer software, Clustal W on Bioedit and maximum likelihood trees were drawn using MEGA version 6. Intra-patient diversity, enumeration of glycosylation sites, and coreceptor usage prediction analysis were also done.

**Results:** The plasma viral loads of the TBM and non-TBM groups did not differ but TBM participants CSF viral loads (median=(5.76 log<sub>10</sub> copies/ml, interquartile range [IQR] 4.85-6.19) were higher than non-TBM CSF viral loads (4.40 log<sub>10</sub> copies/ml [3.46-4.78]) participants (p = 0.0005). Heterogeneous patterns of compartmentalization were observed, 1 TBM and 3 non-TBM patients had equilibration of CSF and plasma sequences, 1 TBM patient had partial intermixing and 2 TBM and 2 non-TBM patients had unique clusters within compartments. These patterns did not seem to depend on TBM versus non-TBM status of patients. TBM CSF viral sequences seem to be more diverse than plasma sequences. Irrespective of the compartment the viruses are R5, with 2 patients displaying some X4 virus. There was no difference in the number of glycosylation sites between compartments and groups.<

**Conclusions:** TB co-infection of the CNS seems to enhance HIV-1 viral replication and evolution .

### 399 Glial Transcriptional Responses Follow HIV-1 Infection in a Humanized Mouse Brain

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**Background:** A humanized mouse brain was created in immune deficient mice. The molecular and biological complexity of this novel organ was subjected to robust cross species histopathologic and molecular analyses for studies of HIV-1 neuropathogenesis. Gene expression analysis of HIV-1 infected patients with co-morbid nervous system disorders was used for comparisons for molecular signatures in this mouse model which combines human hematolymphoid tissue with glial reconstitution.

**Methods:** Human brain glial precursors (GP) were generated from human brain tissue neurospheres. GP were injected into lateral ventricle of animals reconstituted with human CD34+ hematopoietic stem cell. At 6 months of age animals were infected with the macrophage-tropic HIV-1 ADA. Brain tissues from mice were analyzed by immunohistochemistry; peripheral immune profiles and viral load were measured. Animals transplanted with the same donor cells, with near equivalent viral loads (> 10<sup>5</sup> copies/ml) and glial distribution pattern were selected for gene expression analysis. Hippocampus and corpus collosum areas of control unmanipulated, humanized and HIV-1 infected animals were isolated. Deep sequencing was performed with the Illumina HiSeq 2500 analyzer.

**Results:** Brain tissues were anatomically symmetric with both hemispheres showing similar numbers of human glial cells. Glial fibrillary acidic protein stains showed human astrocytes in corpus callosum and periventricular white matter. In comparison with uninfected mice, infected human astrocytes-containing brain samples exhibited unique expression of 45 and 56 human genes in the hippocampus and corpus collosum, respectively. These were linked to inflammatory response and antigen presentation, interferon responses and ubiquitin ligases pathways (PLSCR1, MHC I, IFIT1, IFI44, MX1, STAT1, IGS15, HERC5). The data set surprisingly overlapped with the disease profile in brains of HIV-1 infected humans.

**Conclusions:** Dual reconstituted murine model can readily reflect molecular events in HIV-associated neurocognitive disorders with dysregulated transcriptions related to interferon response genes (IFRGs) and the interferon regulatory factor (IRF), canonical pathways for G protein receptors and cyclic adenosine monophosphate signaling. These results underscore the importance of the model as well as the role astrocytes play in mediating pathophysiological outcomes of human disease.

### 400 CNS Compartmentalization of HIV-1 and Sensitivity to Neutralizing Antibodies

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**Background:** Compartmentalization of HIV-1 has been observed in the cerebrospinal fluid (CSF) of patients with HIV-related neurocognitive disorders (HAND). Compartment specific modifications have been frequently described in the variable loops and the glycosylation sites of the envelope, a known mechanism to escape antibody neutralization. Considering the low permeability of the blood-brain barrier, we wondered if a lower selective pressure by neutralizing antibodies (NAb) could favor the evolution of NAb-sensitive viruses in the CSF.

**Methods:** Single genome amplification (SGA) was used to sequence near full-length HIV-1 envelope variants (453 sequences) from paired CSF and blood plasma samples of 9 subjects with HAND infected by HIV variants of 5 different clades. Dynamics of viral evolution were evaluated with a bayesian coalescent approach for individuals with longitudinal samples (n=4). For 6 subjects, pseudotyped viruses expressing envelope glycoproteins variants representative of the quasi-species present in each compartment were generated, and their sensitivity to autologous neutralization, broadly neutralizing antibodies (bNABs) and sCD4 was assessed.

**Results:** In cross-sectional analyses, significant compartmentalization of HIV populations between blood and CSF were detected in 5 out of 9 subjects by all tests (p < 0,001). Phylogenetic analysis confirmed the presence of monophyletic populations evolving independently within the CSF (aLRT > 0.9). Some of the previously described genetic determinants for neuroadaptation were observed regardless of the HIV-1 clade. There was no difference of sensitivity to autologous neutralization between blood- and CSF-variants, even using sera collected at different time-points. By contrast, in all cases, we observed major differences of sensitivity to sCD4 or to at least one bNAB targeting the N160-V1V2 site, the N332-V3 site or the CD4bs, between blood- and CSF-variants.

**Conclusions:** Our data show that selective pressure by autologous NAB is not the main driver of HIV evolution in the CSF. Given that each of the conserved neutralizing epitopes is linked to a specific property for cell entry, our data suggest that some functional properties of the envelope are responsible for compartmentalization. Considering the possible migration from CSF to blood, CSF could be a reservoir of bNAB resistant viruses, an observation that should be considered for future studies of immunotherapy.

**401 HIV Compartmentalization in the CNS Is Associated With Neurocognitive Impairment**

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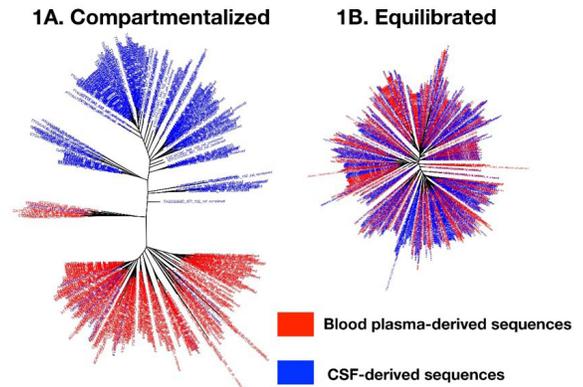
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**Background:** The effect of compartmentalized HIV replication in the central nervous system (CNS) on neurocognitive performance (NP) is still not well characterized. Low level HIV replication in the CNS may sustain inflammation causing lasting neuronal damage. We examined the effect on NP of compartmentalization of HIV between the blood and CNS in a cohort of treatment-naïve patients.

**Methods:** We recruited HIV+ treatment-naïve subjects starting ART with CD4<400. Blood and cerebrospinal fluid (CSF) were collected at entry and 2-4 weeks and 54 weeks after initiating antiretroviral therapy (ART) and analyzed for HIV viral load (VL) and CD4 count. Comprehensive NP assessments were performed at enrollment and 24 weeks and 54 weeks after initiating ART. We genetically characterized virus in CSF and blood at entry to evaluate for compartmentalized lineages in the CNS (see figure). cDNA was generated from viral RNA, and partial *env* genes were amplified and sequenced by Illumina with Primer ID to quantify the number of templates examined and/or full length *env* genes were amplified and sequenced by single genome amplification. Primary outcome was NP. Compartmentalized and equilibrated groups were compared using Wilcoxon rank-sum test and multivariable generalized models.

**Results:** Of 28 subjects whose viral populations were genetically characterized, 10 (36%) had compartmentalized HIV in the CNS pre-ART. Subjects with and without compartmentalization did not differ in demographic variables or mean baseline VL, CSF VL, and CD4 or viral decay rates in the blood and CSF after starting ART. Subjects with CSF compartmentalization had numerically greater NP impairment than subjects lacking compartmentalization pre-ART (global deficit score 1.31 vs. 0.87, p=0.2), and this difference was significant 24 weeks (1.31 vs. 0.63, p=0.04) and 54 weeks (1.25 vs. 0.59, p=0.05) after ART initiation.

**Conclusions:** Compartmentalized HIV lineages in the CNS were common in treatment-naïve subjects. Despite the fact that compartmentalization was not associated with baseline VL, CD4 count, or demographic characteristics, subjects with compartmentalized HIV trended towards impaired NP at baseline compared to non-compartmentalized subjects, and this difference was significant at 24 and 54 weeks after ART initiation. Compartmentalization was associated with NP impairment, and improvement on ART may be blunted in those with compartmentalized virus suggesting that local viral replication may have an impact on the CNS that is less reversible.



**402 Persistent HIV-1 in the CNS During Therapy: Evidence of a Viral Reservoir in the CNS**

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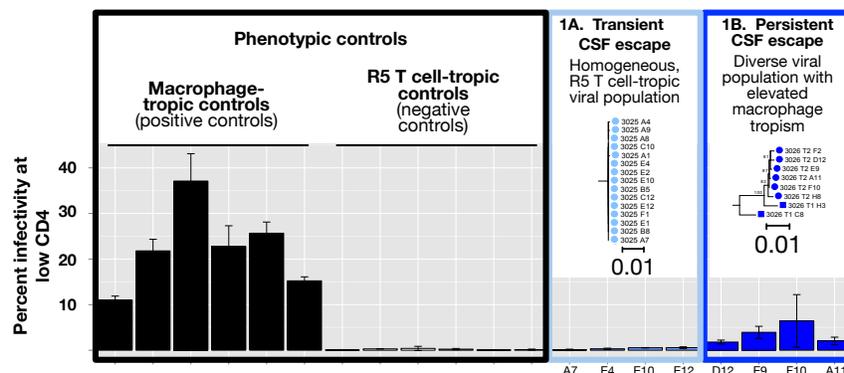
**Background:** In some untreated subjects, HIV-1 forms diverse, CNS-specific lineages with an enhanced ability to infect myeloid-lineage cells. It is unknown whether these viral lineages persist during antiretroviral therapy (ART).

**Methods:** We examined Cerebrospinal fluid (CSF) viral escape in a cohort of 96 HIV-infected subjects on ART for at least one year. All subjects had neurocognitive assessments. CSF and blood plasma samples were collected from each subject and examined for HIV-1 RNA and drug concentrations. CSF escape was defined as having HIV-1 RNA concentrations ≤ 40 cp/ml in blood plasma and ≥ 40 cp/ml in CSF. Subjects whose CSF viral load exceeded 100 cp/ml were examined at a follow-up visit. Viral RNA was isolated from the CSF of these subjects and converted to cDNA. Single genome amplification (SGA) was used to amplify full-length *env* genes for sequencing and cloning. Pseudotyped viruses were generated from the cloned *env* genes and used in entry assays (3 replicates per clone) to determine whether the encoded Env proteins were well-adapted to entering cells expressing low levels of CD4 (i.e. were macrophage-tropic).

**Results:** Four of 96 subjects had CSF escape (4%; 47, 89, 265 and 1295 cp of RNA/ml of CSF). Subjects with and without CSF escape did not differ in their neurocognitive performance (overall global deficit score; Wilcoxon rank-sum, p=0.64). Two subjects had follow-up samples; after 9 months one subject was suppressed (transient escape) but after 8 months the second subject still had 356 cp of RNA/ml of CSF (persistent CSF escape). Analyses of *env* genes amplified from the CSF revealed that the subject with transient CSF escape had a homogeneous viral population that was poorly adapted to entering macrophages (Fig. 1A). In contrast, the subject with persistent CSF escape had a genetically diverse viral population with an elevated ability to infect macrophages (Fig. 1B) at a level that is virtually never detected in blood-derived viruses. There was no association between CSF escape in these subjects and low CSF drug concentration.

**Conclusions:** HIV-1 can persist in the CNS during suppressive antiretroviral therapy. Trafficking of an infected T cell into the CNS or activation of a small number of infected resident cells could generate a transient, homogeneous viral population in the CNS. In contrast, our observation of genetically diverse CSF viral populations eight months apart is consistent with the production of HIV-1 by a persistently infected resident cell population.

**Figure 1:** Phylogenetic and analysis of CD4 entry phenotype of transient (A) and persistent (B) viral populations in the CSF of two subjects with CSF viral escape.



#### 403 Bone Marrow Macrophages Are a Potential Source of CNS Virus With SIV-Encephalitis

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**Background:** The origin of central nervous system (CNS) inflammatory macrophages and source of CNS virus in HIV-associated neurocognitive disorders (HAND) and SIV-encephalitis (SIVE) are not well defined. CD163+ perivascular macrophages are derived from bone marrow (BM) monocyte precursors, comprise SIVE lesions and are productively infected in the CNS. Monocyte expansion and increased plasma soluble CD163 (sCD163) are associated with HAND and SIVE. Blocking monocyte traffic to the CNS with SIV infection reduces the number of inflammatory and productively infected macrophages in the brain. We hypothesized that SIV-infected perivascular macrophage precursors in BM (Mo/MΦ) are a source of CNS virus in SIVE.

**Methods:** Twenty-one rhesus macaques were infected with SIVmac251. Eleven were CD8+ lymphocyte-depleted to expedite AIDS progression and ten were non-depleted. We compared numbers of Mo/MΦ in BM, expansion of monocyte subsets and plasma sCD163 and virus from animals with AIDS and SIVE (n=6) to animals that developed AIDS without SIVE (SIVnoE, n=11), and animals sacrificed without AIDS (n=4). CD68+ Mo/MΦ were extracted from BM using laser capture microdissection. SIV *gp120* sequences from BM CD68+ Mo/MΦ were analyzed using intra-host Bayesian phylogeography and compared to SIV *gp120* from BM tissue, sorted monocytes and CD3+ lymphocytes, plasma virus, dissected meninges and CNS tissues.

**Results:** SIVE animals had higher numbers of Mo/MΦ in BM than SIVnoE animals. Numbers of BM Mo/MΦ correlated with monocyte expansion, plasma sCD163 and virus at necropsy. SIVE animals had higher percentages of CD14+CD16+ pro-inflammatory monocytes from day 20 post-infection to necropsy than SIVnoE animals. We were able to amplify SIV *gp120* cDNA from qPCR of 15 animals from BM. SIV *gp120* from BM Mo/MΦ of SIVE animals shared a most recent common ancestor (MRCA) with sequences from monocytes collected at necropsy. BM was the origin of the MRCA of CNS SIV *gp120* sequences in late stage infection of SIVE animals.

**Conclusions:** The correlation of BM Mo/MΦ with monocyte expansion and plasma sCD163 that are markers of HAND and SIVE highlights the association between increased numbers of BM Mo/MΦ and development of SIVE. The BM ancestry of SIV *gp120* sequences sampled from the CNS suggests a viral migration occurred between BM and brain in late stage infection of SIVE animals. CNS virus seeding from BM and viral migration between BM Mo/MΦ and monocytes in late stage infection underscore the importance of BM Mo/MΦ in neuropathogenesis.

#### 404 Peripheral Immune Activation Modulates HIV RNA Entry to CSF in Early Acute Infection

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**Background:** The mechanisms determining the magnitude of initial HIV entry into the nervous system during acute HIV infection (AHI) are largely unknown. We examined whether peripheral blood and mucosal cellular immune activation were independently associated with the level of HIV RNA detected in cerebrospinal fluid (CSF) during the earliest stages of HIV infection (Fiebig I to III).

**Methods:** Concurrent blood, CSF and sigmoid biopsy samples were obtained at the time of AHI diagnosis (8 Fiebig I, 11 Fiebig II and 19 Fiebig III) in the context of an observational study of AHI in Bangkok, Thailand (RV254/SEARCH 010). CSF and plasma HIV RNA levels were measured by Roche Amplicor HIV-1 Monitor and Roche COBAS TaqMan HIV-1 tests. Multiparameter flow cytometry was performed using frozen and fresh samples to determine systemic and mucosal immune activation (Ki67+ and CD38+/HLA-DR+), respectively. CSF chemokine levels (IP-10 and neopterin) were quantified by ELISA. Mann Whitney U test was used for comparisons and linear regression and Pearson's Correlation to evaluate associations.

**Results:** Among 38 early AHI subjects, 90% were MSM, the median age was 29 years, mean CD4 count was 438 cells/mm<sup>3</sup>, and estimated duration since exposure was 15 days. During early AHI, plasma (p<0.001) and CSF HIV RNA (p<0.001), CSF neopterin levels (p=0.003) and the frequency of activated CD8+ T cell in blood (p=0.002) and sigmoid mucosa (p<0.001) increased with progression from Fiebig I to Fiebig III. In univariate analyses of the overall group, CSF HIV RNA was associated with CSF IP-10 levels (r=0.37, p=0.04), CSF neopterin levels (r=0.61, p<0.001), the frequency of CD8+Ki67+ T cells in the blood (r=0.56, p=0.001) and the frequency of CD8+Ki67+ (r=0.48, p=0.008) and CD8+CD38+HLA-DR+ (r=0.46, p=0.01) T cells in the mucosa. Moreover, when adjusting for levels of plasma HIV RNA, the frequency of peripheral CD8+Ki67+ T cells remained a significant predictor of CSF HIV RNA (adjusted r=0.78, p<0.001; Figure 1).

**Conclusions:** During early AHI, CSF inflammation and peripheral and mucosal immune activation are present and increase with progression of Fiebig stage from I to III. The correlation between CSF HIV RNA and frequency of activated CD8+ T cells in the blood independent from plasma HIV RNA supports the hypothesis that peripheral immune activation modulates the amount of HIV entering the CNS during this earliest stage of infection.

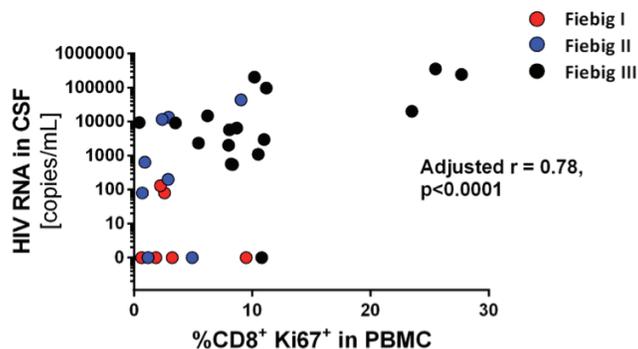


Figure 1: Multivariate correlation between CSF HIV RNA and the frequency of peripheral CD8+Ki67+ T cells adjusted for plasma HIV RNA.

**405 Early CSF Viremia and CNS T-Cell Infiltrate in a Nonaccelerated SHIV Infection Model**

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**Background:** Recent studies describe CNS inflammation and viremia during the earliest stages of HIV infection. We sought to develop a non-accelerated model to characterize these effects in rhesus macaques.

**Methods:** Macaques were infected with a single SHIV1157ipd3N4 challenge intrarectally (9 males) or intravaginally (3 females). Weekly plasma SHIV RNA and CD4+ T cell counts were quantified by RT-PCR and flow cytometry. Immune activation markers (IP-10, MCP-1 and IL-15) were measured by Luminex at weeks 2 and 12 post infection (W2 and W12) in plasma and W12 in CSF. At W12 necropsy, brain sections from 6 SHIV+ and 6 uninfected control animals were stained by immunohistochemistry (IHC) with CD3, CD4, and CD68 antibodies and quantified as immunoreactive cells per 40 HPF.

**Results:** Plasma SHIV RNA mimicked early human HIV infection with mean peak and W12 set-point viremia at 6.4 and 5.1 log<sub>10</sub> copies/mL, respectively. CSF viremia was detectable in the 4 animals with highest W12 plasma viral load. Plasma IL-15, MCP-1 and IP-10 at W2 were significantly elevated over baseline (19.7 vs 9.8 pg/mL, p=0.0002; 436 vs 215 ng/mL, p<0.0001; 384 vs 70 pg/mL, p=0.0001, respectively), and normalized by W12. However, while there was no elevation of IL-15 in the W12 CSF, MCP-1 and IP-10 W12 CSF levels were significantly elevated over W12 plasma (284 vs 136 ng/mL, p<0.0001; 282 vs 112 pg/mL, p=0.0005) and over normal control CSF (284 vs 126 pg/mL, p=0.03; 282 vs 117 pg/mL, p=0.004). The W12 CSF/serum albumin ratio was <5 x 10<sup>-3</sup> in all 12/12 animals, consistent with an intact blood brain barrier. IHC revealed no evidence of CD68+ or CD4+ infiltrate in midbrain, frontal cortex, or basal ganglia. A subset of SHIV-infected animals had evidence of a mild CD3+ T cell infiltrate with qualitative paving along the vascular epithelium, and clustering in the brain parenchyma. Interestingly, CD4+ T cells were increased in the meninges of a subset of SHIV-infected animals vs controls, in the absence of CD68+ cells.

**Conclusions:** Early CSF viremia and IP-10 and MCP-1 elevations reflect a discrete neurovirologic process consistent with published human studies. This is accompanied by a mild, non-specific CD3+, CD4- infiltrate in the brain parenchyma and CD4+ T cells in the meninges in some SHIV+ animals in the absence of macrophages. The SHIV1157ipd3N4 non-accelerated challenge model reflects pathophysiologic changes characteristic of acute/early HIV infection in humans, and can serve as a model for future interventional studies.

**406 Relative Frequency of Drug Resistance Mutations on Individual HIV-1 Genomes in HAND**

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**Background:** The ability of the CNS to act as sanctuary may result in discordant drug-resistance mutations (DRMs) in CSF and plasma. Compartmentalization and independent evolution of DRMs in CSF HIV-1 variants has been described. We hypothesized that in those with HAND differences in the relative frequency of DRMs in the CSF and plasma would be observed.

**Methods:** We used SGA to study paired CSF and plasma samples of 12 HIV+ subjects from the CHARTER study with no neurocognitive impairment (NCN) (N=5), asymptomatic neurocognitive impairment (ANI) (N=4), mild neurocognitive disease (MND) (N=2) and HIV-associated dementia (HAD) (N=1). Subjects were viremic and on treatment at the time of sampling. The Stanford HIVdb program and phylogenetic analyses were used to identify HIV-1 DRMs on each SGA-derived genome and determine viral compartmentalization respectively.

**Results:** On average, 25 SGS were analyzed per compartment per subject (N=621). We found statistical evidence of compartmentalization between CSF- and plasma-derived viruses (p<0.05) in individuals with NCN (2/5=40%) and HAND (5/7=71%). HIV-1 DRMs were commonly found, with 11/12 (92%) subjects with at least 1 DRM in the CSF or plasma (median 7, range 1-18). Multiple DRMs were commonly identified on SGA-derived genomes (range 0-6). For all subjects with NCN, the relative frequency of each DRM was statistically similar between compartments. In contrast, in subjects with HAND, 57% (4/7) of individuals demonstrated statistically significant differences in the relative frequency of at least one DRM in the CSF and plasma (Bonferroni corrected p-value thresholds from p<0.0028 to p<0.0125). This represents a trend toward a statistical difference in the relative frequency of discordant DRMs in the CSF and plasma in those with NCN and HAND (p=0.08 by Fisher's exact test). When identified, the relative difference in DRMs reflects a higher percentage of DRMs in the plasma as opposed to the CSF (21 events vs. 1, p<0.0001 by Fisher's exact test). 3 subjects with HAND (3/7=43%) demonstrated one DRM in the CSF that was not identified in paired plasma. Longitudinal analyses of 2 subjects with HAND reveal the development of significant differences in the relative frequency of DRMs in the CSF and plasma over time - both in the presence (N=1) and absence (N=1) of changes in the cART regimen.

**Conclusions:** Using SGA, statistically significant differences in the frequency of DRMs in the CSF and plasma are readily found in those with HAND.

**407 Dual Role of Activated and HIV-Specific CD8 T Cells in CSF During Acute HIV Infection**

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**Background:** HIV enters the CNS compartment as early 8 days post estimated exposure in humans. Infiltration of CD8 T cells into the CNS is a recognized feature of many neurodegenerative diseases and is seen in HIV-associated dementia. The number of CD8 T cells present in the cerebrospinal fluid (CSF) during HIV infection is elevated compared to other CNS diseases; however, the role of these CD8 T cells and whether they are serving a detrimental or protective effect during acute HIV infection is unknown.

**Methods:** We analyzed CD8 T cells from CSF in a unique cohort enrolled during the earliest stages of acute HIV infection (RV254/SEARCH010 cohort, n=26) compared to chronic HIV patients (SEARCH011, n=9, cART naive) and uninfected controls (RV304/SEARCH013, n=8). We analyzed the absolute number and phenotype of CD8 T cells from the CSF and blood by flow cytometry. Phenotypic analyses were completed; we then correlated these with CSF HIV RNA and inflammatory markers from CSF. After *in vitro* expansion of the CSF CD8 T cells, we examined Vbeta repertoire usage by flow cytometry; HIV-specificity was determined by intracellular cytokine staining using peptide pools covering Env, Pol, Gag and Nef.

**Results:** The total numbers and frequency of activated CSF CD8 T cells were elevated in chronic and acute HIV-infected participants compared to controls (P=0.04 and P<0.0001, respectively). Activated CD8 T cells correlated with CSF HIV RNA (P=0.001), and with markers of CNS inflammation [neopterin (P=0.0005), IP-10 (P=0.004), CD163 (P<0.0001), and sCD14 (P=0.02)]. CD8 T cells in the CSF harbored a more restricted Vbeta repertoire compared to total and activated CD8 T cells from peripheral blood. During acute infection, different Vbeta families were expanded in the CSF and peripheral blood, suggesting local specific expansions. Finally, HIV-specific CD8 T cells were detected during acute infection in CSF and activated CD8 T cells in blood. These CD8 T cells were found to target different HIV proteins in the CSF compared to peripheral blood.

**Conclusions:** These results highlight the dual role of activated CD8 T cells in CSF during HIV infection. Their increase in the CSF early in HIV infection and their correlation to neuro-inflammatory markers suggest that they could play a role in the development of HIV-associated neuroinflammatory disorders. Their unique T cell repertoire and HIV-specificity in the CSF in acute infection suggest that they could also play a role in controlling viral replication in the CNS compartment.

**408 CD4/CD8 Ratio Decline and Risk of Neurocognitive Deterioration**

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**Background:** Persistent immune activation is among suspected causes of HIV-associated neurocognitive disorders (HAND) in the cART era. The CD4/CD8 ratio has been recently identified as a marker of immune activation and HAND. Our aim was to investigate a potential impact of CD4/CD8 ratio decline over time on neurocognitive deterioration

**Methods:** Randomly selected HIV-infected patients were included in the Neuradapt study and followed for neuropsychological (NP) testing during a follow-up period of almost 2 years. Tests were adjusted for age, gender and education. Patients were divided into 5 groups: Normal tests (NT), neuropsychological deficit (ND, 1 impaired cognitive domain), asymptomatic neurocognitive disorders (ANI), mild neurocognitive disorders (MND) and HIV-associated dementia (HAD). Risk factors for neurocognitive deterioration were analyzed.

**Results:** 256 patients underwent NP tests and 94 completed follow-up. The two groups were comparable. Mean duration of follow-up was 22.4 months. At inclusion, 75 patients were male (80%), mean age was 46.3 years (SD=10.8), mean time since HIV infection was 12.1 years, mean CD4 cell count 552.1 cc/mm<sup>3</sup> (SD=273.7), mean nadir of CD4 cells was 262.3 cc/mm<sup>3</sup> (SD=183.1), mean CD4/CD8 ratio was 0.73, 69 subjects (73%) had HIV-RNA below 200 copies/ml and 23 (25%) were HCV co-infected. Upon neuropsychological retesting, 6 patients showed clinical improvement, 30 had worsened and 58 were stable, resulting in 42 patients presenting with HAND (45%). The majority of HAND cases consisted of ANI (26%) and MND (16%).

Patients who worsened their NP performances had lower CNS penetration effectiveness (CPE 2010) score at inclusion (7.13 vs 8.00,  $p=0.003$ ) and higher frequency of CD4/CD8 decrease (60% vs 31%,  $p=0.008$ ) than those who were stable or improved. Multivariate analysis confirmed that the CPE score at inclusion (OR 0.59, [CI 0.37-0.86],  $p=0.015$ ) and the slope of the CD4/CD8 ratio over time (OR 3.7 [CI 1.45-9.87],  $p=0.007$ ) were independent risk factors for cognitive decline.

**Conclusions:** A decrease of the CD4/CD8 ratio in a longitudinal follow-up of randomly selected HIV-infected patients was independently associated with cognitive decline, suggesting an inflammatory pathway for brain injury. Monitoring the trend in CD4/CD8 ratio could contribute to identifying patients at higher risk of neurocognitive deterioration.

**409 Novel CSF Biomarker Associations With HIV-Associated Neurocognitive Disorder (HAND)**

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**Background:** Dysregulated iron transport may promote glial-cell activation and angiogenesis, which are implicated in blood-brain-barrier compromise and some neurodegenerative disorders. We hypothesized that CSF ceruloplasmin (CP), a ferroxidase involved in iron transport, haptoglobin (HP), an iron-binding protein and ligand for the macrophage-monocyte scavenger receptor CD163, and vascular endothelial growth factor (VEGF) are associated with HAND.

**Methods:** CP, HP, and VEGF were quantified by bead suspension array immunoassay in CSF from 405 participants enrolled in the CHARTER cohort who underwent comprehensive neuropsychiatric and neuromedical evaluations. CSF biomarker associations with HAND, defined by a global deficit score (GDS)  $\geq 0.5$  (GDS impairment) or by Frascati criteria, and with GDS as a continuous measure (cGDS), were evaluated for tertile (T)3 vs. T1, due to non-normality of biomarker distributions, adjusting for antiretroviral therapy, nadir CD4, genetic ancestry, and comorbidity status (contributing vs. incidental to HAND). Analyses stratified by comorbidity and excluding subjects with multiple biomarker values  $\geq 2$  SD above the mean were also performed.

**Results:** Higher CSF VEGF was associated with GDS impairment and cGDS in univariate analyses [odds ratio (OR) 2.17 and  $\beta=0.12$ , respectively; both  $p<0.05$ ] and remained associated with GDS impairment after adjustment (OR 2.00,  $p<0.05$ ). GDS impairment was associated with higher CSF CP in adjusted analyses (OR 1.77,  $p<0.05$ ) and with higher CP and HP in 252 persons with only minimal comorbidities (ORs 2.37 and 2.13, respectively; both  $p<0.05$ ). In the subset with minimal comorbidities, higher CP and HP levels were also associated with HAND by Frascati criteria (ORs 2.44 and 2.08, respectively, both  $p<0.05$ ), and increased CP was associated with higher cGDS values (more impairment,  $p<0.01$ ). Finally, CP and HP were associated with GDS impairment (ORs 5.57 and 2.96, respectively; both  $p<0.01$ ) and HAND by Frascati criteria (both  $p<0.01$ ) in persons with undetectable plasma viral load and minimal comorbidities. Weak correlations of CP, HP, and VEGF with concurrently measured IL-6, IL-8, CXCL10, and TNF- $\alpha$  were observed.

**Conclusions:** CSF HP and CP are associated with HAND in HIV-infected persons, especially those with minimal comorbidities; VEGF levels are also linked to HAND. Future studies might explore interventions aimed at disordered iron transport and angiogenesis for preventing or treating HAND.

**410 (1 $\rightarrow$ 3)- $\beta$ -D-Glucan Levels Correlate With Neurocognitive Functioning in HIV Infection**

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<sup>1</sup>Univ of California San Diego, San Diego, CA, USA; <sup>2</sup>Rsr Lab, Associates of Cape Cod, Inc, Falmouth, MA, USA

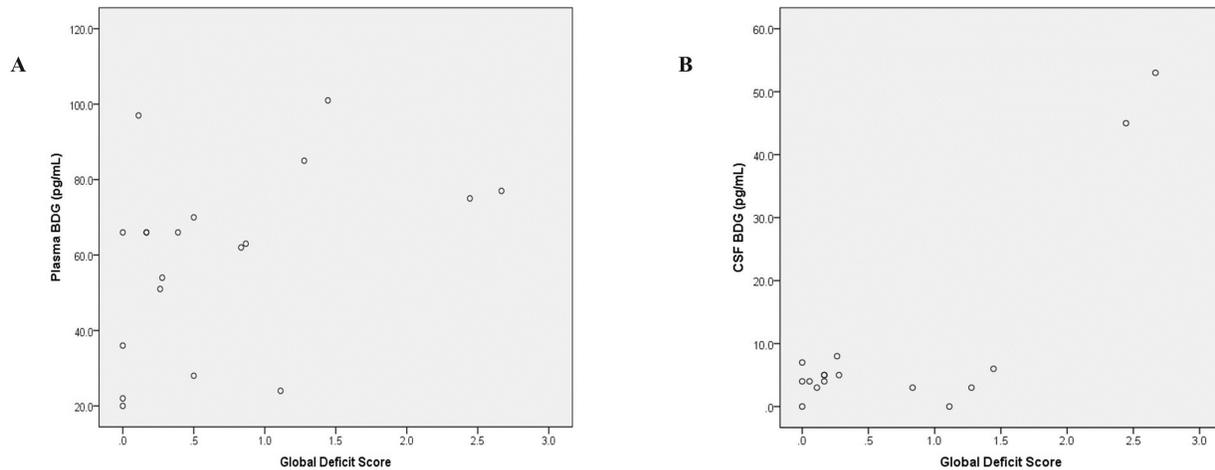
**Background:** Microbial translocation from the gastrointestinal tract is associated with persistent inflammation, and might play a role in the pathogenesis of neurocognitive dysfunction during HIV-infection, even in people treated early after seroconversion. (1 $\rightarrow$ 3)- $\beta$ -D-Glucan (BDG) is a polysaccharide component of most fungal species cell walls including *Candida* spp. In the absence of an active fungal infection, increased blood BDG levels may be an indicator of gut mucosal barrier disruption and microbial translocation. The objective of this study was to evaluate whether higher blood BDG levels correlate with worse neurocognitive functioning (evaluated by global deficit score [GDS]) in a cohort of HIV+ adults on suppressive antiretroviral therapy (ART).

**Methods:** We measured levels of BDG in paired plasma and CSF samples, and compared levels with GDS, soluble (s)CD14 and Interleukin-8 (IL-8) in a cohort of 19 adults with acute/early HIV diagnosis, early treatment and suppressed levels of HIV RNA in blood plasma throughout treatment. Study samples were collected prospectively between December 2013 and June 2014 at the University of California, San Diego. 19 plasma and 16 CSF samples were stored at -80°C on the day of collection. BDG testing of plasma and CSF supernatant samples was performed in June 2015 at Associates of Cape Cod, Inc., research laboratories using the Fungitell assay.

**Results:** Median GDS was 0.39 (range 0 - 2.67;  $>0.5$  is considered at least mild cognitive impairment). Median plasma BDG level was 66 pg/mL (range: 20-101 pg/mL), median CSF BDG level was 5 pg/mL (range: 0-53 pg/mL). Higher levels of plasma BDG were associated with more severe cognitive impairment as measured by the GDS (Spearman  $r=0.47$ ;  $p=0.04$ , Figure A). Correlation of other markers with GDS scores were as follows: IL-8 ( $r=0.55$ ;  $p=0.014$ ), sCD14 ( $r=0.4$ , n.s.), nadir CD4 count ( $r=0.01$ , n.s.). We found no significant correlation between plasma BDG and IL-8 ( $r=0.12$ , n.s.) and plasma BDG and plasma sCD14 ( $r=0.38$ , n.s.).

Two CSF samples presented elevated BDG levels (45 and 53 pg/mL; Figure B), while all other samples had BDG levels  $< 10$  pg/mL. Interestingly, these two samples originated for the two subjects with the highest GDS scores of the cohort.

**Conclusions:** BDG may be an indicator of gut mucosal barrier disruption and a promising independent biomarker associated with neurocognitive functioning in virologically suppressed HIV infected individuals with high CD4 counts diagnosed and treated



**411 Use of Mass Cytometry to Study Cellular Subsets in CSF and Blood in ART-Treated HIV**

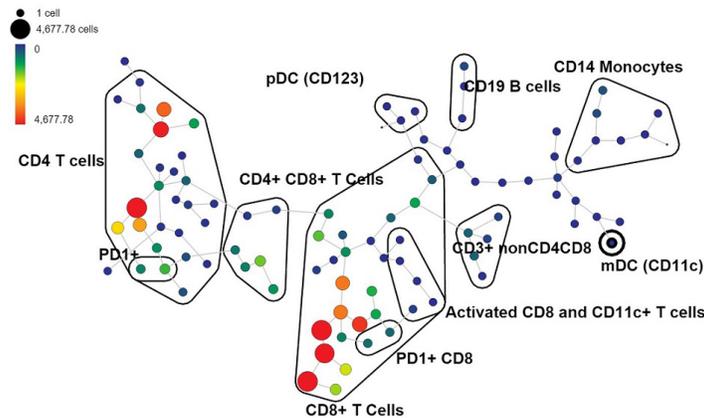
**Shang-Lin Chung**; Diane Trotta; Leah Le; Laurie Andrews; Jennifer Chiarella; Khadir Raddassi; Serena S. Spudich; Brinda Emu  
*Yale Univ Sch of Med, New Haven, CT, USA*

**Background:** HIV leads to immunological abnormalities in the blood and cerebrospinal fluid (CSF) that persist despite effective antiretroviral therapy (ART). Novel methods are needed to comprehensively characterize these changes. Mass cytometry (CyTOF) uses metal-conjugated antibodies to label cells allowing for larger number of markers compared to flow cytometry. We used both CyTOF and flow cytometry to examine cell surface markers in blood and CSF in participants on ART, despite low CSF white blood cell counts (WBC).

**Methods:** Fresh specimens of blood and CSF were analyzed by CyTOF (n=4) and flow cytometry (n=4) from HIV infected participants on ART with plasma viral suppression, and HIV-uninfected individuals (n=3). For CyTOF, 20 markers were analyzed in a single panel (CD3, CD4, CD8, CD11c, CD14, CD16, CD19, CD27, CD28, CD25, CD38, CD45, CD45RO, CD56, CD123, CD127, CCR7, HLA-DR, PD-1, cisplatin) to identify T cells (including exhaustion, activation, maturation, regulatory phenotypes), monocytes, NK cells, and dendritic cells. For flow cytometry, two different panels were employed (A) CD3, CD4, CD8, CD27, CD28, CD38, CD45RA, CCR7, HLA-DR, PD-1; and (B) CD3, CD11c, CD14, CD16, CD19, CD45, CD56, CD123, and HLA-DR.

**Results:** CSF WBC was < 5 cells/mcl except for a single patient with 25 cells/mcl. Total CSF cell count captured on CyTOF ranged between 1981-111443 events (median 5679). Phenotypically-related cell clusters were generated based on a hierarchical algorithm through SPADE (see figure for representative analysis), allowing for evaluation of T and non-T cell subsets. In analysis of CyTOF data, a unique effector memory (EM) subset comprised the largest proportion of CSF CD4+ and CD8+ T cells (CD45RO+CCR7-CD27+CD28+), which was higher than that seen in blood. Data from flow cytometry confirmed that this EM population was higher in CSF compared with blood in CD4+ (65.1% vs 24.9%, p=0.0001) and CD8+ T cells (53.3% vs 20.4%, P=0.0004). This EM subset in CSF had a higher proportion of CD38+/HLA-DR+ (14.1 vs 4.1%, P=0.03 in CD4; 23.3 vs 3.5%, P=0.03 in CD8) and PD-1 (58.3% vs 34.7%, P=0.003) expression as compared to blood by flow.

**Conclusions:** This pilot study has for the first time demonstrated meaningful use of CyTOF to analyze immune cells in CSF. Our results and methodology provide opportunity for future studies to reveal intricate CSF cellular phenotypic patterns of numerous disorders, including neurocognitive disorders associated with treated HIV.



**412 CNS Drug Distribution and CSF Inflammation During Suppressive Antiretroviral Therapy**

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**Background:** Antiretroviral therapy (ART) drugs differ in their distribution into the CNS. Greater estimated ART distribution into the CNS is associated with lower HIV RNA levels in CSF but the relationship with inflammation in the CNS during viral suppression is relatively unknown. The objective of this analysis was to assess this using a training-validation approach with the hypothesis that higher CNS penetration-effectiveness (CPE) values would be associated with lower levels of inflammation-associated biomarkers in CSF.

**Methods:** We selected 268 HIV+ adults who were assessed in the CHARTER study and who were taking 3-drug ART; underwent lumbar puncture; and had HIV RNA in plasma and CSF  $\leq 50$  copies/mL. CXCL10, TNF- $\alpha$ , and IL-6 were quantified in CSF by bead suspension array immunoassay in 2 independent samples: the Training Set (TS, n=144) and the Validation Set (VS, n=124). Data were analyzed using conventional parametric, non-parametric, and multivariable regression methods.

**Results:** The two sets were similar in age, self-reported race/ethnicity, sex, body mass index, AIDS status, current and nadir CD4<sup>+</sup> T-cell count, self-reported ART duration, and CPE values. The most common ART regimen was efavirenz-tenofovir-emtricitabine (n=49). All HIV RNA levels were  $\leq 50$  copies/mL. In both groups, higher CPE values correlated with lower levels of CXCL10 (TS:  $r=-0.30$ ,  $p<0.001$ ; VS:  $r=-0.30$ ,  $p<0.001$ ) and TNF- $\alpha$  (TS:  $r=-0.22$ ,  $p=0.01$ ; VS:  $r=-0.19$ ,  $p=0.03$ ) but not IL-6 (TS:  $r=-0.11$ ,  $p=0.23$ ; VS:  $r=0.07$ ,  $p=0.48$ ). Multivariable models combined data from both groups and adjusted for other statistically significant correlates of each biomarker as well as correlates of CPE. These models confirmed the associations between higher CPE values and either lower CXCL10 (model  $R^2=0.18$ ,  $p<0.001$ ) or TNF- $\alpha$  levels (model  $R^2=0.05$ ,  $p=0.04$ ).

**Conclusions:** During suppressive ART, regimens that are estimated to have better distribution into the CNS are associated with less inflammation in CSF, as indicated by levels of CXCL10 and TNF- $\alpha$ . This may reflect better suppression of HIV RNA in the CNS. Estimated ART drug distribution into the CNS did not correlate with IL-6 levels in CSF, which is consistent with findings that IL-6 can both remain elevated during suppressive ART and be associated with HAND (e.g., JAIDS 2015 68:281-8). While cross-validation is a strong approach, longitudinal analyses are needed to further strengthen confidence in these findings.

#### 413 Role of HCV Coinfection on CSF Biomarkers in HIV Patients

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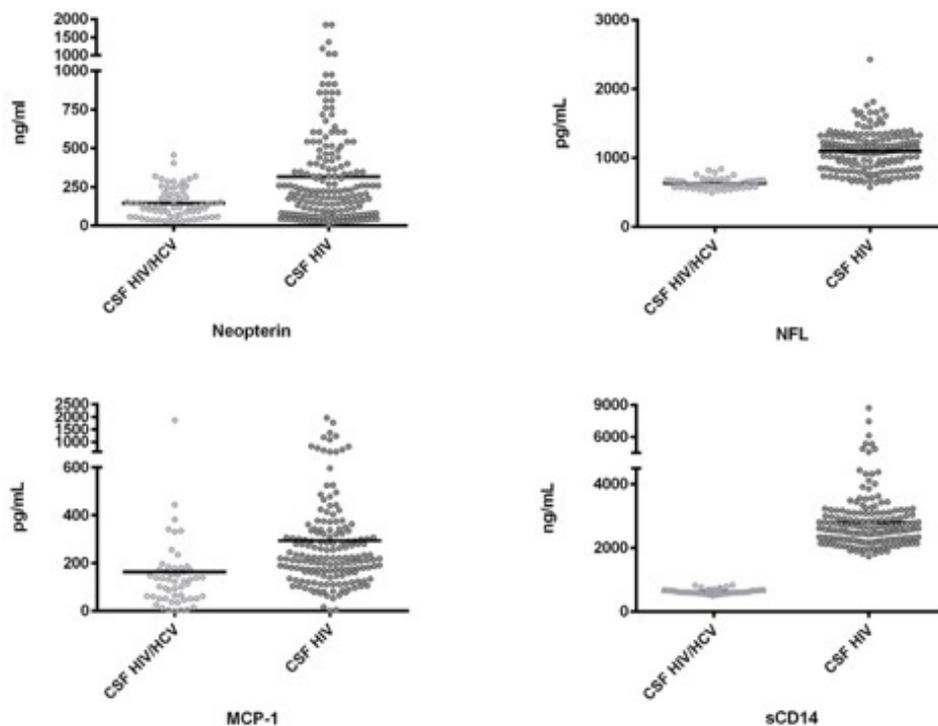
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**Background:** The effect of HCV co-infection on neurological injury has not been explored. Aim was to evaluate changes in CSF biomarkers of neurodegeneration and inflammation and to analyse contribution of HCV to neuroinjury.

**Methods:** Retrospective analysis of neopterin, neurofilament (NFL), sCD14 (ELISA assays), MCP-1 (Luminex assay) concentrations in CSF/plasma paired samples from HIV-infected patients (pts). Lumbar puncture (LP) achieved for neurological signs/symptoms, CNS staging of lymphoma, or neurological disease. By fitting a multivariate linear regression model, factors independently associated with CSF biomarkers were identified.

**Results:** 238 CSF/plasma pairs from 234 pts were included: 79% male, median age 47 (IQR, 39-51), HIV transmission: heterosexual 34%, MSM 21%, IVDU 27%; 79% in CDC C. Neurological signs/symptoms in 37.8%. At LP, median CD4 was 184/mm<sup>3</sup> (IQR 93-408), nadir 75/mm<sup>3</sup> (IQR 21-156); 173 pts (72.7%) were on ARV, with median log<sub>10</sub> HIV-RNA of 2.2 (IQR 1.6-4.4) in plasma and  $<1.7$  (IQR 1.6-3.2) in CSF. In 51% plasma HIV-RNA was  $<50$  cp/mL. Median log<sub>10</sub> HCV-RNA in plasma and CSF was 5.8 and 1.0. Detectable HIV-RNA in both plasma and CSF was independently associated with higher levels of neopterin ( $p=0.001$ ) and NFL ( $p=0.002$ ), while CD4 $>350$ /mm<sup>3</sup> with lower levels of MCP-1 ( $p=0.022$ ) and of sCD14 ( $p=0.009$ ), when compared with CD4 $<200$ . Pts with neurological disease showed a higher concentration of sCD14 ( $p=0.013$ ). 67 pts were HIV+/HCV+, with detectable plasma HCV-RNA in 52 (29%) (median log<sub>10</sub> HCV-RNA in plasma: 5.7); 13 (25%) of them had detectable CSF HCV-RNA (median log<sub>10</sub> 1.04). At multivariable analysis, higher plasma HCV-RNA was associated to a lower concentration of CSF neopterin ( $p<0.001$ ), NFL ( $p<0.001$ ), MCP-1 ( $p=0.001$ ) and sCD14 ( $p=0.001$ ). Differences in biomarkers are shown in Fig. 1. In a multivariate analysis restricted to HCV-RNA+ pts, higher CSF HCV-RNA was associated with increased CSF neopterin ( $p=0.010$ ), MCP-1 ( $p<0.001$ ), sCD14 ( $p=0.005$ ). CSF values of neopterin, MCP-1, sCD14 and NFL in HIV+ remained significantly higher than those observed in HIV+/HCV+.

**Conclusions:** HIV-RNA contemporary detectable in plasma and CSF is associated to increased values of neuroinflammatory and neuroinjury biomarkers. Interestingly, HCV-RNA in CSF independently correlates with increased neuroinflammation and/or neuroinjury, while this effect seems to be reversed in case of HCV-RNA present only in plasma. The clinical implications of these findings are warranted.



**414 Psychiatric Symptoms Are Common in Acute HIV and Correlate With Disease Biomarkers**

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**Background:** To assess the trajectory of anxiety and depression in acute HIV infection over six months following HIV diagnosis, and assess correlations between affective symptoms and blood and cerebrospinal fluid (CSF) disease biomarkers prior to treatment.

**Methods:** A total of 123 participants were enrolled during acute HIV infection (Fiebig I-V) through the SEARCH010/RV254 cohort in Bangkok, Thailand. Participants completed the Hospital Anxiety and Depression Scale (HADS) and the Patient Health Questionnaire (PHQ-9) at baseline prior to combination antiretroviral therapy (cART) and again at weeks 4, 12, and 24 following diagnosis. Depression was defined as a score  $\geq 8/21$  on HADS-D or  $\geq 10/27$  on PHQ-9, with anxiety defined as a score  $\geq 8/21$  on HADS-A. Disease biomarkers of plasma and CSF HIV RNA levels, CD4 count, and plasma and CSF neopterin were obtained at entry, as was magnetic resonance spectroscopy (MRS).

**Results:** At diagnosis, 46% of subjects met the clinical cutoff for depression on the PHQ-9 and 41% on the HADS-D, with 66% meeting the threshold for anxiety on the HADS-A. Affective symptoms decreased from baseline to week 12 without significant change from week 12 to week 24 (8% frequency on HADS-D, 18% frequency on PHQ-9, 17% frequency on HADS-A at the final visit). At baseline, higher average  $\log_{10}$  plasma HIV RNA was observed in participants experiencing depression on the HADS-D (5.9 vs. 5.7;  $p=0.006$ ), or on the PHQ-9 (6.0 vs. 5.6;  $p=0.010$ ) compared to those not experiencing significant depression. Similarly, those with baseline depression on the PHQ-9 had a lower CD4 count (330 vs. 414 cells/mm<sup>3</sup>;  $p=0.024$ ) and higher plasma neopterin, a marker of macrophage activation (3150 vs. 2118 pg/mL;  $p=0.026$ ). CSF neopterin was higher on average in those experiencing baseline anxiety on the HADS-A (3218 vs. 1545 pg/mL;  $p=0.011$ ;  $n=19$ ). Examined as continuous variables, CSF neopterin correlated with PHQ-9 depression scores ( $r=0.47$ ;  $p=0.044$ ) and plasma neopterin correlated with HADS-A anxiety scores ( $r=0.304$ ;  $p=0.037$ ). No differences between groups were seen on MRS indices.

**Conclusions:** In acute HIV infection, anxiety and depression are tightly linked to disease biomarkers, including indicators of plasma and intrathecal immune activation. These results confirm high rates of anxiety and depression in acute HIV that decrease with time in the setting of early cART, stabilizing at weeks 12 and 24.

**415 Neurologic Signs and Symptoms Frequently Manifest in Acute HIV Infection**

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**Background:** To determine the incidence, timing, and severity of neurologic findings in pre-antibody seroconversion acute HIV infection, as well as persistence after early combination antiretroviral therapy (cART).

**Methods:** A prospective cohort of participants identified through laboratory screening at an HIV/STD testing center in Bangkok, Thailand with Fiebig I-V acute HIV underwent structured neurologic evaluations, immediately initiated cART, and were followed with neurologic evaluations at 4 and 12 weeks after diagnosis. Concurrent viral and inflammatory markers in the blood and cerebrospinal fluid (CSF) were obtained, as was magnetic resonance imaging (MRI).

**Results:** For the 139 participants, median estimated HIV infection duration at baseline evaluation was 19 days (range: 3-56). Seventy-three participants (53%) experienced one or more neurologic finding in the 12 weeks after diagnosis, with one developing a fulminant neurologic manifestation (Guillain-Barre syndrome). A total of 245 neurologic findings were noted, reflecting cognitive symptoms (33%), motor findings (34%), and neuropathy (11%). Nearly half of the neurologic findings ( $n=121$ , 49%) occurred at diagnosis, prior to cART initiation, and most of these ( $n=110$ , 90%) remitted concurrent with one month on treatment. Only 9% of neurologic findings ( $n=22$ ) persisted at 24 weeks on cART. Nearly all neurologic findings ( $n=236$ , 96%) were categorized as mild in severity. Participants with neurologic findings had a higher mean plasma  $\log_{10}$  HIV RNA at diagnosis compared to those without neurologic findings (5.9 vs. 5.4;  $p=0.006$ ), but no differences in markers of immune activation in blood or CSF. Four subjects with neurologic findings referable to the central nervous system (CNS) had undetectable CSF HIV RNA at diagnosis. No structural neuroimaging abnormalities were observed.

**Conclusions:** Acute HIV infection is commonly associated with mild neurologic findings that largely remit while on treatment, and may be mediated by direct viral factors. Severe neurologic manifestations are infrequent in acute and early HIV in the setting of immediate treatment.

**416 Male/Female Differences in Cognitive Function in HIV+ Individuals**

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**Background:** Converging evidence suggests that HIV+ women are more vulnerable to cognitive impairment compared to HIV+ men. Historically, comparisons of neurocognitive function between men and women living with HIV/AIDS have been difficult to interpret because of limited sample sizes and confounding by education, ethnicity, and socioeconomic status.

**Methods:** We completed a longitudinal investigation of the independent and interactive effects of sex and HIV serostatus on neurocognitive measures including speed of information processing, executive function, and fine motor skills in HIV-infected and HIV-uninfected women from the Women's Interagency HIV Study (WIHS) and men from the Multicenter AIDS Cohort Study (MACS) who were individually matched on race/ethnicity, HIV status, education, and age. The average number of visits for WIHS participants was 6.2 and for MACS was 9.2. Results were adjusted for other potential confounding factors including income, depression, and substance use.

**Results:** The cohort consisted of 710 (429 HIV+) female and 710 male (429 HIV+) (67% NonHispanic-Black; 53% high school or less). In the multivariable analyses, we detected significant Sex by HIV Serostatus interactions on the Symbol Digit Modalities Test (SDMT) and both of the Trail Making Tests (TMT). Among HIV-uninfected individuals, women showed enhanced SDMT performance compared to men; however, among HIV-infected individuals that difference was eliminated ( $P = .007$ ). On TMT Parts A and B, HIV-infected women performed significantly worse than HIV-infected men; however, among HIV-uninfected individuals the difference between women and men was smaller (Part A) or did not exist (Part B).

**Conclusions:** These results suggest that HIV-infected women have greater cognitive vulnerabilities than HIV-infected men in the domains of processing speed and executive function. Our study also demonstrates that direct comparisons of neurocognitive outcomes among large cohorts of HIV+ men and women are feasible and important to identify possible sex-specific profiles of neurocognitive impairments in HIV.

**417 CNS Safety of Simplification to ATV/r+3TC in Virologically Suppressed HIV+ Patients**

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**Background:** A concern of simplification to dual therapies is their low CNS penetrance. We explored the evolution of neurocognitive performance (NP) at week 48(w48) after treatment simplification to ATV/r+3TC versus maintaining 3-drugs ATV/r-based cART.

**Methods:** ATLAS-M (NCT 2011-001060-21) is a phase IV, multicenter, open-label, randomized, non-inferiority trial. Patients (pts) on ATV/r+2NRTIs, without previous virological failure, with HIV-RNA <50copies/mL and CD4>200cells/mm<sup>3</sup> for >6months were randomized to switch to ATV/r+3TC(dual therapy, DT) or to maintain the 3-drug regimen(triple therapy, TT). At baseline(BL) and W48, NP was evaluated. Exclusion criteria for NP testing were: active psychiatric disorders, alcohol/drug abuse, and linguistic difficulties for non native pts. Raw scores obtained at each task were Z-transformed using normative data. A composite Z-score was calculated to explore global NP. Cognitive impairment was defined according to Frascati criteria. Factors associated with the evolution of NP were identified by linear regression analysis.

**Results:** A total of 266 pts (78% males, median age 44 yrs, median CD4 603cells/μL) were enrolled. W48 NP data were available in 151 pts, of which 81 and 70 in DT and TT arms, respectively. Pts with an available NP testing showed shorter time from HIV diagnosis(p=0.024) and cART initiation(p=0.049), higher CD4 nadir(p=0.04), lower proportions of females(p=0.003), HCV co-infection(p=0.008) and past IDU(p=0.004) compared to the 115 participants not included in the sub-study. At BL, pts in the DT and TT arms did not differ for the main characteristics and showed a similar proportion of cognitive impairment(16% vs 21%,p=0.41). Overall, no change in the prevalence of cognitive impairment was observed at W48(p=0.39). DD and TT confirmed a comparable global NP(-0.04 vs -0.003,p=0.78); also in all cognitive domains we observed no difference. However, both groups showed a decreased memory performance when compared to BL: mean change DT -0.42(p<0.001) and TT -0.27(p=0.01); no difference were observed between the two arms in the mean memory change(p=0.29). Analyzing factors associated with the evolution of the global NP (W48-BL composite z-score), we found no significant effect of DT compared with TT(β=0.10;p=0.11), after adjusting for time on cART(β=-0.01;p=0.09) and CD4 nadir(β=0.04 for 100 cells increase;p=0.04).

**Conclusions:** Simplification to DT was apparently CNS safe. Cognitive evolution will be further investigated at the scheduled W96 follow-up.

#### 418 Neurocognitive Improvement With NRTI-Sparing Treatment in Acute HIV Infection

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**Background:** HIV accesses the CNS within days of infection and persistent neurocognitive impairment (NI) may occur despite viral suppression due to ongoing low level viral replication causing persistent immune activation. Therefore, early antiretroviral therapy (ART) in Acute HIV infection (AHI) may improve neurocognitive function by limiting CD4 nadir.

**Methods:** In a single arm, open-label pilot study, AHI patients were treated within 30 days of diagnosis with ritonavir-boosted darunavir 800mg once daily plus etravirine, 400mg once daily or 200mg twice daily for 48 weeks. Neuropsychological performance (NP) was assessed at baseline, week 24 and week 48 in the following domains: Premorbid/language, Learning, Memory, Speed of Processing, Attention, Fine motor, and Executive functioning. Best available demographically corrected normative data were utilized to create z scores and then deficit scores for impairment ratings.

**Results:** Between August 2009 and November 2012, 15 AHI patients started treatment, 13 (87%) of whom achieved HIV RNA <200 copies/mL by week 24 of ART. Among 12 patients retained through week 48, 9 (75%) suppressed to <50 copies/mL by week 48. Median time from ART initiation to HIV RNA <200 copies/mL and <50 copies/mL was 59 days and 86 days, respectively. 13 patients completed 32 NP assessments; 8 patients at all 3 time points, 2 at baseline and week 24 or 48 and 2 at baseline only. 61% were impaired at baseline, 33% at 24 weeks and 30% at 48 weeks. There was a statistically significant improvement in overall neurocognitive performance (NP) over time (F(2,17)= 4.23, p= 0.03), with most improvement occurring from baseline to week 24 (baseline mean z = -0.69, (.14), week 24 mean z = -0.40 (15), and week 48 mean z = -0.45 (.15)). 2 of 3 persons who did not achieve improvement in overall neurocognitive function also did not suppress HIV RNA by week 24. There was no association with CD4 nadir and NP (r=-.19, ns). Self-report of current cognitive or physical problems at baseline was noted for 23%, but not there was no association with overall NP (r=-.22, ns).

**Conclusions:** Most AHI patients achieving HIV RNA suppression on ART experienced neurocognitive improvement in contrast to those who did not achieve HIV suppression, 2/3 of whom remained impaired. While psychological distress of HIV infection was noted in some, there was no significant relationship to NP. Early institution of ART during AHI may improve overall neurocognitive function and reduce the risk of subsequent neurocognitive impairment.

#### 419 HIV Associated Neurocognitive Impairment: A Randomized Controlled Trial of Lithium

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**Background:** HIV-associated neurocognitive disorders (HAND) remain highly prevalent despite effective anti-retroviral therapy (ART). The incidence of severe HAND has decreased but with longer life expectancy and associated cerebro-vascular risk factors, the overall prevalence is probably rising. Severe HAND is associated with high rates of morbidity and mortality. There is an urgent need to identify suitable neuroprotective adjunctive treatments for HAND.

Lithium is a low-cost drug and widely available in public service settings in low and middle-income countries. Two pilot studies investigating lithium in HAND have been conducted. Lithium improved neurocognitive impairment in one study and neuronal integrity in both studies. However, these studies were limited by the lack of a comparator arm, and the short duration of lithium treatment.

**Methods:** We conducted a 24 week randomized placebo-controlled trial to study lithium as an adjunctive treatment in patients with severe HAND [Global Deficit Score (GDS) > 0.5] stabilised on ART for at least 6 months with suppressed viral loads. The primary objective was to measure the change in neuropsychological function as determined by the GDS from baseline to week 24.

**Results:** We randomized 66 Xhosa patients to lithium (n=34) or placebo (n= 32) and 61 completed the study (lithium arm = 30; placebo arm = 31). Fifty eight were women and 8 men who at enrolment had a mean age of 39.3 and 40.6 years and a mean CD4+ T-cell count of 502 and 498 cells/μL respectively in the lithium and placebo arms. The median GDS for both the lithium and placebo arms at enrolment were 1.11.

The median change in GDS score between baseline and week 24 for the lithium and placebo arms were -0.57 (95% CI -0.77, -0.32) and -0.56 (-0.69, -0.34) respectively, with a mean difference of -0.054 (-0.26, 0.15); p = 0.716.

The study drug was well tolerated with no statistically significant difference (p = 0.413) in adverse events between the 2 study arms. Six serious adverse events occurred but none were considered related to the study drug. Four participants were withdrawn with 1 loss to follow-up.

**Conclusions:** We found that adjunctive lithium in virologically suppressed patients with HAND was well tolerated but had no additional benefit on neurocognitive impairment.

#### 420 Week48 Cognitive Improvement in HAND After Switch to HAART Based on CHARTER Score +3

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**Background:** Neuro+3 is a pilot open-label study which evaluates the cognitive status in patients with HAND (HIV-Associated Neurocognitive Disorders), despite effective ARV therapy for more than one year. After inclusion, the initial ARV treatment (T) was changed to a new combination with enhanced Charter score (+3) and the same battery of cognitive tests was planned after 48 and 96 weeks. Week 48 data are analyzed in the present study.

**Methods:** 63 pts were screened with BREF≤15/18 or mHIVDS≤10/12 in 8 investigational centers. 31pts were included with at least 2 ability domains >1SD for the following tests after depression assessment using Beck Depression Inventory BDI II: Grooved Pegboard (d and nd), Verbal Fluency, CVLT, Digit span, PASAT, Digit symbol, Wisconsin Card Sorting Test (6 domains).

**Results:** Median range characteristics of the 31 enrolled pts were: 26 men, 54 years (33-64), HIV duration 20 years (2-29), Nadir CD4 165/mm<sup>3</sup> (4-1465), and at screening CD4 count was 622/mm<sup>3</sup> (125-2130). Plasma HIV-RNA was <20 copies/ml at screening except in 2pts (31 and 79 copies/ml), and at baseline except in 2 other pts (24 and 22 copies/

ml) and HIV-RNA in CSF at baseline was <20 copies/ml except 6pts (38;41;47;48;64;78 copies/ml). All pts had a negative serology for HBsAg and HCV. At baseline, the median genotypic susceptibility Charter score was 6 (3-8), with NRTI 90%, NNRTI 26%, PI 61%, II 36%; after T modification, the new score based on Charter+3 was 10 ( $\geq 9$  in all pts except 1), with NRTI 84%, NNRTI 45%, PI 55%, II 97%, R5 inhibitor 32%. The average number of drugs in initial T was 3.1 [2-5] and 3.8 [3-5] after change. At week 48, significant improvements over baseline was observed (Wilcoxon exact Signed-Rank tests) on GDS (Global Dementia Score)(median 0.9 vs 1.4,  $p=0.03$ ), with a GDS reduction for 55% of pts (17/31, 95%CI=[36.0%;72.7%]), number of altered cognitive domains (3 vs 4,  $p=0.02$ ), and on Score of Cognitive Complaints (2 vs 4,  $p=0.0001$ ). At baseline, according to the criteria of AAN, there were 7 ANI, 8 MND, and 16 HAD. At week 48, they became 12 ANI, 5 MND, 8 HAD and 6 pts with only one altered ability domain: the proportion of pts improving category of HAND was 15/31 (48.4%, 95%CI=[30.2%;66.9%]).

**Conclusions:** In this little cohort of 31 pts with HAND, the gap of 48 weeks between tests prevents the learning effect. The treatment intensification by NNRTI, II, and/or R5 inhibitor was associated with a higher CHARTER score, leading to a statistically significant improvement in cognitive tests.

#### 421 Statin or ACE/ARB Effects on Neurocognitive Function of HIV-Infected Adults

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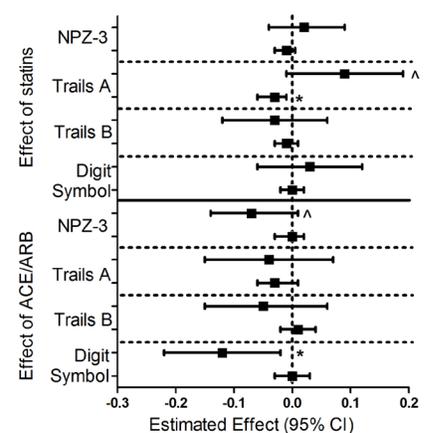
**Background:** Due to anti-inflammatory and anti-oxidant effects, we hypothesized that statins and angiotensin converting enzyme-inhibitors (ACE) or angiotensin-II receptor blockers (ARB) would protect neurocognitive function (NCF) among HIV+ adults on ART.

**Methods:** Eligible participants from the ACTG ALLRT longitudinal cohort study were not on a statin or ACE/ARB within 30 days of first neurologic assessment (baseline), and had assessments by NPZ-3 (z-score of averaged Trailmaking [Trails] A and B, and digit symbol test [DST] adjusted for race, language, education, age) from  $\geq 2$  measurements, collected every 48 weeks. Persons with known impaired NCF, current/prior CNS opportunistic infections, or major psychiatric illness were excluded; baseline diabetes also was excluded from ACE/ARB models. Marginal structural models estimated the causal effect of statin or ACE/ARB initiation on NCF change; initial constant slope was assumed during the first year of treatment and a second constant slope thereafter. Demographics, CD4 count, HIV RNA, and cardiovascular risk factors were included as confounders of treatment. ACE/ARB was included as a confounder in statin models and statins as a confounder in ACE/ARB models.

**Results:** 3949 (of 5972) ALLRT participants were included. At baseline, 53% of participants were <40 years old, male (82%), white (56%), non-smokers (62%), with CD4 count >200 cells/ $\mu$ L (79%) and HIV-1 RNA <400 copies/mL (73%). Statins were initiated in 611 (15.5%) and ACE/ARB in 377 (9.6%) participants; 182 participants (4.6%) received both. Overall, small increases in the mean NPZ-3 and component scores were seen (NPZ-3: 0.064 [95% CI: 0.059, 0.068]; Trails A: 0.079 [0.073, 0.085]; Trails B: 0.070 [0.065, 0.076]; DST 0.036 [0.031, 0.041]; all SD/year). Statin therapy was associated with a positive effect in Trails A during year 1 of exposure (Figure; estimate 0.09 [-0.01, 0.19]  $p=0.077$ ) and a negative effect (-0.03 [-0.06, -0.01]  $p=0.007$ ) with each subsequent year. Statins did not have a statistically significant effect on NPZ-3, Trails B, or DST. ACE/ARB had a negative effect on NPZ-3 scores (estimate -0.07 [-0.14, 0.01],  $p=0.078$ ) and DST (-0.12 [-0.22, -0.02]  $p=0.023$ ) during year 1 but not in subsequent years or on other neurologic function tests.

**Conclusions:** Contrary to our hypothesis, statins and ACE/ARB were not associated with sustained neurocognitive improvement in HIV+ adults. The site of drug action (macro vs microvascular) or uncontrolled factors could account for the findings.

Effect of Statin or ACE/ARB on Neurocognitive Function within Year 1 and Each Subsequent Year of Therapy



Effects of Yr 1 of therapy are shown in the 1st bar and each subsequent year in the 2nd bar for each neurocognitive test. Effects size units are reported as the standard deviation on the respective test. \* p-value <0.05; ^ p-value 0.05-0.10

#### 422 Liver Fibrosis Linked to Cognition in HIV and HCV: The Women's Interagency HIV Study

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**Background:** Cognitive disorders persist in up to one-half of people living with HIV despite access to combination antiretroviral therapy (cART). Minimal Hepatic Encephalopathy (MHE) occurs in cirrhotic patients with or without HIV infection and is thought to be associated with inflammation. Since HIV impairs gut barriers to pathogens, we hypothesized that HIV-infected adults are vulnerable to MHE in the absence of cirrhosis.

**Methods:** We completed a cross-sectional investigation of associations between liver fibrosis severity, using the aspartate aminotransferase to platelet ratio index (APRI), and neuropsychological testing performance in 1,479 women from the Women's Interagency HIV Study (WIHS). A subset underwent liver transient elastography (n=303). We evaluated associations to neuropsychological testing performance on a one-hour testing battery.

**Results:** We evaluated 1479 women (mean (SD) age of 46 (9.3) years): 770 (52%) only HIV-infected, 73 (5%) only HCV-infected, 235 (16%) HIV and HCV co-infected and 401 (27%) HIV- and HCV uninfected. 1221 (83%) had an APRI  $\leq 0.5$  (no or only mild fibrosis), 206 (14%) had an APRI >0.5 and  $\leq 1.5$  (moderate fibrosis) and 52 (3%) had an APRI >1.5 (severe fibrosis). Having moderate or severe fibrosis (APRI >0.5) was associated with deficits in learning, executive function, memory, psychomotor speed, fluency, and fine motor skills. In these models that adjusted for fibrosis, smaller associations were found for HIV (learning and memory) and HCV (executive functioning and attention). Similarly, the severity of fibrosis, measured by liver transient elastography, was associated with deficits in attention, executive functioning, and fluency.

**Conclusions:** Independent of HCV and HIV, liver fibrosis has distinct contributions to cognitive performance in the era of cART. In adjusted models, HCV and HIV had only limited associations when fibrosis is included. These data highlight the heterogeneous contributions to cognitive impairment in the era of combination antiretroviral therapy.

#### 423LB A Randomised Controlled Trial of Maraviroc-Intensified bPI ART on Cognitive Function

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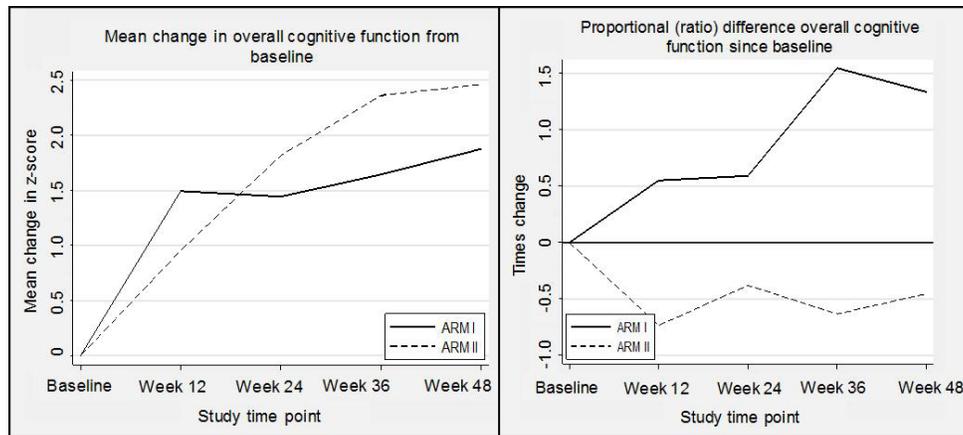
**Background:** Maraviroc-intensified ART may have anti neuroinflammatory properties which could result in cognitive benefits. However intensified ART may be associated with increased neurotoxicity.

**Methods:** Therapy naïve, neurologically asymptomatic, HIV-positive patients were randomly allocated on a 1:1 basis to a standard boosted-protease-inhibitor (bPI) ART regimen (Arm1; tenofovir-emtricitabine plus atazanavir/r) or a maraviroc 150mg once daily intensified bPI regimen (Arm2: abacavir-lamivudine plus darunavir/r/maraviroc). A detailed assessment of 11 cognitive function (CF) tests was undertaken at baseline and after 12, 24, 36 and 48 weeks and cerebral metabolites measured using proton magnetic resonance spectroscopy in the right frontal white matter (FWM) at baseline. Mean and proportional changes in CF, changes in individual domains between treatment arms and factors associated with changes in CF were assessed.

**Results:** Of 60 subjects randomised (30 Arm1 and 30 Arm2), 58 were male and 44 of white ethnicity. Treatment groups had similar disease characteristics including overall mean (SD) baseline CD4+ count 425 (246) and 434 (229) cells/uL and plasma HIV RNA (IQR) 44K (16 to 69K) and 53K (27 to 81K) copies/mL in arms1 and 2, respectively. At week 48 plasma HIV RNA was <200 copies/mL in all and mean (SD) CD4+ count was 586 (209) and 611 (249) cells/uL in arms1 and 2, respectively.

CF improved over 48 weeks (mean change z-score 0.19 Arm1 and 0.24 Arm2, figure). No statistically significant differences in changes in individual domains or overall CF were observed between study treatment arms however proportional change in overall CF from baseline was numerically greater in arm1. In a multivariate model, greater improvements in overall CF was associated with greater baseline CF performance (coef 0.82,  $p < 0.005$ ) and higher FWM neuronal metabolites (n-acetyl aspartate/creatine ratio, coef 0.51,  $p < 0.05$ ).

**Conclusions:** Maraviroc-intensified ART had no demonstrable benefit on CF. In subjects with higher baseline cerebral function markers (higher CF performance and neuronal metabolites), greater improvements in CF was observed. We hypothesise a greater reversibility of underlying disease processes may occur in such individuals.



#### 424LB Neurocognitive Safety After 96-Wks on ATV/r+3TC: Results of the Randomized SALT Trial

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**Background:** Due to its low CNS penetrance, there are concerns about the capacity of non-conventional ART to preserve neurocognitive performance (NP).

**Methods:** The SALT study is a multicentre, open-label, non-inferiority clinical trial that compares whether 3TC+ATV/r (dual therapy; DT) is non-inferior to 2NRTI+ATV/r (triple therapy; TT), in HIV+ patients on a stable 3-drug regimen. The SALT NP sub-study (per protocol analysis) was performed to evaluate the effects over NP of DT vs. standard TT. A global deficit score (GDS) of 5 neurocognitive tasks selected following AAN-2007 criteria were used to assess NP at baseline (BL), W48 and W96. Changes in the neurocognitive impairment rates (NI) (GDS  $\geq 0.5$ ) and NP (GDS value) were determined at W48 and W96. The effect of DT over GDS at W96 adjusted by GDS at BL, GDS at W48 and significant confounders that change the relation between DT and GDS at W96  $>20\%$  was determined using ANCOVA.

**Results:** Per protocol analysis included 92 participants (DT: 47 vs. TT 45). All BL characteristics were comparable in both groups, including mean [95% CI] GDS change: DT 1.2 [0.9 – 1.5] vs. TT 1.1 [0.8 – 1.5];  $p=0.80$  and rate of NI (DT 66% vs. TT 62.2%; 0.71). At W48 and W96, GDS changes (W48: DT -0.3 [-0.5 to -0.1] vs. TT -0.2 [-0.4 to 0.0];  $p=0.39$ . W96: DT -0.3 [-0.5 to -0.1] vs. TT -0.2 [-0.4 to -0.1];  $p=0.471$ ) and rates of NI (W48: DT 55.3% vs. TT 51.1%;  $p=0.69$ . W96: DT 51.1% vs. TT 51.1%;  $p=0.99$ ) were similar. This absence of differences was also observed in all cognitive tasks. At W96, two participants in each group developed NI (incidence rate: DT 12.5% vs. TT 11.8%;  $p=0.95$ ). DT use did not impact NP change: GDS at W96 adjusted by BL and W48 GDS and significant confounders (none) was -0.26 [-0.39 to -0.12] on DT vs. -0.27 [-0.41 to -0.13] on TT ( $p=0.90$ ).

**Conclusions:** Neurocognitive performance remained stable after 96 weeks both in ATV/r+3TC arm as in the 2NRTI+ATV/r arm provided HIV-suppression was maintained. In this trial, ATV/r + 3TC showed a safe neurocognitive profile through 96 weeks.

#### 425 HIV-1 Maturation Inhibitor BMS-955176: Pharmacokinetic and Exposure-Response Analysis

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**Background:** BMS-955176 is a second-generation HIV-1 maturation inhibitor that targets HIV-1 Gag, inhibiting the final protease cleavage event between capsid protein p24 and spacer peptide-1, resulting in the production of immature, non-infectious virions. In a proof-of-concept (POC) study, 10 days of BMS-955176 monotherapy led to maximum median declines in HIV-1 RNA that plateaued at  $\sim 1.64 \log_{10} \text{c/mL}$  at doses of 40–120mg once daily (QD). BMS-955176 showed similar antiviral activity in subjects with either wild-type HIV-1 or HIV-1 with Gag polymorphisms and in subjects with either HIV-1 subtype B or subtype C. Exposure-response (ER) analyses were performed to guide dose selection for further development.

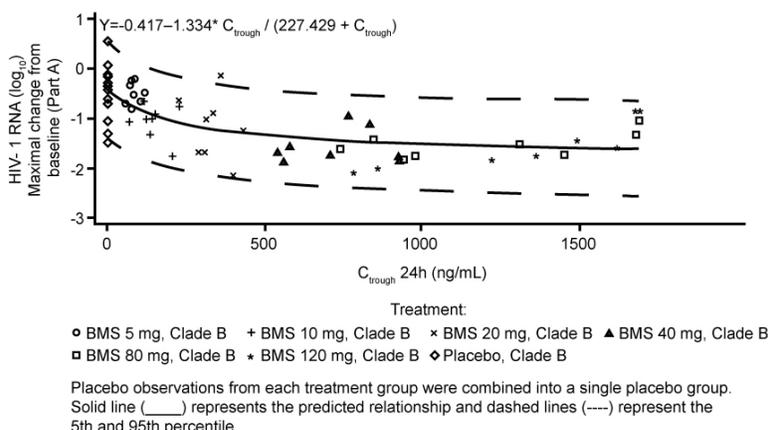
**Methods:** AI468002 (NCT01803074) was a Phase 2a, randomized, multipart trial. In Part A, 60 HIV-1, subtype B-infected subjects (HIV-1 RNA  $\geq 5000 \text{c/mL}$ ; CD4+ T-cell counts  $\geq 200 \text{cells/uL}$ ) received an oral suspension of BMS-955176 (5, 10, 20, 40, 80 and 120 mg doses) or placebo (8:2 for each dose group) QD for 10 days. Blood samples for noncompartmental PK analysis were collected on Days 1 and 10, and trough samples on Days 2, 4, 6, and 8. Dose proportionality was assessed using a power model. Blood samples for HIV-1 RNA were collected on Days 1–14, 17, 19 and 24. The ER relationship was assessed using a non-linear, three-parameter sigmoid  $E_{\text{max}}$  equation,  $Y = E_0 + E_{\text{max}} * C / (EC_{50} + C)$ .

**Results:** Following multiple dose administration of BMS-955176 under fasted conditions, BMS-955176 systemic exposures ( $C_{\text{max}}$ ,  $AUC_{\text{TAU}}$  and  $C_{24}$ ) increased  $\sim 2$ -fold from first dose (Day 1) to last dose (Day 10). Day 10 BMS-955176 exposures increased in a generally dose-proportional manner at 5–40mg QD, but were less than dose proportional at 40–120mg QD. Steady state was achieved by Day 6. The ER relationship between Day 10 BMS-955176  $C_{24}$  and response (maximum decline in HIV-1 RNA from baseline) (Figure) was described by

an  $E_{max}$  equation with an intercept,  $E_0$  of  $-0.417 \log_{10} \text{c/mL}$ ,  $E_{max}$  of  $-1.334 \log_{10} \text{c/mL}$ , and  $EC_{50}$  of  $227 \text{ng/mL}$ . A short-term response plateau was achieved at  $BMS-955176 C_{24} \geq 500 \text{ng/mL}$ . Consistent with clinical results, predicted maximum response ( $E_0 + E_{max}$ ) was  $\sim 1.7 \log_{10} \text{c/mL}$  decline in HIV-1 RNA.

**Conclusions:** This ER analysis from the Phase 2a POC study demonstrated maximum viral load decline at  $BMS-955176 C_{24} \geq 500 \text{ng/mL}$  with 10 days of monotherapy. These results informed dose selection for the ongoing Phase 2b program, which assesses  $BMS-955176$  safety/efficacy at exposures similar to oral suspension doses  $\geq 40 \text{mg}$ .

Figure. Maximal change from baseline in plasma  $\log_{10}$  HIV-1 RNA levels versus  $C_{trough}$  (24h) plasma concentration (AI468002 Part A)



**426 Predicting Drug Discontinuation in TDF-Tolerant Patients: A Prospective PK/PK Study**

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**Background:** Proximal tubular toxicity has been reported in tenofovir disoproxil fumarate (TDF) receiving HIV-positive patients; demographic, therapeutic, pharmacokinetic and pharmacogenetic variables might predict such toxicity. Aim of the study was to describe the prevalence of tubulopathy and the impact on risk of drug discontinuation.

**Methods:** Adult HIV-positive patients on TDF-containing HAARTs (>6 months), with estimated creatinine clearance (eCrCl) >60 ml/min and no significant comorbidity (diabetes or urinary tract abnormalities) were included. Tenofovir (TFV) plasma and urinary concentration were measured through validated HPLC/MS-MS methods. Urinary retinol binding protein (uRBP) was measured (ELISA) and age-defined urinary creatinine corrected values were used (uRBP/uCr <130 in patients aged <50 years and <172 in older ones) to identify tubulopathy. Single nucleotide polymorphisms in the following genes were analysed through real-time PCR: *ABCB1*, *ABCC2*, *ABCC4*, *ABCC10*, *SLC22A6*, *SLC28A2*. Patients were followed prospectively and drug discontinuations were categorized as renal toxicity (eCrCl <60 ml/min, nephrolithiasis, persisting 24-hour urine abnormalities) or others.

**Results:** 310 patients (73.2% male, 84.8% Caucasian) were enrolled. Age, BMI and eCrCl were 45.5 years (39.1-52.2), 23.5 kg/m<sup>2</sup> (21.7-26.2) and 90.9 ml/min (79.6-107.3). Abnormal uRBP/uCr was observed in 145/288 patients (50.3%) while 24-hour proteinuria (>300 mg) in 10/92 patients (10.9%); both were associated with low TFV urinary output (p=0.008 and p=0.005). Over a median follow up of 20.6 months (16.8-26.8) in 24 patients (out of 32, 75%) TDF was interrupted for renal toxicity: at univariate log-rank analysis it was associated with increasing age (p=0.031), male gender (p=0.004), PI use (p=0.001), eCrCl (p=0.055), tenofovir urinary/plasma ratio <350 (p=0.018), *ABCB1* TT genotype (p=0.035) and *SLC28A2* T allele (p=0.059). Multivariate step-wise Cox regression identified age (p=0.005), male gender (p=0.047), PI use (p=0.002) and *ABCB1* TT genotype (p=0.0042) as independent predictors of tenofovir discontinuation.

**Conclusions:** Subclinical tubular toxicity was detected in half of otherwise TDF-tolerant patients and was associated with low TFV urinary output; however, it did not predict further worsening of glomerular or tubular function requiring TDF withdrawal. Age, male gender, PI use and pharmacogenetic markers predicted TDF discontinuation for renal toxicity.

**427 Random Lopinavir Concentrations Predict Resistance on Lopinavir-Based ART**

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**Background:** Considering that most patients who experience virologic failure (VF) on lopinavir-based antiretroviral therapy (ART) fail due to poor adherence rather than resistance, an objective adherence measure could limit costs by rationalizing the use of genotype antiretroviral resistance testing (GART) in countries with access to third line ART.

**Methods:** We conducted a cross-sectional study on patients at two large ART clinics in Durban, South Africa experiencing VF (HIV RNA >1000 copies/mL) on lopinavir-based ART who had GART done. An association of major protease inhibitor (PI) resistance mutations with random lopinavir concentrations was assessed.

**Results:** 134 patients, including 31 children, were included in the analysis. The median age in years was 35.0 (IQR: 24.6 – 41.2) and the median duration on lopinavir was 24.8 months (IQR: 12.8 – 34.2). The predominantly used NRTI backbone was AZT and DDI (50.8%). The median log viral load at time of genotype was 4.6 (log<sub>10</sub> copies/mL). The prevalence of  $\geq 1$  major PI mutation was 21%. On multivariate analysis, the following were associated with the presence of a major PI resistance mutation: random lopinavir concentration above the recommended minimum trough of 1mg/mL and male sex.

**Conclusions:** Random lopinavir concentrations are strongly associated with the presence of major PI mutations. Access to costly GART could be restricted to patients with lopinavir concentrations above the recommended minimum trough.

**Factors associated with major protease inhibitor mutations at virologic failure on lopinavir-based antiretroviral therapy**

	Univariate		Multivariate	
	OR (95% CI)	P value	OR (95% CI)	P value
Age at genotype (per 10 yr increase)	1.26 (0.90-1.75)	0.174	1.27 (0.91-1.77)	0.163
Sex	males	3.90 (1.64-9.28)	3.19 (1.22-8.33)	0.018
	females	referent		
VI > 100000 copies/mL	0.93 (0.40-2.27)	0.916	0.93 (0.34-2.54)	0.892
Duration on lopinavir	> 2years	2.20 (0.95-5.12)	2.17 (0.84-5.62)	0.111
	≤ 2 years	referent		
Lopinavir concentration $\geq 1 \mu\text{g/mL}$	5.59 (2.09-14.93)	0.001	5.81 (2.04-16.50)	0.001

**428 Inflammation Investigated as a Source of Atazanavir Pharmacokinetic Variability**Charles Venuto<sup>1</sup>; Peter W. Hunt<sup>2</sup>; Grace A. McComsey<sup>3</sup>; Gene D. Morse<sup>4</sup>; Susan Messing<sup>1</sup><sup>1</sup>Univ of Rochester Med Cntr, Rochester, NY, USA; <sup>2</sup>Univ of California San Francisco, San Francisco, CA, USA; <sup>3</sup>Case Western Reserve Univ, Cleveland, OH, USA; <sup>4</sup>State Univ of New York at Buffalo, Buffalo, NY, USA

**Background:** Inflammation is associated with the downregulation of drug metabolizing enzymes and transporters including cytochrome P450 3A (CYP3A) and P-glycoprotein (P-gp). Thus, the chronic inflammatory state associated with HIV-infection may be a source of pharmacokinetic variability of antiretrovirals that are substrates or inhibitors of CYP3A or P-gp. We analyzed plasma atazanavir (ATV) and inflammatory marker concentration obtained from AIDS Clinical Trials Group (ACTG) protocols A5202 and A5224s to determine if changes in inflammatory markers were associated with ATV clearance.

**Methods:** ACTG protocol A5202 randomized treatment-naïve adults to ATV/ritonavir (ATV/r) or efavirenz, with blinded tenofovir disoproxil fumarate/emtricitabine or abacavir/lamivudine. Plasma ATV clearance for each individual was estimated by population PK modeling. Several biomarkers were measured at weeks 0, 24, and 96: high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), tumor necrosis factor alpha and its soluble receptors (TNF- $\alpha$ , sTNFR-I, -II), soluble vascular cellular and intracellular adhesion molecules (sVCAM-1, sICAM-1), and total bilirubin. Statistical analysis was performed by a matrix of Spearman correlation coefficients between ATV clearance and biomarkers at weeks 24 and 96, and changes from baseline.

**Results:** There were 107 participants with both available ATV clearance and biomarker concentrations. Significant correlations were observed between ATV clearance and changes in sTNFR-II from weeks 0 to 24 ( $\rho = -0.24$ ,  $P = 0.01$ ), in sVCAM-1 from weeks 0 to 96 ( $\rho = -0.24$ ,  $P = 0.02$ ) and in bilirubin from weeks 0 to 96 ( $\rho = 0.22$ ,  $P = 0.04$ ). Bilirubin concentrations were inversely correlated with each of the inflammatory markers at weeks 24 and 96 ( $\rho: -0.17$  –  $-0.51$ ,  $P \leq 0.002$ ) except hsCRP ( $P = 0.45$ ).

**Conclusions:** The clearance of ATV was not significantly correlated with the majority of measured inflammatory biomarker changes. Inflammatory-mediated inhibition of CYP3A may have been attenuated due to ATV-associated increases of bilirubin, which has known anti-inflammatory properties and indeed was negatively correlated with most inflammatory markers in this study. The effects of chronic inflammation due to HIV infection on the pharmacokinetics of antiretrovirals should be further assessed in CYP3A metabolized agents that do not alter bilirubin concentrations.

**429 Recommended Efavirenz Concentration for Therapeutic Drug Monitoring Is Too High**Catherine Orrell<sup>1</sup>; Andrzej Bienczak<sup>1</sup>; Karen Cohen<sup>1</sup>; David R. Bangsberg<sup>2</sup>; Robin Wood<sup>1</sup>; Gary Maartens<sup>1</sup>; Paolo Denti<sup>1</sup><sup>1</sup>Univ of Cape Town, Cape Town, South Africa; <sup>2</sup>Harvard Med Sch, Boston, MA, USA

**Background:** The therapeutic range for efavirenz plasma concentrations is unclear, with some studies failing to find a correlation with virologic outcomes. EFV concentrations are highly variable, driven in part by polymorphisms in cytochrome P450 (CYP) 2B6, which make it an attractive candidate for therapeutic drug monitoring (TDM) if therapeutic ranges could be established. We hypothesised that EFV mid-dosing concentrations, together with cytochrome P450 (CYP) 2B6 metaboliser genotype status, could predict virological outcomes in an ART-naïve cohort.

**Methods:** ART-naïve participants from a South African outpatient antiretroviral therapy (ART) clinic were monitored for the first 48 weeks on EFV-based ART. CD4 cell count and HIV-RNA were determined at baseline, week 16 and 48. Samples for EFV concentrations at mid-dose interval were drawn at weeks 16 and 48. CYP 2B6 genotyping (516G→T and 983T→C) data was used to determine metaboliser status. Cox proportional hazards modelling was used to predict virological outcome. Comparison of Akaike Information Criterion (AIC) values was used to determine the most predictive lower limit of mid-dosing EFV concentration.

**Results:** 180 participants with both EFV concentrations and HIV-RNA available were included in the study. Median EFV concentrations were 2.3mg/L (IQR1.6–4.6) and 2.21mg/L (IQR1.5–3.9) at weeks 16 and 48. 49 (27.2%), 84 (46.7%) and 39 (21.7%) participants had extensive, intermediate and slow or ultra-slow CYP2B6 metaboliser genotype respectively. Only  $\log_2$  EFV concentrations [adjusted hazard ratio (aHR) 0.81, 95% confidence interval (95%CI) 0.72–0.92] and baseline CD4 cell count [aHR 0.99, 95% CI 0.99–1.0] were predictive of virological outcome. For every doubling in drug concentration there was a 21% decrease in the hazard of viral failure ( $p=0.001$ ); and for every 50 cell increase in baseline CD4 count there was a 31% reduction in the hazard of non-suppression. The most predictive lower limit for mid-dosing EFV concentration was 0.7 mg/L.

**Conclusions:** Mid-dosing EFV concentrations are predictive of virological outcome, but the currently recommended lower therapeutic limit (1 mg/L) for TDM is higher than our finding. Knowledge of CYP2B6 metaboliser genotype is not required for prediction of virological outcomes, suggesting that adherence is the key determinant.

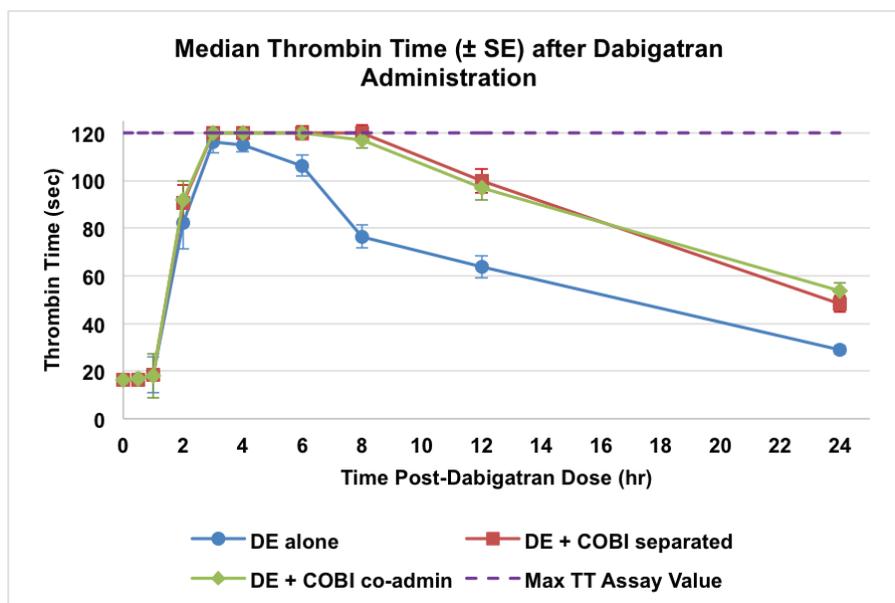
**430 Cobicistat Increases the Effects of Dabigatran on Thrombin Time in Healthy Volunteers**Kristina M. Brooks<sup>1</sup>; Colleen Hadigan<sup>2</sup>; Lori A. Gordon<sup>3</sup>; Scott Penzak<sup>4</sup>; Anela Kellogg<sup>5</sup>; Khanh Nghiem<sup>6</sup>; Jay Lozier<sup>6</sup>; James Mikula<sup>5</sup>; Parag Kumar<sup>1</sup><sup>1</sup>Clinical Pharmacokinetics Rsr Lab, Clinical Cntr, NIH, Bethesda, MD, USA; <sup>2</sup>NIAID, NIH, Bethesda, MD, USA; <sup>3</sup>Xavier Univ of Louisiana Coll of Pharm, New Orleans, LA, USA; <sup>4</sup>Univ of North Texas System Coll of Pharm, Fort Worth, TX, USA; <sup>5</sup>Leidos Biomed Rsr, Inc, Bethesda, MD, USA; <sup>6</sup>Clinical Cntr, NIH, Bethesda, MD, USA

**Background:** Dabigatran etexilate (DE) is an oral direct thrombin inhibitor and substrate of intestinal Permeability-glycoprotein (P-gp) and renal multidrug and toxin extrusion-1 (MATE-1) transporters. Cobicistat (COBI) is a pharmacokinetic (PK) enhancer and inhibitor of P-gp and renal MATE-1 transporters, which may result in increased DE exposure when co-administered. Using thrombin time (TT), we sought to characterize the pharmacodynamic (PD) effects of COBI on DE, and if this potential interaction may be mitigated by separated administration.

**Methods:** This was a single-center, open-label, fixed sequence, intra-subject study conducted in healthy volunteers. The study was comprised of 3 phases: (1) DE 150 mg x1 alone (day 0), (2) DE 150 mg x1 two hours prior to COBI 150 mg (day 19±1), and (3) DE 150 mg x1 with COBI 150 mg (day 26±1). Subjects underwent a 5-day washout period following phase 1, followed by COBI 150 mg once daily throughout Phases 2 and 3 (days 5 - 26±1). Blood was collected at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours post-dose on DE dosing days. TT was determined using STA®-Thrombin reagent (Diagnostica Stago, Asnières-sur-Seine, France). Noncompartmental methods were used to derive DE PD parameters (TT area-under-the-effect-curve [AUEC] and thrombin time at 24 hours [TT-last]). Geometric mean ratios (GMR) with 90% confidence intervals [CI] were compared between phases and p-values were calculated using a two-tailed paired t-test.

**Results:** A total of 13 subjects have been enrolled, with 11 completing the study and 2 without phase 3 data due to lack of study compliance. There was a 32% and 33% increase in AUEC GMR between Phase 2 and Phase 1 ( $p<0.0001$ , 90% CI [1.21, 1.43]), and Phase 3 and Phase 1 ( $p<0.001$ , 90% CI 1.21–1.44), respectively. Significant increases in TT-last GMR of 57% and 29% were also observed between Phase 2 and Phase 1 ( $p<0.001$ , 90% CI [1.34, 1.72]) and Phase 3 and Phase 1 ( $p<0.001$ , 90% CI [1.37, 1.88]), respectively. No significant differences were noted between Phase 3 and Phase 2 in GMR of AUEC ( $p=0.9466$ , 90% CI [0.92, 1.10]) or TT-last ( $p=0.6685$ , 90% CI [0.91, 1.21]).

**Conclusions:** COBI co-administration resulted in significant increases in TT AUEC and TT-last compared to DE alone. These effects were preserved despite separating COBI and DE administration by 2 hours, supporting the putative mechanisms of P-gp and MATE-1 inhibition by COBI. Further analyses of changes in DE PK-PD relationships are needed to explore the clinical implications of this interaction.



**431 Pharmacokinetics of Crushed Elvitegravir Combination Tablet Given With Drip Feed**

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**Background:** If HIV-patients are unconscious or cannot swallow tablets for other reasons, antiretroviral medication is often crushed and solved prior to administration. Currently, there is no information about crushing the fixed-dose combination of elvitegravir/cobicistat/emtricitabine/tenofovir (E/C/E/T). Crushing can influence pharmacokinetics (PK) leading to altered drug exposure, possibly leading to treatment failure, development of resistance or toxicity. Therefore crushing of E/C/E/T is not recommended. A possible PK interaction between elvitegravir (EVG) and drip feed is expected, based on the interaction between EVG and antacids. No interaction occurs between other pH-increasing drugs (omeprazole) and EVG, therefore the interaction is most likely caused by complexation between EVG and cations.

**Methods:** An open-label, 3-period, randomized, cross-over, trial in 24 healthy volunteers was conducted. Subjects randomly received a single dose of STB with a 7-day washout period. Reference treatment A: E/C/E/T whole tablet with breakfast (350 kcal), intervention treatments B: crushed and suspended E/C/E/T with breakfast and C: crushed and suspended E/C/E/T with drip feed (350kcal). To show bioequivalence between reference A versus B and C a 32-h PK profile was measured for EVG, COBI (cobicistat), FTC (emtricitabine) and TDF (tenofovir). Geometric mean ratios (GMR) with 90% confidence interval (CI) for AUC and Cmax were calculated. Safety and tolerability were evaluated.

**Results:** 24 healthy volunteers (23 Caucasian and 1 mixed-race, 12 female), 37(20-54) years and BMI of 24(19-29) (median (range)) were included in the trial. The GMR (90% CI) of Cmax and AUC of EVG were 117% (106-129) and 109% (99-120) for B vs A, 104% (94-115) and 104% (94-114) for C vs A. GMR of Cmax and AUC for COBI were 83% (76-91) and 89% (82-97) for B vs A and 101% (92-110) and 102% (94-111) for C vs A. For FTC the GMR of AUC and Cmax were 89% (83-97) and 99% (95-104) for B vs A and 96% (89-104) and 101% (97-106) for C vs A. For TDF the GMR of AUC and Cmax were 81% (71-92) and 100% (92-113) for B vs A and 94% (83-107) 105% (97-108) for C vs A.

No SAEs were reported during the trial.

**Conclusions:** AUCs fell within the bioequivalence ranges for all compounds. For Cmax the 90% CI were just outside the bioequivalence range, but this was considered not clinically relevant. E/C/E/T can be crushed and suspended and given with drip feed.

**432 Minimal Removal of Dolutegravir by Hemodialysis in HIV-Infected Patients**

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**Background:** Dolutegravir can be safely administered to HIV-infected patients with advanced kidney disease (GRF <30 ml/min). However, little is known about dolutegravir removal by hemodialysis in patients with end-stage renal disease (ESRD). Our objective, therefore, was to evaluate the effect of hemodialysis on dolutegravir clearance in five anuric ESRD HIV-infected patients undergoing routine hemodialysis.

**Methods:** Exploratory clinical trial including 5 HIV-infected patients on stable antiretroviral treatment with ESRD undergoing routine hemodialysis. After enrolment (day 1), dolutegravir (Tivicay, Viiiv) 50 mg once daily was added to antiretroviral treatment for five days. Blood samples were collected at the beginning and at the end of the dialysis session on day 5. Additionally, paired blood samples going into (predialyzer) and out (postdialyzer) of the dialyzer membrane, as well as a dialysate sample were collected during the dialysis session. Dolutegravir concentrations in plasma and in dialysate were determined by LC-MS/MS.

**Results:** Five patients were included in the study. Two patients underwent conventional hemodialysis sessions while the three remaining underwent online hemodiafiltration (HDF-OL). Blood flows were held constant at 300 ml/min for patients on conventional hemodialysis, and at 400 ml/min for patients on HDF-OL. Dialysate flow was held constant at 500 ml/min for all participants. Dolutegravir concentrations in plasma and in dialysate are depicted in the table. Mean dolutegravir concentrations in plasma at the end of the dialysis session remained 30 fold above the protein binding-adjusted 90% inhibitory concentration.

**Conclusions:** Our results show minimal removal of dolutegravir by hemodialysis. Dolutegravir dosage adjustments seem, therefore, to be unnecessary in HIV-infected patients with ESRD undergoing hemodialysis.

ID	Type of hemodialysis	Dolutegravir concentration (ng/ml)		
		Predialyzer	Postdialyzer	Dialysate
1	Conventional	1468	1428	62
2	Conventional	2126	2589	37
3	HDF-OL	472	983	17
4	HDF-OL	1514	2725	22
5	HDF-OL	1905	3901	30

### 433 A Comparison of the Pharmacokinetics of Efavirenz During Pregnancy and Postpartum

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**Background:** Efavirenz (EFV) 600mg is currently recommended by WHO as a first-line antiretroviral agent in HIV infected adults. A dose reduction to 400mg EFV has been proposed because of concerns regarding toxicity. EFV is widely used during pregnancy in those countries where HIV infection is most common. Pregnancy can reduce exposure to antiretroviral agents with a corresponding risk of poor maternal virologic control and PMTCT. Pharmacokinetics (PK) of EFV 600 mg have been previously studied in pregnancy with contradictory results. The aim of this multinetwork study was to further investigate the PK of EFV 600 mg in pregnant women.

**Methods:** HIV-infected pregnant women treated with EFV 600 mg once daily were recruited by the P1026s network (N=10) and PANNA network (N=13). Intensive PK profiles were obtained during 2<sup>nd</sup> (2T) and 3<sup>rd</sup> trimester (3T) and at least two weeks postpartum (PP). 2T and 3T PK parameters were compared with PP. Where possible cord blood and maternal delivery blood samples were obtained.

**Results:** Seven, 19 and 22 women completed 2T, 3T, and PP PK evaluations. Median (range) age was 33 (20-40) years. 21 subjects were Black, 2 mixed race. The geometric means (GM) (95% CI) for AUC<sub>0-24h</sub>, C<sub>max</sub> and C<sub>min</sub> in 3T were 60 (49-74) mg\*h/L, 4.6 (3.7-5.5) mg/L and 1.8 (1.4-2.3) mg/L, respectively. The GM during PP for AUC<sub>0-24h</sub>, C<sub>max</sub> and C<sub>min</sub> were 63 (50-80) mg\*h/L, 4.3 (3.4-5.3) mg/L and 1.9 (1.4-2.6) mg/L, respectively. When comparing 3T to PP (N=19), GM ratios (90% CI) were 1.01 (0.92-1.10), 1.11 (0.94-1.29), and 0.97 (0.82-1.16) for AUC<sub>0-24h</sub>, C<sub>max</sub>, and C<sub>min</sub>. Similar results were found when comparing 2T to PP (N=5). Two patients had a C<sub>min</sub> below the suggested threshold of 1.0 mg/L during 3T, but not PP. One patient had a C<sub>min</sub> below 1.0 mg/L only PP. Three patients used concomitant rifampicin, but no obvious deviations were observed and C<sub>min</sub> levels were >1.0 mg/L.

Median (range) gestational age at delivery was 39 wks(33-42); birth weight was 3310 (1875-4150) gm. All of the children for whom HIV-infection status was available were not infected as of the last HIV test. The median (range) ratio of cord to maternal concentrations (n=4), was 0.81 (0.65-0.95).

**Conclusions:** No significant effects of pregnancy on EFV PK parameters were observed and EFV 600mg led to adequate exposure during pregnancy. The absence of a significant pregnancy-related effect on EFV PK in this study suggests that a prospective evaluation in pregnant women of the proposed EFV dose reduction to 400mg is warranted.

### 434 Contamination of Herbal Medicines With ARVs and Widespread Use by PLWH in Nigeria

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**Background:** Nigeria has an estimated 3.1 million people living with HIV (PLWH), accounting for nearly 10% of global HIV burden. Use of traditional medicines is high in the general population. Here, we sought to evaluate the use of herbal medicines amongst (PLWH), and undertake a country-wide analysis of herbal medicines used by PLWH for possible contamination with antiretrovirals (ARV).

**Methods:** Part A) large questionnaire-based survey of patients attending health centres in mixed rural and urban centres across 4 states. Data were collated and analysed using SPSS. Part B) country-wide (Fig. 1) collection of herbal samples for drug analysis. Investigators followed a standard protocol i) street vendors from a mixture of urban and rural settings were approached ii) a request for herbals for treatment of general pains, hepatitis, UTI, and febrile ailments on a background of HIV iii) herbals sold as powders or liquids were purchased iv) instructions for use, date and site were recorded. Analysis for efavirenz, nevirapine, lopinavir, darunavir, ritonavir, atazanavir, emtricitabine, tenofovir and lamivudine using validated LC-MS/MS methods was performed at the University of Liverpool.

**Results:** Of 742 PLWH aged 2-91y, prevalence of herbal medication use was 41.8% (310). Use of herbals was significantly associated with educational attainment (31.8% in patients with little or no education, vs 43.8% and 44.8% in patients with secondary and tertiary education respectively; P= 0.037) and with employment status (44.9% of the employed vs 36.7% of unemployed patients used herbals; P= 0.01). Of those who took herbals, 45% did so prior to commencement of ARVs, 53.9% did so to cure HIV (46% with little effect while 5.1% believed themselves cured); influence of family or friends contributed to herbal intake in 75%. Of 138 herbal samples collected across 8 States, 3 (2%; all from large cities) contained measurable antiretrovirals. One sample contained tenofovir (0.2ng/mg powder) and emtricitabine (0.0065ng/mg powder), while two samples contained tenofovir (0.2 and 1.6ng/mg powder) and emtricitabine (0.123 and 0.00049ng/mg powder), with one of these also containing lamivudine (0.25ng/mg powder)

**Conclusions:** Herbal use amongst PLWH is widespread, poorly recorded and often precedes ARV therapy. Contamination with ARVs is worrying (particularly in untreated patients) given the potential for drug resistance.

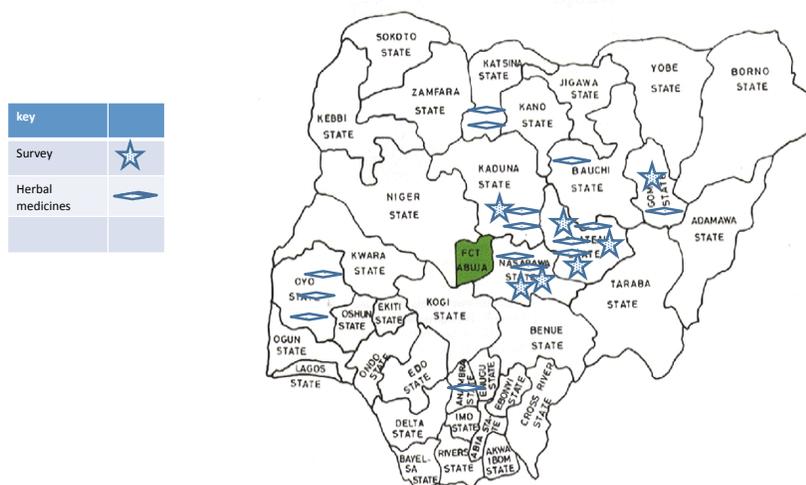


Fig 1:Map of Nigeria showing Regions where survey was conducted and herbal samples were collected

**435 TDF/EVG Nanoparticle Formulation: Plasma Pharmacokinetics in Humanized Mice****Christopher Destache**; Subhra Mandal*Creighton Univ, Omaha, NE, USA*

**Background:** One of the highest priorities of HIV research is to have access to long-acting pharmaceutical formulations for HIV treatment and prevention. Our research aims at fabrication of combination antiretroviral (cARV) drugs loaded nano-system for PReP. Presently we report on the pharmacokinetics of tenofovir disoproxil fumarate (TDF) and elvitegravir (EVG) loaded nanoparticles (TDF+EVG NP) in humanized mice (huNSG).

**Methods:** Formulation of cARV loaded nanoparticles (NP) by oil-in-water emulsion. Poly(lactic-co-glycolic acid) (PLGA), a biocompatible polymer was used to formulate TDF+EVG drugs loaded NPs (TDF+EVG NPs) using standard methods. For pharmacokinetic studies, female humanized NSG mice (Jackson Labs, Inc.) were treated with TDF+EVG NPs (50 mg/kg administered SubQ in 1 ml D5W). At specific time points (1, 2, 4, 7, 10, and 14 days post-SubQ administration), mice (n=3/time point) were euthanized, blood harvested, centrifuged, and plasma was analyzed for tenofovir (TNF) and EVG drug levels. The plasma concentrations of the drugs were measured by high-performance liquid chromatography (HPLC). Intra-day and inter-day variability was < 10%. Plasma concentration-vs-time was modeled using non-compartmental modeling. Peak plasma levels and time to peak were determined by graph inspection.

**Results:** TDF+EVG NP size averaged 136.7 nm and drug entrapment efficiency was 41.7% and 39.7%, respectively. Mean ( $\pm$  SD) peak plasma TNF and EVG levels were  $1.2 \pm 0.28$  and  $0.2 \pm 0.25$  mcg/mL and time to peak was 6.3 and 4.3 days after SubQ administration. Using non-compartmental modeling, plasma PK parameters for TNF and EVG from NP formulation (TDF/EVG NP), respectively, were  $AUC_{0-24h}$  (TNF)  $13.5 \pm 3.1$  and  $EVG 2.2 \pm 2.9$  mg x h/L. Elimination half-life for TNF and EVG averaged  $14.9 \pm 15.1$  and  $15.2 \pm 14.9$  days, respectively from the NP formulation. Apparent volume of distribution (Vd) averaged 0.027 and 0.497 L/kg and total body clearance (Cl) averaged 0.002 and 0.052 L/day/kg for TNF and EVG, respectively. Based on modeling, a dosing interval of one SubQ dose every 28 days would provide detectable drug in plasma throughout the dosing interval above 50 mcg/mL.

**Conclusions:** The simultaneous administration of TAF and EVG in single NP formulation shows prolonged PK parameters of TNF and EVG. Further tissue concentrations in colon and female reproductive tract are needed to determine if this will be an appropriate prevention option. This is the first report of using NRTI+ISTI in NP formulation for PReP.

**436 Antiretroviral Drug Use in a Cross-sectional Population Survey in Africa: HPTN 043****Jessica M. Fogel**<sup>1</sup>; William Clarke<sup>1</sup>; Michal Kulich<sup>2</sup>; Estelle Piwowar-Manning<sup>1</sup>; Glenda Gray<sup>3</sup>; Linda Richter<sup>4</sup>; Heidi van Rooyen<sup>5</sup>; Thomas Coates<sup>6</sup>; Susan H. Eshleman<sup>1</sup>; for the HPTN 043 (NIMH Project Accept) Study Team<sup>1</sup>*Johns Hopkins Univ Sch of Med, Baltimore, MD, USA;* <sup>2</sup>*Charles Univ, Prague, Czech Republic;* <sup>3</sup>*South African Med Rsr Council, Cape Town, South Africa;* <sup>4</sup>*Univ of the Witwatersrand, Johannesburg, South Africa;* <sup>5</sup>*Human Scis Rsr Council, Durban, South Africa;* <sup>6</sup>*Univ of California Los Angeles, Los Angeles, CA, USA*

**Background:** HIV Prevention Trials Network 043 (NIMH Project Accept) was a community-randomized trial in Africa and Thailand that assessed the impact of behavioral interventions on HIV incidence. Interventions were delivered over 3 years. HIV incidence was then assessed in a cross-sectional survey of >50,000 randomly-sampled adults (survey period: 2009-2011). At the African sites (34 communities at Soweto and KwaZulu-Natal, South Africa; Tanzania; Zimbabwe). HIV incidence was 1.52% and 1.81% in intervention and control communities, respectively (P=0.082). We evaluated antiretroviral drug (ARV) use by HIV-infected adults at these sites.

**Methods:** Plasma samples from 7,352 (99.9%) of the 7,354 HIV-infected adults in the cross-sectional survey were tested for 20 ARV drugs using a qualitative assay (6 nucleoside/nucleotide reverse transcriptase inhibitors [NRTIs], 3 non-nucleoside reverse transcriptase inhibitors [NNRTIs]; 9 protease inhibitors; maraviroc; raltegravir; lower limit of detection: 10 ng/mL for all drugs).

**Results:** Test results were obtained for 7,347 (99.9%) of the samples. ARVs were detected in 2,011 (27.4%) of the samples (88.1% had 1 NNRTI +/- 1-2 NRTIs; 62.3% had efavirenz; 57.3% had lamivudine). ARV detection was strongly associated with female sex, pregnancy; age (>24 years), and study site (KwaZulu-Natal > Soweto > Tanzania and Zimbabwe), P<0.0001 for all comparisons. ARV detection was also more frequent in widowed compared to married or single adults (P=0.006) and in unemployed compared to employed adults (P=0.02). It was not associated with CD4 cell count, socioeconomic status or education level. ARVs were detected more frequently in the intervention communities compared to the control communities during the first 6 months of the survey (9/2009-3/2010; P=0.02). ARV detection increased over time in intervention communities (from 22.3% in the first 6 months of the survey to 37.7% after 3/2011) and control communities (from 18% in the first 6 months of the survey to 32.4% after 3/2011).

**Conclusions:** The availability of a low-cost, high-throughput multi-drug assay allowed a population-level assessment of ARV use in this large clinical trial. This analysis identified demographic factors associated with ARV use and changes in ARV use over time. Increased ARV use in the control communities, which may have reflected increased HIV testing, referral to care, or access to ARV treatment, could have lowered HIV incidence, which may have impacted the results of this large intervention trial.

**437LB A Single Monotherapy Dose of MK-8591, a Novel NRTI, Suppresses HIV for 10 Days****Evan Friedman**<sup>1</sup>; Dirk Schuermann<sup>2</sup>; Deanne J. Rudd<sup>3</sup>; Sabrina Fox-Bosetti<sup>3</sup>; Sandra Zhang<sup>3</sup>; Martine Robberechts<sup>3</sup>; Andreas Hueser<sup>4</sup>; Daria J. Hazuda<sup>2</sup>; Marian Iwamoto<sup>6</sup>; Jay Grobler<sup>5</sup>; for the HIV Early Development Team<sup>1</sup>*Merck & Co, Inc, Rahway, NJ, USA;* <sup>2</sup>*Charité Rsr Organisation GmbH, Berlin, Germany;* <sup>3</sup>*Merck & Co, Inc, Kenilworth, NJ, USA;* <sup>4</sup>*Charité Universitätsmedizin Berlin, Germany, Rsr Hosp, Berlin, Germany;* <sup>5</sup>*Merck & Co, Inc, West Point, PA, USA;* <sup>6</sup>*Merck & Co, Inc, Kenilworth, NJ, USA*

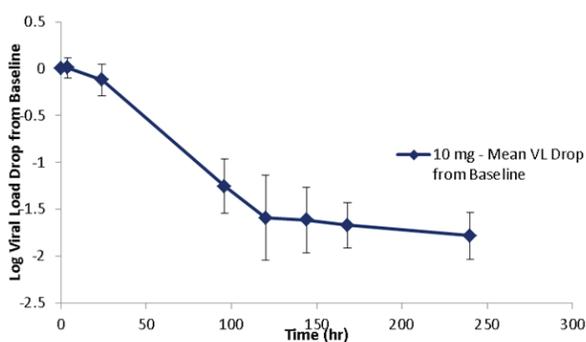
**Background:** MK-8591 is a nucleoside reverse transcriptase translocation inhibitor (NRTTI) in early clinical development. MK-8591-triphosphate (TP), the active phosphorylated anabolite of MK-8591, exhibited protracted intracellular persistence in human and monkey peripheral blood mononuclear cells (PBMCs) in vitro. In preclinical experiments, MK-8591 administered once-weekly in an SIV rhesus macaque model demonstrated potent antiviral efficacy. Clinically, MK-8591-TP exhibited a half-life of ~150-160 hrs in human PBMCs with  $C_{168h}$  exceeding projected efficacious concentrations at doses of  $\geq 10$  mg. A Phase 1b monotherapy proof-of-concept efficacy study is currently underway to assess the potential for once weekly oral dosing in the clinic. The antiviral potency, human pharmacokinetics (PK), and physical properties of MK-8591 have the potential to open new paradigms for extended duration HIV treatment and prophylaxis approaches.

**Methods:** In an open label study in HIV-1 infected subjects naïve to antiretroviral treatment (ART), subjects are being administered a single dose of MK-8591 across a range of doses. Doses were chosen based on PK/PD simulations. Blood samples are being collected for viral load (VL), MK-8591 PK, and MK-8591-TP PK at prespecified time points up to 10 days postdose. Following completion of Day 10 procedures, subjects are being offered standard of care ART. Safety, PK, and VL data from the 10-mg dose (N=6) are available.

**Results:** A single 10 mg dose of MK-8591 was associated with a rapid and robust reduction in VL. At 168 hours postdose, a mean (95% CI) placebo adjusted VL reduction of 1.67 log<sub>10</sub> (1.47, 1.87) was observed. Mean VL continued to decline through Day 10 with a mean reduction of 1.78 log<sub>10</sub> (1.59, -1.98) and no evidence of recrudescence. The 10-mg dose was generally well tolerated with a limited number of mild/moderate adverse experiences reported. MK-8591 plasma and MK-8591-TP PBMC PK were similar to previously reported data in healthy subjects.

**Conclusions:** MK-8591 suppressed HIV replication for at least ten days when administered as a single 10 mg dose. The low dose and potent antiviral effect of MK8591 provides a platform for extended duration oral and parenteral formulations.

### MK-8591: Time versus Log10 Viral Load Reduction (N=6)



#### 438 Dolutegravir Pharmacokinetics in HIV-Infected Pregnant and Postpartum Women

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**Background:** Dolutegravir (DTG), an integrase strand transfer inhibitor, is metabolized by UGT1A1 and CYP3A4. It has not been studied in pregnant women or infants. This study described DTG exposure during pregnancy compared to postpartum and in infant washout samples after delivery.

**Methods:** IMPAACT protocol P1026s is an ongoing, nonrandomized, open-label, parallel-group, multi-center phase-IV prospective study of antiretroviral pharmacokinetics (PK) in HIV-infected pregnant women. Intensive steady-state 24 hour PK profiles of DTG 50 mg once-daily were performed during the 2<sup>nd</sup> trimester (2T), 3<sup>rd</sup> trimester (3T) and 6-12 weeks postpartum (PP). Infant DTG washout samples were collected if birth weight > 1000 grams and there were no severe malformations or medical conditions. Dolutegravir was measured by validated LC-MS/MS with a quantitation limit of 0.005 mcg/mL. A two-tailed Wilcoxon signed rank test ( $\alpha = 0.10$ ) was employed for paired within-subject comparison.

**Results:** Thirteen subjects from the United States were enrolled – 9 black, 3 white, 1 American Indian / Alaskan Native with a median 3T age of 32 years (range 22 – 40). DTG PK data were available for 5, 11 and 4 women in 2T, 3T and PP. PK parameters are represented as median (interquartile range) in the table below.  $AUC_{0-24}$  and  $C_{24h}$  appeared to be lower in the 3T compared to PP, while clearance appeared to be higher, but no significant differences for any PK parameters were found in paired comparisons between 3T and PP ( $n = 4$ ). Washout DTG PK data were available for 5 infants; elimination half-life was 35 hours (range 32 – 55). Viral load at delivery was < 50 copies/mL for 13 of 13 women (100%). Median infant gestational age at birth was 38.9 weeks. Thirteen of 13 infants were HIV-negative based on best available data.

**Conclusions:** DTG exposure may be lower in pregnancy compared to postpartum. Infant elimination half-life was over twice that of maternal subjects and historical non-pregnant adult controls. More PK, safety and outcome data in pregnant women are needed before DTG can be recommended for clinical use during pregnancy.

**Table 1. Maternal Dolutegravir Pharmacokinetic Parameters, Median (IQR)**

Parameter	2 <sup>nd</sup> Trimester n = 5	3 <sup>rd</sup> Trimester n = 11	Postpartum n = 4	Historical Control <sup>1</sup>
$AUC_{0-24}$ (mcg*hr/mL)	58.4 (47.6 - 64.5)	49.7 (44.6 - 57.6)	72.6 (68.6 - 90.1)	53.6 (27)
$C_0$ (mcg/mL)	0.88 (0.88 - 1.18)	1.01 (0.88 - 1.42)	1.77 (1.50 - 2.12)	-
$C_{max}$ (mcg/mL)	4.59 (3.89 - 5.22)	3.97 (3.56 - 4.44)	5.10 (4.72 - 6.09)	3.67 (20)
$T_{max}$ (hr)	2 (2 - 2)	4 (2 - 4)	3 (1.75 - 4.50)	2 to 3
$C_{24}$ (mcg/mL)	0.86 (0.69 - 1.31)	0.91 (0.74 - 1.21)	1.74 (1.59 - 2.84)	-
$C_{min}$ (mcg/mL)	0.86 (0.69 - 1.18)	0.91 (0.74 - 1.13)	1.73 (1.45 - 2.12)	1.11 (46)
CL/F (L/hr)	0.86 (0.78 - 1.05)	1.01 (0.87 - 1.12)	0.69 (0.60 - 0.73)	1
$T_{1/2}$ (hr)	11.5 (9.2 - 12.6)	11.2 (9.9 - 13.9)	12.6 (9.2 - 14.9)	14

<sup>1</sup>Historical data from Tivicay<sup>TM</sup> (Dolutegravir) package insert, represented as geometric mean (%CV)

#### 439 Rilpivirine Female Genital Tract Concentrations in Pregnant and Postpartum Women

Mark Mirochnick<sup>1</sup>; **Brookie M. Best**<sup>2</sup>; Angela Kashuba<sup>3</sup>; Craig Sykes<sup>3</sup>; Amanda Schauer<sup>3</sup>; Jiajia Wang<sup>4</sup>; Alice Stek<sup>5</sup>; Elizabeth Smith<sup>6</sup>; Nahida Chakhtoura<sup>7</sup>; Edmund Capparelli<sup>2</sup>

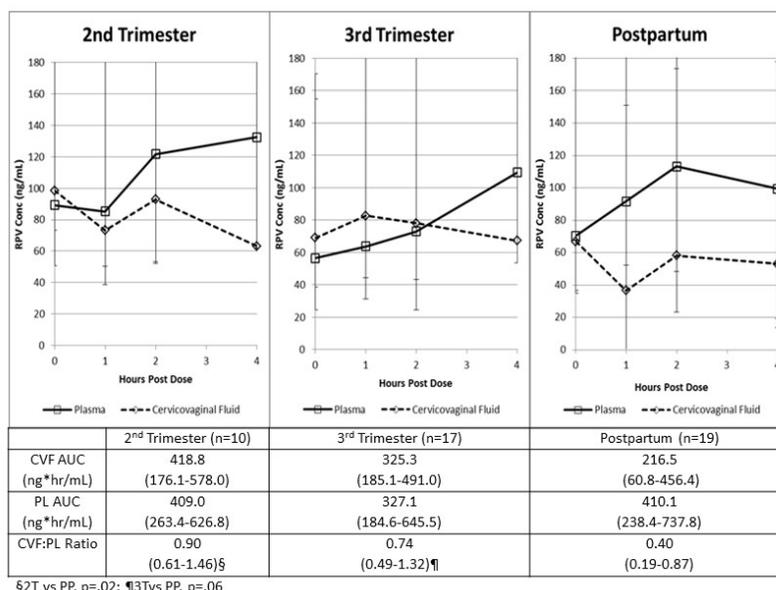
<sup>1</sup>Boston Univ Sch of Med, Boston, MA, USA; <sup>2</sup>Univ of North Carolina San Diego, San Diego, CA, USA; <sup>3</sup>Univ of North Carolina at Chapel Hill, Chapel Hill, NC, USA; <sup>4</sup>Harvard Sch of PH, Boston, MA, USA; <sup>5</sup>Univ of Southern California, Los Angeles, CA, USA; <sup>6</sup>NIAID, NIH, Bethesda, MD, USA; <sup>7</sup>Eunice Kennedy Shriver NICHD, Bethesda, MD, USA

**Background:** Genital tract (GT) concentration (conc) of antiretrovirals (ARVs) may play a key role in the success of oral ARVs used for PrEP in HIV uninfected women and for prevention of intrapartum MTCT in HIV infected pregnant women. Female GT conc with chronic oral rilpivirine (RPV) dosing have not previously been described. The impact of pregnancy on RPV ARV secretion into the female GT is unknown.

**Methods:** International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) Protocol 1026s (P1026s) is an ongoing, multicenter, non-blinded prospective study evaluating the pharmacokinetics of ARVs in pregnant HIV-infected women that included a cohort of pregnant women receiving RPV 25mg once daily as part of clinical care. Plasma (PL) and cervicovaginal fluid (CVF) samples were collected predose and 1, 2 and 4 hours post dose during the 2<sup>nd</sup> trimester (2T), 3<sup>rd</sup> trimester (3T) and 6-12 weeks postpartum (PP). RPV conc were measured using LC-MS/MS. PL and CVF lower limit of quantitation (LLQ) were 5 and 2 ng/mL, respectively. Area under the conc time curve (AUC) through 4 hours after dosing was estimated using the trapezoidal rule. Pairwise comparisons for PL AUC, CVF AUC and their ratio within each subject between sampling periods were performed using a two-sided Wilcoxon signed rank test with  $p < 0.05$  considered statistically significant. Median (IQR) summary statistics are reported.

**Results:** RPV CVF and PL samples were collected from 24 US women (12 Black, 11 Hispanic, 1 White). For all samples collected, RPV conc was 70.3 ng/mL (22.9-120.60) in CVF and 92.0 ng/mL (48.9-147.4) in PL, and their ratio was 0.68 (0.28-1.38). Samples were adequate to calculate CVF and PL AUC for 10 2T subjects, 17 3T subjects and 19 PP subjects. CVF and PL conc-time plots and AUCs for each sampling period are presented in the figure. Compared to PP, CVF:PL AUC ratio was lower during 2T ( $p=.02$ ) and 3T ( $p=.06$ ).

**Conclusions:** These data demonstrate that with chronic oral RPV dosing, CVF RPV conc is similar to PL, and is likely to achieve inhibitory concentrations. Our data also suggest that CVF conc of RPV may be greater during pregnancy than postpartum.



#### 440 Nevirapine Dosing for Treatment in the First Month of Life

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<sup>1</sup>Boston Univ Sch of Med, Boston, MA, USA; <sup>2</sup>Univ of California Los Angeles, Los Angeles, CA, USA; <sup>3</sup>Hosp Geral de Nova Iguaçu, Rio de Janeiro, Brazil; <sup>4</sup>Makerere Univ, Kampala, Uganda; <sup>5</sup>Wake Forest Univ, Winston Salem, NC, USA; <sup>6</sup>Univ of Massachusetts Med Sch, Worcester, MA, USA; <sup>7</sup>Univ of California San Diego, San Diego, CA, USA

**Background:** NVP clearance (CL) is low in term neonates and further decreased in preterm infants due to immaturity in CYP2B6 and CYP3A4 activity. NVP autoinduces its own CL but the extent of autoinduction on immature enzyme systems is unknown. While pharmacokinetic (PK) studies have been done to determine NVP dosing regimens for treatment of HIV infection (trough conc target 3.0 ug/mL) in infants after 1 month of life, NVP PK studies under age 1 month are limited to evaluations of dosing regimens for prophylaxis against HIV infection (trough conc target 0.1 ug/mL). Population modeling of these PK data and simulations can be used to evaluate proposed NVP dosing regimens to achieve treatment target conc in term and late preterm infants (34-37 weeks gestation) from birth through 6 months of life.

**Methods:** We developed a NVP population PK model using NONMEM that incorporated data from 192 infants (1121 plasma NVP conc) from US, Africa and Brazil under age 1 yr in 5 PACTG or HPTN protocols. Prematurity effects were estimated from the published literature (de Waal 2014). Dosing regimens from birth through 6 months of age were evaluated using simulations. Simulated NVP doses included 6 mg/kg BID for term infants and 4mg/kg BID for 1 week followed by 6 mg/kg BID for late preterm infants. Proposed PK target was NVP trough conc > 3.0 ug/mL.

**Results:** A one compartment model with first order absorption was used. CL was scaled allometrically and volume of distribution (Vd) was scaled linearly for weight. CL was modeled to mature exponentially with age. Autoinduction of CL was modeled as a linear function of dose. The effects of prematurity and maturation of CYP2B6 and CYP3A4 activity on NVP CL were imputed from published studies. Typical CL (L/hr/kg) in term infants increased by nearly 6 fold from birth to 6 months due to maturation and by an additional 79% due to induction. Final simulations used term infant doses of 6 mg/kg BID and late preterm infant doses of 4mg/kg BID for 1 week followed by 6 mg/kg BID. In these simulations, the dosing regimens achieved NVP targ

**Conclusions:** NVP CL is low immediately after birth and increases dramatically over the 1st months of life. Appropriate NVP dosing regimens in neonates must take into account the impact of maturation, auto-induction and prematurity on NVP CL. The dosing regimens supported by these simulations and NVP PK in preterm infants are being studied in the IMPAACT 1115 and 1106 protocols.

#### 441 Prediction of Infant Exposure to Maternal Drugs From Breast Milk Using PBPK Modeling

**Adeniyi Olagunju**<sup>1</sup>; Rajith Kumar Reddy Rajoli<sup>1</sup>; Oluseye Bolaji<sup>2</sup>; David J. Back<sup>1</sup>; Saye Khoo<sup>1</sup>; Andrew Owen<sup>1</sup>; Marco Siccardi<sup>1</sup>

<sup>1</sup>Univ of Liverpool, Liverpool, UK; <sup>2</sup>Obafemi Awolowo Univ, Ile-Ife, Nigeria

**Background:** Therapeutic drug use during lactation is widespread despite a paucity of data on breast milk excretion and the extent of breastfed infants' exposure for many drugs. Physiologically-based pharmacokinetic (PBPK) modeling is increasingly used in paediatric studies, with significant regulatory support. Here we present for the first time the development and validation of a PBPK model to predict infant exposure to maternal therapeutic drugs through breast milk.

**Methods:** A bespoke breastfeeding model integrating a whole-body PBPK maternal model with a whole-body PBPK infant model was developed. The model included mathematical descriptions of system and drug-specific parameters regulating absorption, distribution, metabolism and excretion, and breastfeeding. Virtual populations of nursing mothers-infant pairs (n = 100 per infant age group: 10 days-1 month, 1-3 months, 3-6 months, and 6-12 months) were simulated. Simulated mothers received 600 mg efavirenz and infants were exclusively breastfed until 6 months. Suckling rate was assumed to be equal to milk production rate and was kept constant after 6 months to reflect mixed feeding. Pharmacokinetic parameters were obtained at steady state. Model building and simulation was conducted using SimBiology on MATLAB 2014b. Previously published clinical data on efavirenz in a cohort of nursing mother-infant pairs was used for model validation.

**Results:** Key anatomical and physiological parameter predictions were within 50% difference of available clinical data. The model adequately described efavirenz pharmacokinetics, with over 90% of all individual observed data points (n = 29) within the predictive interval. Compared with clinical data, all parameters were within 50% difference. Predicted versus observed breast milk AUC<sub>0-24h</sub>, C<sub>max</sub> and C<sub>min</sub> were 78.2 (15.3-335) versus 68.5 (26.3-257) µg.hr/mL, 4.65 (1.15-18.0) versus 5.39 (1.43-18.4) µg/mL, and 2.19 (0.283-13.0) versus 1.68 (0.316-9.57) µg/mL, respectively. Model-predicted infant efavirenz dose from breast milk and the resulting plasma concentrations were within 50%

difference of clinical data. The average infant plasma concentration was highest in 10 days to 1 month and lowest in 6 to 12 month old infants at 0.22 (0.061-0.77) and 0.12 (0.026-0.60)  $\mu\text{g/mL}$ , respectively (Table 1).

**Conclusions:** The application of PBPK modeling creates opportunities for expanding our understanding of infant exposure to maternal drugs through breast milk.

**Table 1** Predicted versus observed pharmacokinetic parameters of efavirenz in breast milk and plasma of mother-infant pairs (median, range)

Parameters	Predicted (n = 400 <sup>a</sup> )	Observed (n = 29 <sup>b</sup> )
Breast milk AUC <sub>(0-24)</sub> ( $\mu\text{g}\cdot\text{hr/mL}$ )	78.2 (15.3-335)	68.5 (26.3-257)
Breast milk C <sub>max</sub> ( $\mu\text{g/mL}$ )	4.65 (1.15-18.0)	5.39 (1.43-18.4)
Breast milk C <sub>min</sub> ( $\mu\text{g/mL}$ )	2.19 (0.283-13.0)	1.68 (0.316-9.57)
Plasma AUC <sub>(0-24)</sub> ( $\mu\text{g}\cdot\text{hr/mL}$ )	70.7 (21.0-336)	60.7 (26.8-177)
Plasma C <sub>max</sub> ( $\mu\text{g/mL}$ )	4.26 (1.71-17.5)	4.63 (2.05-9.76)
Plasma C <sub>min</sub> ( $\mu\text{g/mL}$ )	1.97 (0.371-12.6)	2.03 (0.755-6.74)
Average infant EFV dose from breast milk ( $\mu\text{g/kg/day}$ )	412 (82.3-2170)	428 (164-1610)
Maximum infant EFV dose from breast milk ( $\mu\text{g/kg/day}$ )	571 (131-2430)	809 (215-2760)
Infant plasma EFV conc., 10 days-1 month old ( $\mu\text{g/mL}$ ) <sup>c</sup>	0.22 (0.061-0.77)	0.19 (0.071-0.705)
Infant plasma EFV conc., 1-3 months old ( $\mu\text{g/mL}$ ) <sup>c</sup>	0.19 (0.037-0.81)	0.18 (0.036-0.504)
Infant plasma EFV conc., 3-6 months old ( $\mu\text{g/mL}$ ) <sup>c</sup>	0.15 (0.035-0.52)	0.15 (0.052-0.33)
Infant plasma EFV conc., 6-12 months old ( $\mu\text{g/mL}$ ) <sup>c</sup>	0.12 (0.026-0.60)	0.12 (0.038-0.590)

<sup>a</sup>A total 400 mothers and 100 infants per group were simulated; <sup>b</sup>Observed maternal data were obtained from 29 mothers and 96 infants (n = 10, 26, 29 and 29, respectively; infants < 10 days and > 12 months old were excluded); <sup>c</sup>Predicted infant plasma EFV concentrations did not change significantly during the dosing interval and average predicted values are presented.

#### 442 Predicting Utility of Long-Acting Injectables in Paediatric Patients With PBPK Models

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**Background:** The use of long-acting (LA) ARVs in children and adolescents could be a valuable pharmacological option to simplify regimens, reduce drug costs and improve adherence. Dose optimisation in paediatric patients is complex and physiologically-based pharmacokinetic (PBPK) modelling represents a predictive tool to identify promising dosing strategies. The aim of this study was to simulate the pharmacokinetics (PK) of LA ARVs in children and adolescents and to identify optimal doses using PBPK modelling.

**Methods:** *In vitro* PK data for cabotegravir (CBV) and rilpivirine (RPV) were integrated into PBPK models using MATLAB, R2013b. The models were validated against available clinical data (800 mg CBV and 900 mg RPV) for the LA formulations in adults. Paediatric patients were simulated using a mathematical description of covariance between demographics and tissue size, expression of metabolic enzymes and processes regulating drug distribution. The weight band categories were selected according to the World Health Organisation recommendations. ARV PK was simulated for 200 paediatric patients for each weight band following IM administration of LA CBV and RPV. LA doses were optimised to obtain C<sub>trough</sub> values above the protein-binding-corrected IC<sub>95</sub> (PBIC<sub>95</sub>) or clinical cut-offs.

**Results:** The simulated PK parameters for a long-acting CBV and RPV in adults were in agreement with previously published clinical data. The mean values of AUC were 4467 vs. 5257  $\mu\text{g}\cdot\text{h/mL}$ , C<sub>max</sub> 3.3 vs. 3.54  $\mu\text{g/mL}$  and C<sub>trough</sub> 1.1 vs. 1.2  $\mu\text{g/mL}$  for 800 mg CBV quarterly intramuscular administration. The mean values of AUC for 900 mg IM RPV monthly administration were 74,420 vs. 91,087 ng.h/mL, C<sub>max</sub> 168 vs. 168.7 ng/mL and C<sub>trough</sub> 79.1 vs. 78.3 ng/mL. The models predicted optimal ARV doses resulting in at least 95% of the patients achieving C<sub>trough</sub> over the cut-off values for quarterly or monthly administration of CBV or RPV, respectively (table 1).

**Conclusions:** These data suggest that existing LA nanoformulations may potentially be used in children and adolescents by adjusting the dose based on weight, thus broadening the usage of LA ARVs and providing alternative strategies for treatment simplification.

Table 1: Summary of doses required in milligram for CBV and RPV formulations for different weight classes of children/adolescents and cut-off limits

Age (years)	Weight (Kg)	Rilpivirine		Cabotegravir	
		mg	mg	mg	mg
3 - 5.75	14 - 19.9	180	720	30	110
5.75 - 7.75	20 - 24.9	190	720	30	130
7.75 - 9.4	25 - 29.9	190	730	35	150
9.4 - 10.75	30 - 34.9	200	735	35	160
10.75 - 11.9	35 - 39.9	200	770	45	170
11.9 - 12.8	40 - 44.9	210	790	45	180
12.8 - 13.7	45 - 49.9	220	810	50	190
13.7 - 14.75	50 - 54.9	225	825	50	200
14.75 - 15.75	55 - 59.9	230	840	55	210
15.75 - 17.25	60 - 64.9	230	860	55	220
17.25 - 19.5	65 - 69.9	240	880	60	240
Duration		1 month		3 months	
Cut-off limit (ng/ml)		20.3	80	166	664

**443 Phenome-Wide Association Study (PheWAS) Using Data From ACTG Clinical Trial A5202**Anurag Verma<sup>1</sup>; Yuki Bradford<sup>1</sup>; Shefali Verma<sup>1</sup>; Sarah Pendergrass<sup>2</sup>; Eric Daar<sup>3</sup>; Charles Venuto<sup>4</sup>; Gene D. Morse<sup>5</sup>; Paul McLaren<sup>6</sup>; Marylyn D. Ritchie<sup>1</sup>; **David W. Haas**<sup>7</sup><sup>1</sup>The Cntr for Systems Genomics, Pennsylvania State Univ, University Park, PA, USA; <sup>2</sup>Geisinger Hlth System, Danville, PA, USA; <sup>3</sup> Harbor Univ of California Los Angeles Med Cntr, Torrance, CA, USA; <sup>4</sup>Univ of Rochester Med Cntr, Rochester, NY, USA; <sup>5</sup>State Univ of New York at Buffalo, Buffalo, NY, USA; <sup>6</sup>PH Agency of Canada, Winnipeg, MB, Canada; <sup>7</sup>Vanderbilt Univ, Nashville, TN, USA**Background:** Clinical trials datasets likely contain as yet undiscovered genetic associations, some of which are context dependent. Phenome-wide association studies (PheWAS) explore whether human genetic polymorphisms (SNPs) are associated with any trait (phenotype) across the “phenome”. This pilot PheWAS used data from antiretroviral therapy (ART)-naïve subjects who were randomized to initiate ART regimens in AIDS Clinical Trials Group (ACTG) protocol A5202.**Methods:** In A5202, 1858 HIV-infected subjects were randomized to tenofovir DF/emtricitabine or abacavir/lamivudine, with either atazanavir/ritonavir (ATVr) or efavirenz (EFV). We analyzed phenotypes across 4 clinical domains: immunology derived from CD4 counts; virology derived from HIV-1 RNA data (<200 copies/mL); metabolism derived from fasting LDL cholesterol and triglycerides (TG); and pharmacology derived from EFV and ATV pharmacokinetics (PK). From these, we derived 774 phenotypes based on context at baseline (sex, race/ethnicity, age category, randomized ART regimen, individual ART drug, HIV RNA, CD4 count, and body mass index strata). We considered absolute values and change from baseline. Using Illumina Human1M-Duo data from 1181 subjects we imputed ~5.8 million SNPs, from which we assessed for association with 2374 SNPs in 761 genes. These SNPs were selected from PharmGKB based on published association with any drug. Statistical models with  $\geq 100$  subjects excluded SNPs with minor allele frequency (MAF) <0.05, and models with <100 subjects excluded SNPs with MAF <0.10. Analyses controlled for age, sex, and 3 principal components. By permutation testing, the majority of SNP-phenotype associations with  $p < 1.5 \times 10^{-4}$  were not by chance alone.**Results:** Within each domain, we observed SNP-phenotype pairs with  $p < 1.5 \times 10^{-4}$ . Some associations were not unexpected, including *CYP2B6* rs3745274 with EFV PK phenotypes ( $p = 1.1 \times 10^{-28}$ ), *APOE* rs7412 with LDL phenotypes ( $p = 2.9 \times 10^{-10}$ ), and rs17482753 (near *LPL*) with TG phenotypes ( $p = 2.3 \times 10^{-6}$ ). We also observed potentially novel associations, such as rs1799964 (near *TNF*) with CD4 counts ( $p = 2.0 \times 10^{-6}$ ), *ABCC4* rs57270423 with ATV PK ( $p = 2.9 \times 10^{-5}$ ), and *CDKN2B-AS1* rs7865618 with HIV RNA control ( $p = 6.2 \times 10^{-7}$ ).**Conclusions:** PheWAS efficiently identified both expected and potentially novel SNP-phenotype associations across multiple clinical domains in a large, randomized clinical trial. These results encourage continued application of PheWAS to HIV clinical trials datasets.**444 Effect of CYP2B6 Gene Variants on Levonorgestrel PK When Combined With EFV-Based ART****Megan Neary**<sup>1</sup>; Mohammed Lamorde<sup>2</sup>; Adeniyi Olagunju<sup>1</sup>; Kristin M. Darin<sup>3</sup>; Pauline Byakika-Kibwika<sup>4</sup>; Concepta Merry<sup>4</sup>; David J. Back<sup>1</sup>; Marco Siccardi<sup>1</sup>; Andrew Owen<sup>1</sup>; Kimberly K. Scarsi<sup>5</sup><sup>1</sup>Univ of Liverpool, Liverpool, UK; <sup>2</sup>Infectious Diseases Inst, Makerere Univ, Kampala, Uganda; <sup>3</sup>Northwestern Univ, Chicago, IL, USA; <sup>4</sup>Makerere Univ Coll of Hlth Scis, Kampala, Uganda;<sup>5</sup>Univ of Nebraska, Omaha, NE, USA**Background:** The subdermal levonorgestrel (LNG) implant is a highly effective and desirable method for contraception. Our group described a significant drug interaction between LNG and efavirenz (EFV)-based antiretroviral therapy (ART), resulting in suboptimal LNG concentrations and a high rate of unintended pregnancy. Herein we describe the association between pharmacogenetic variants and LNG pharmacokinetic (PK) parameters in these study participants.<**Methods:** In this prospective PK evaluation of HIV-infected Ugandan women receiving EFV-based ART (n=20), a LNG implant was inserted at study entry and a whole blood sample was collected for pharmacogenetic analysis. At each study visit over 48 weeks, a plasma sample was collected to assess LNG and mid-dose EFV PK. The primary endpoint was LNG PK at week 24. Follow-up was interrupted for 9 subjects between weeks 36-44 after 3 pregnancies were identified. LNG and EFV concentrations were analysed by a validated LC-MS/MS and HPLC method, respectively. SNPs in *CYP2B6* (rs3745274, rs28399499, rs4803419), *CYP2A6* (rs28399433, \*9B rs8192726), *NR1I2* (rs2472677) and *NR1I3* (rs2307424, rs3003596) were analysed using TaqMan assays. Associations between patient genotype and LNG PK were determined through univariate and backwards multivariate linear regression.**Results:** All women were Black African and received EFV for a median of 10 (range 5-66) months prior to entry. Allele frequencies for associated SNPs in *CYP2B6* (rs3745274, 516G>T; rs4803419, C>T) are described in Table 1. *CYP2B6* rs4803419 ( $X^2 = 20.62$ ,  $P = < 0.001$ ) and *NR1I3* rs2307424 ( $X^2 = 12.36$ ,  $P = < 0.001$ ) were not in Hardy-Weinberg equilibrium, which compromises their interpretation. The presence of a T allele for *CYP2B6* rs3745274 was associated with lower  $\log_{10}$  LNG  $C_{max}$  ( $P = 0.02$ ,  $\beta = -0.20$ ). The presence of a T allele for *CYP2B6* rs4803419 was associated with lower  $\log_{10}$  LNG  $AUC_{0-24wk}$  ( $P = 0.006$ ,  $\beta = -0.18$ ) and  $\log_{10}$  LNG  $AUC_{0-last}$  ( $P = 0.007$ ,  $\beta = -0.25$ ). LNG and EFV PK results summarized by *CYP2B6* variant are presented in Table 1. No other genetic associations were observed; however, the sample size was too low to robustly assess the *CYP2B6* 983T>C variant (rs28399499).**Conclusions:** These data demonstrate that pharmacogenetic variations in *CYP2B6* influence LNG pharmacokinetics when combined with EFV-based ART. This supports the need for further investigation in a larger population to assess whether a pharmacogenetic approach could be used to identify patients on EFV who are at highest risk of suboptimal LNG concentrations.

	CYP2B6 rs3745274			CYP2B6 rs4803419	
	GG (n=8)	GT (n=11)	TT (n=1)	CC (n=5)	CT (n=15)
LNG $C_{max}$ ( $\log_{10}$ pg/mL)	2.73 (2.67-3.11)	2.69 (2.34-3.15)	2.30	2.73 (2.62-3.11)	2.69 (2.3-3.15)
LNG $C_{last}$ ( $\log_{10}$ pg/mL)	2.35 (2.28-2.61)	2.34 (2.16-3.15)	2.09	2.48 (2.39-2.58)	2.32 (2.09-3.15)
LNG $T_{max}$ (h)	1.00 (1-36)	1.00 (1-48)	0.60	1.00 (1-36)	1.00 (1-48)
LNG $AUC_{0-24wk}$ ( $\log_{10}$ pg*wk/mL)	3.92 (3.89-4.27)	3.89 (3.62-4.04)	3.56	4.00 (3.87-4.27)	3.89 (3.56-4.11)
LNG $AUC_{0-last}$ ( $\log_{10}$ pg*wk/mL)	4.125 (4.1-4.59)	4.11 (3.77-4.41)	3.72	4.19 (4.13-4.59)	4.10 (3.72-4.41)
EFV $C_{12-14h}$ (pg/mL)	2.11 (1.31-2.91)	2.63 (1.50-17.13)	8.31	2.38 (1.39-2.63)	2.58 (1.31-17.13)

**445 Transporter Genetics and TFV-DP/FTC-TP Cellular Pharmacology In Vivo****Sharon M. Seifert**<sup>1</sup>; Xinhui Chen<sup>2</sup>; Carolyn W. Clayton<sup>3</sup>; Taylor Alford<sup>1</sup>; Amie L. Meditz<sup>4</sup>; Jose R. Castillo-Mancilla<sup>5</sup>; Lane R. Bushman<sup>5</sup>; Christina Aquilante<sup>1</sup>; Samantha MaWhinney<sup>3</sup>; Peter L. Anderson<sup>5</sup><sup>1</sup>Univ of Colorado Skaggs Sch of Pharm and Pharmaceutical Scis, Aurora, CO, USA; <sup>2</sup>Univ of Colorado, Aurora, CO, USA; <sup>3</sup>Colorado Sch of PH, Aurora, CO, USA; <sup>4</sup>Beacon Cntr for Infectious Diseases, Boulder, CO, USA; <sup>5</sup>Univ of Colorado, Denver, CO, USA**Background:** The intracellular anabolites, tenofovir-diphosphate (TFV-DP) and emtricitabine-triphosphate (FTC-TP), exhibit high inter-individual variability in vivo, but the sources of this variability have not been fully elucidated. The purpose of this study was to assess the association between single nucleotide polymorphisms (SNPs) in candidate

membrane transporters (MDR1; MRP2, 4, 7; OAT1; NPT1; ENT1, 3; CNT2, 3) with the intracellular concentrations of TFV-DP and FTC-TP in peripheral blood mononuclear cells (PBMC) and red blood cells (RBC).

**Methods:** HIV-negative and HIV-positive participants were enrolled in a pharmacokinetic (PK) study of daily TFV/FTC (plus efavirenz in HIV-positive) over 30 days. This analysis focused on day 30 PK. Human genomic DNA samples were genotyped for 19 SNPs using PCR-pyrosequencing methods. Blood was collected at 1, 2, 4, 8, and 24 hours and C<sub>ss</sub>(ave) was determined for PBMC with non-compartmental methods (AUC<sub>(0-24)</sub>/tau). For RBC, the 2 hour post-dose sample was used for the analyses. Associations were evaluated graphically to determine grouping for heterozygotes. PK outcomes were log transformed for normality. T-tests were used for univariate analyses with no adjustment for multiple comparisons. For PK outcomes that demonstrated significance (p<0.05) in the univariate analysis, multivariable linear regression was utilized to adjust for race.

**Results:** Day 30 data were available from thirty-one adults (19 men, 13 HIV-positive). In univariate analysis, significant relationships were identified between CNT2 and ENT3 with TFV-DP, and MDR1 with FTC-TP (Table). After adjusting for race, TFV-DP in PBMC was 24% lower (95% CI: 5.1%, 38.8%; p=0.025), 27% lower (7.0%, 43.3%; p=0.013), and 26% lower (1.3%, 44%; p=0.041) in CNT2\_rs1060896, CNT2\_rs11854484, and ENT3\_rs1099976 homozygous variant carriers, respectively; TFV-DP in RBC was 46% higher (7.6, 99.1; p=0.017) according to ENT1\_rs324148 variant carriage; and FTC-TP was 24% higher (5.1%, 38.8%; p=0.017) in those with 0 or 1 copies of the MDR1 (1236/2677/3435)-CGC haplotype versus 2 copies of the CGC haplotype.

**Conclusions:** This study found novel associations between TFV-DP in PBMC and RBC with SNPs in CNT2, ENT3 and ENT1, respectively, as well as SNPs in MDR1 for FTC-TP. We hypothesize that polymorphisms in these transporters are a source of PK variability for TFV-DP and FTC-TP in PBMC and RBC in vivo, and that accurate prediction of individual PK will require accounting for these pharmacogenetic profiles.

SNP	Genotype <sup>a</sup> (n)	Drug Conc <sup>b</sup>	P value
<b>TFV-DP in PBMC (fmol/10<sup>6</sup> cells)</b>			
CNT2_rs1060896 C>T	WT (5) + HET (16)	102 (88, 117)	0.012
	VAR (10)	73 (58, 93)	
CNT2_rs11854484 T>C	WT (4) + HET (16)	103 (89, 119)	0.006
	VAR (11)	73 (59, 91)	
ENT3_rs1099976 C>T	WT (8) + HET (13)	101 (89, 115)	0.014
	VAR (10)	74 (56, 97)	
<b>TFV-DP in RBC (fmol/10<sup>6</sup> cells)<sup>c</sup></b>			
ENT1_rs324148 C>T	WT (22)	80 (68, 95)	0.009
	HET (7) + VAR (1)	119 (100, 143)	
<b>FTC-TP in PBMC (pmol/10<sup>6</sup> cells)</b>			
MDR1_1236 C>T, 2677 G>T/A, 3435 C>T	0 (8) or 1 (13) CGC copies	5.9 (5.4, 6.5)	0.012
	2 (10) CGC copies	4.6 (3.7, 5.7)	

<sup>a</sup>WT=wild-type, HET=heterozygous, VAR=homozygous variant

<sup>b</sup>geometric mean and 95% confidence interval for univariate analysis

<sup>c</sup>one day 30 value was missing

#### 446 CYP2B6 Genotype Effects on Neurocognitive Impairment in HIV+ Patients on Efavirenz

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**Background:** Blood-brain barrier active efflux transporters of the ATP-binding cassette (ABC) gene family and CYP enzymes are involved in distribution and elimination of antiretroviral drugs in the brain and periphery. Single nucleotide polymorphisms (SNPs) in *CYP2B6* and *ABCB1* have been associated with peripheral exposure of efavirenz. The impact of genetic profiles of transporters and CYP2B6 on central nervous system (CNS) exposure of efavirenz or on neurocognitive (NC) performance has not been assessed.

**Methods:** Adult HIV-positive patients receiving efavirenz (EFV)-containing cART for at least six months (n=44 from CHARTER, n=23 from China neuroAIDS) were included. Single random plasma or paired plasma and CSF samples were collected and analyzed using LC/MS-MS methods. Standardized comprehensive neurocognitive testing was performed. SNPs in the following genes were extracted from an SNP array: *ABCB1*, *ABCC4*, and *CYP2B6*. Results are expressed as median and interquartile range (IQR).

**Results:** The median EFV plasma and CSF concentrations were 2335 ng/mL (1707-3510) and 13.6 ng/mL (5.9-19.1), respectively. CSF-to-plasma ratios ranged from 0.00003 to 0.073 (median 0.0053, IQR 0.0023-0.0082). CSF concentrations significantly correlated with plasma levels (Pearson rho = -0.28, P = 0.04). *ABCB1* 3435 C>T (rs1045642) had no significant impact on CSF concentrations [TT 12.9 (4.96-18.5) vs. CC/CT 13.8 (10.7-15.5) ng/mL, p = 0.391]. *CYP2B6* 516G>T (rs3745274) TT carriers had significantly higher plasma and CSF concentrations [TT 27.2 (12.6-33.6), GT 15.6 (2.63-20.3), GG 7.45 (3.27-19.3) ng/mL, p = 0.047]. Significantly higher incidence of global neurocognitive impairment was noted among *CYP2B6* TT carriers (81% vs. 26%, p=0.02).

**Conclusions:** The interpatient variability of CNS exposure of EFV is large. *CYP2B6* 516G>T is associated with plasma and CSF concentrations as well as global neurocognitive impairment, suggesting the relationship between genetic factors and CNS exposure may be one of the underlying mechanisms for the development of NC impairment.

#### 447 Multispecies ARV Distribution in Intestinal Tissue by Mass Spectrometry Imaging (MSI)

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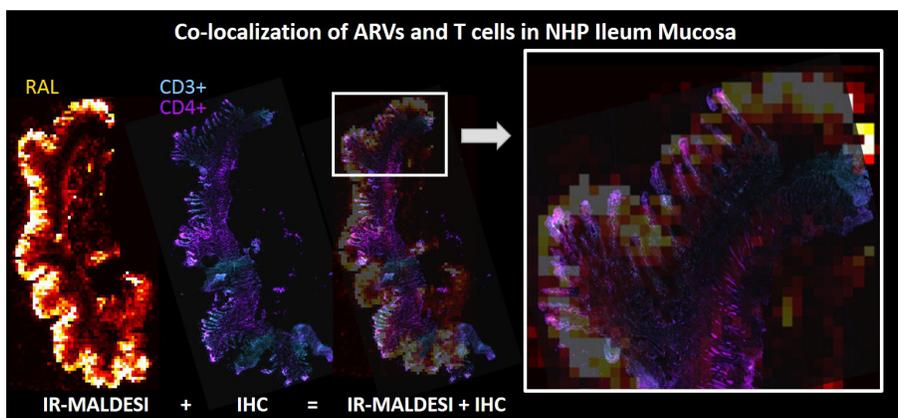
**Background:** Methods to accurately evaluate drug distribution within tissues are needed to design effective eradication strategies. Here, we characterize the spatial distribution of emtricitabine (FTC), tenofovir (TFV), and raltegravir (RAL) within the upper and lower intestine (ileum and colorectum) and evaluate differences between ARV accumulation in three animal models for HIV infection using MSI.

**Methods:** Iliac (IL) and colorectal (CR) tissue was removed at necropsy from uninfected rhesus macaques (NHP, n=3) and from two humanized mouse models (BLT, n=3; hu-HSC-Rag, n=3) dosed to steady-state with FTC+TFV+RAL. MSI of snap frozen cryosections (10mm) were analyzed using an infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) source. Response was calibrated by ARV standards on blank tissue, with a limit of detection of 250 fg/voxel (24 ng/g tissue). Serial sections of tissue were utilized to validate MSI results by LC-MS/MS, and stained to correlate observed ARV response with CD3, CD4, and CD8+ cells. LC-MS/MS concentrations are reported in micrograms/g tissue.

**Results:** All ARVs were observed to penetrate NHP intestinal tissues, increasing in concentration from FTC to TFV to RAL for both IL (1.0±1.0, 2.2±1.2, 6.5±4.8, respectively) and CR (2.8±2.6, 4.0±3.7, 71.4±9.2, respectively). Relative to plasma, tissue concentrations for FTC, TFV, and RAL were 3.7±1.1, 3.4±0.6, 3.8±0.2 log units higher, respectively, in IL

and  $4.8 \pm 0.9$ ,  $3.8 \pm 0.7$ ,  $6.3 \pm 0.7$  log units higher, respectively, in CR. Heterogeneous drug exposure was seen by MSI, with highest accumulation for FTC, TFV, and RAL in the mucosa and lamina propria of the IL and CR, corresponding to high density of CD3+, CD4+, and CD8+ T cells. Only TFV was detected in BLT (IL:  $2.5 \pm 1.5$ ; CR:  $3.0 \pm 1.9$ ) and hu-HSC-Rag (IL:  $11.4 \pm 10.3$ ; CR:  $60.0 \pm 26.2$ ). IL and CR concentrations were  $2.8 \pm 0.9$  and  $3.0 \pm 1.9$  log units higher than plasma, respectively, for BLT and were  $4.4 \pm 0.9$  and  $6.4 \pm 0.5$  log units higher than plasma for hu-HSC-Rag. MSI quantitative response was similar to LC-MS/MS.

**Conclusions:** This study is the first to map the biodistribution of multiple ARVs across intestinal tissue from different animal models. Observed differences in tissue concentrations cannot be extrapolated solely from plasma. By differentiating and quantifying ARV exposure within and across compartments, IR-MALDESI MSI can provide key information to evaluate ARV penetration into putative reservoir tissues and guide selection of optimal interspecies scaling of therapy.



**448 Single-Dose Maraviroc Provides High Drug Levels in All Sites: No Gender Differences**

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**Background:** Oral pre-exposure prophylaxis (PrEP) is an effective prevention strategy against HIV-1 transmission. All completed PrEP clinical trials have tested ARV acting post-viral entry in the target cell. Maraviroc (MVC) showed no protection *ex vivo* following stat dosing [CROI 2105] and daily dosing studies are ongoing. Understanding the drugs levels achieved by stat dosing which failed to show *ex vivo* protection will help to inform future clinical trials of daily or "on demand" MVC PrEP.

We present results of a PK dosing study of single oral dose MVC 300mg in all HIV exposure compartments and compare men and women, in a phase 2, multi-site, open label, randomised controlled clinical trial.

**Methods:** 56 healthy adult female (n=26) and male participants (n=30) were randomized to a control arm (Arm A n=6 with tissue samples taken at two time points one month apart) or to one of 4 intervention arms (n=12 per arm) where a single oral MVC 300 mg dose was taken at two time points prior to sampling, one month apart (Arm B: first sampling 2 h post first dose and second sampling 24 h post second dose; Arm C: 4 h and 36 h; Arm D: 6 h and 48 h; Arm E: 12 h and 72 h). Sampling to determine MVC concentrations included blood, saliva and rectal fluid (RF) for all subjects. In addition, men provided a urethral swab and rectal tissue (RT) and women provided cervico-vaginal fluid (VF) and vaginal tissue (VT). MVC drug concentrations were measured by validated LC-MS/MS.

**Results:** MVC Cmax was reached within 4 hours in all compartments, and exceeded suggested MEC (25 ng/ml). The highest Cmax level was in urethra (median 4 hr compartment-to-plasma ratio =116), RF (99 males and 102 females), RT (9.7 males), VT (3.6) and vaginal fluid (2.6): only saliva (0.22 males and 0.17 females) levels were lower than plasma at Cmax. MVC concentrations remained above the MEC of 25ng/ml until the following times: saliva (2h), VF 12h, plasma 8h, VT 24h, urethra 60h, RF >72h, RT >72h. All drug levels were above EC90 of 0.5ng/ml for 72 h except saliva; most samples were <0.4 ng/ml at 72 h).

At 72 h drug concentrations in compartments were higher than plasma: saliva (males 2 0.617) correlated in males and VF swab and VT (0.182) correlated in females.

**Conclusions:** MVC concentrations greater than the EC90 occurred in multiple sites of HIV acquisition after single oral 300mg MVC. This suggests that MVC may be a suitable candidate for PrEP. However, the lack of inhibition in rectal and vaginal tissue previously reported suggests that either the ex

Table 1: Drug levels over a 72h duration following single oral dosing of Maraviroc 300mg

	Correlation with plasma R <sup>2</sup>	Duration levels above MEC (hours)	Cmax (4hr) compartment-to-plasma ratio	Cmax compared to plasma	72h level compared to plasma	AUC Ratio Compartment to plasma
Plasma	-	8	-	-	-	-
Saliva (Males)	0.8149	0	0.2	4.6x lower	<LLQ	0.22
Saliva (Females)	0.7687	4	0.17	5.8x lower	1.3x higher	0.2
Urethra	0.2442	60	116	116x higher	1.7x higher	157.7
VF (Swab)	0.2955	12	2.6	2.6x higher	24x higher	3.6
VF (Aspirate)	0.0927	6	0.4	2.5x lower	12x higher	1.24
VT	0.66	24	3.6	3.6x higher	26x higher	4.55
RF (Males)	0.0058 (NS)	72	99	99x higher	718x higher	427.1
RF (Females)	0.00007(NS)	72	102	102x higher	13754x higher	470.2
RT (Males)	0.114	72	9.7	9.7x higher	60x higher	31.8

#### 449 PK of FTC, TFV and 3TC in Ugandan and Nigerian Breastfeeding Mother-Infant Pairs

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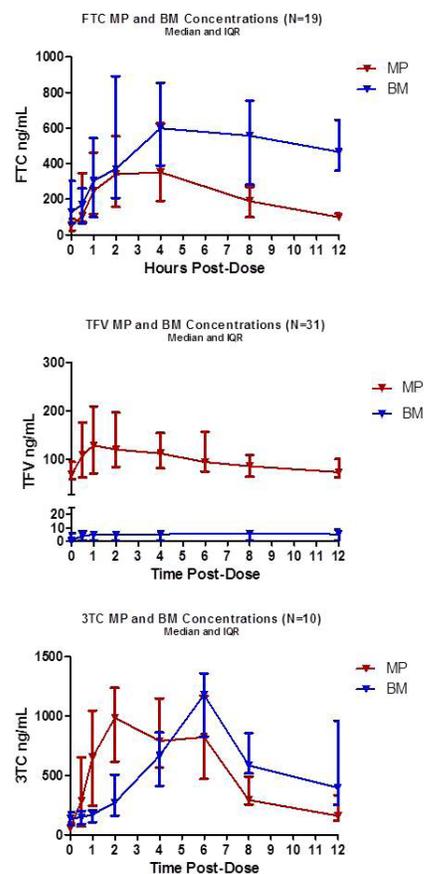
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**Background:** Under WHO Options B and B+, increasing numbers of HIV-positive women receive antiretroviral therapy (ART) during breastfeeding. The pharmacokinetics (PK) of nucleoside reverse transcriptase inhibitors (NRTIs) to breastfed infants are incompletely understood. We present intensive PK profiles of emtricitabine (FTC), tenofovir (TFV) and lamivudine (3TC) in maternal plasma (MP), breast milk (BM) and infant plasma (IP) from Ugandan and Nigerian cohorts.

**Methods:** Breastfeeding mothers receiving a once-daily efavirenz or nevirapine-based ART regimen were enrolled, together with their exclusively breastfed infants. Paired dried blood spots (DBS; maternal and infant) and dried breast milk spots (DMS) were collected pre-dose and serially up to 12 h post-dose. All three NRTIs were quantified using a validated simultaneous LC-MS/MS assay. Non-compartmental PK analysis was performed using WinNonLin and milk-to-plasma (M:P) ratios were calculated arithmetically.

**Results:** 21 Ugandan and 27 Nigerian mother-infant pairs were enrolled. Populations were similar for mean maternal age (30 years) and weight (60 Kg), and infant age (100 days) and weight (6 Kg).  $T_{max}$  of FTC was 4 h in MP and 5.1 h in BM, reaching median  $C_{max}$  of 493 (IQR 467-627) and 933 (716-1238) ng/mL, respectively. The  $AUC_{0-12}$  of FTC was 2492 (511-3260) and 4134 (824-7286) ng.h/mL in MP and BM, with a M:P AUC ratio of 2.13 (SD 1.77). FTC was detected in 18.7% of exposed infants with a median concentration of 18.5 (SD 3.4) ng/mL. TFV had a  $T_{max}$  of 1 h in MP and 4 h in BM, reaching  $C_{max}$  of 186 (109-240) and 7.3 (5.5-9.6) ng/mL in these compartments, respectively. The  $AUC_{0-12}$  was 1014 (738-1394) and 41.5 (23.2-56.1) ng.h/mL in MP and BM, giving a M:P AUC ratio of 0.034 (SD 0.09). No infant had measurable TFV. 3TC had a  $C_{max}$  of 991 (574-1129) ng/mL in MP and 572 (386-710) ng/mL in BM. The  $AUC_{0-12}$  of 3TC in MP and BM was 3916 (2985-6780) and 4001 (1951-4577) ng.h/mL, respectively, with a M:P AUC ratio of 1.02 (SD 0.79). 3TC was detectable in 41% of exposed infants with a median concentration 16.4 (SD 8.5) ng/mL.

**Conclusions:** This is the first report of full PK profiles of FTC and TFV in plasma and BM of breastfeeding mother-infant pairs, indicating higher concentration of FTC in BM compared to MP but transfer to IP only in a minority. TFV is measurable in BM but is not detectable in IP. Consistent with previous studies, 3TC levels in BM were comparable to MP with transfer to IP in almost half the infants.



#### 450 Multispecies Differences in Drug Transporter Expression and Localization in GI Tissue

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**Background:** HIV replication may persist during treatment within tissue reservoirs, including the gastrointestinal (GI) tract. Differences in drug transporter expression and localization may alter ARV exposure and confound the ability to translate study results between animal models, and to humans. Here, we characterize the expression and localization of transporters relevant to ARVs in 3 animal models.

**Methods:** Three cohorts of uninfected animals (rhesus macaques (NHP, n=3); humanized mice (BLT, n=6 and hu-HSC-Rag, n=18)) were dosed to steady-state with a combination of ARVs including tenofovir (TFV), emtricitabine (FTC), and raltegravir (RAL). Ileum and rectum were collected at necropsy and analyzed for gene (qPCR) and protein (Western blot) expression and localization (immunohistochemistry (IHC)) of ARV efflux (ABCB1, ABCG2, ABCB1, ABCB2, and ABCC4) and uptake (SLC29A1, SLC02A1, SLC22A3) transporters. Tissue concentrations were analyzed by LC-MS and normalized to plasma. Species comparisons were performed using ANOVA. Data are reported as mean fold expression vs GAPDH.

**Results:** In the ileum, gene expression differed significantly between BLT and hu-HSC-Rag mice for ABCB1 ( $5.02$  vs  $23.4 \times 10^{-4}$ ;  $p=0.005$ ), ABCB2 ( $0.11$  vs  $0.19$ ;  $p=0.01$ ), and ABCC4 ( $4.56$  vs  $1.91 \times 10^{-6}$ ;  $p=0.005$ ). Protein expression did not differ between these 2 models ( $p>0.05$ ). Iliac NHP gene expression was increased over mouse for ABCB1 & ABCC4 ( $1.7$  &  $3.3 \log$ ;  $p<0.01$ ) and decreased for SLC22A3 ( $3.2 \log$ ;  $p<0.01$ ). In the colorectum, gene differences were again observed between BLT and hu-HSC-Rag mice for ABCB1 ( $2.08$  vs  $26.3 \times 10^{-4}$ ;  $p<0.001$ ), SLC29A1 ( $0.07$  vs  $0.04$ ;  $p=0.019$ ), and ABCG2 ( $0.11$  vs  $0.05$ ;  $p=0.001$ ). No interspecies differences were seen. In all species, IHC showed MDR1 localization on the luminal surface of ileac and rectal mucosa and a lack of MRP2 expression. hu-HSC-Rag TFV tissue concentrations were 13 & 8-fold greater than BLTs and NHPs ( $p<0.05$ ).

**Conclusions:** This is the first study comparing the expression and localization of these transporters across animal models. Observed variability in expression suggests model-dependent ARV tissue penetration (e.g. decreased ABCB1 and increased ABCC4 expression in BLT mice explain the observed decrease in TFV exposure). Multi-log increases in ABCB1 expression between NHP and mice may impact the disposition of many ARVs that use ABCB1 for transport (e.g. TFV and RAL). Ultimately, these data can be coupled with ARV exposure data to inform inter-species drug scaling for targeting HIV reservoirs.

#### 451 Antiretroviral Use and Implications for DAA Therapy in HIV/HCV Coinfection

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**Background:** Interferon-free directly-acting antiviral (DAA) regimens for HCV provide a major advance in clinical management, including in HIV/HCV co-infection. Drug-drug interactions (DDI) with combination antiretroviral therapy (cART) will require consideration. The aim of this analysis was to characterise the cART regimens in HIV/HCV co-infected individuals and their suitability for co-administration with the following DAA HCV regimens: sofosbuvir/ledipasvir, paritaprevir/ritonavir/ombitasvir and dasabuvir, grazoprevir/elbasvir and sofosbuvir plus daclatasvir.

**Methods:** CEASE-D is a prospective cohort of HIV/HCV co-infected individuals in Sydney, Australia. This analysis included all individuals enrolled between July 2014 and August 2015 (n=191). The primary endpoint was the proportion of individuals receiving suitable cART for co-administration with approved or investigational (Phase III) interferon-free DAA regimens.

**Results:** In individuals receiving cART with HCV genotype (GT) 1 and 4 (n=92), no clinically significant DDI were expected in 41% with sofosbuvir/ledipasvir, 25% with paritaprevir/ritonavir/ombitasvir plus dasabuvir, 37% with grazoprevir/elbasvir and 61% with sofosbuvir plus daclatasvir. DDIs requiring DAA or antiretroviral dose adjustment or additional monitoring for toxicity were noted in 34% with sofosbuvir plus daclatasvir, 7% with paritaprevir/ritonavir/ombitasvir plus dasabuvir and 26% with sofosbuvir/ledipasvir. Contraindicated antiretroviral agents were noted in 47% with paritaprevir/ritonavir/ombitasvir plus dasabuvir and 45% with grazoprevir/elbasvir, but only 5% with sofosbuvir/ledipasvir and 0% with sofosbuvir plus daclatasvir. In individuals receiving cART with HCV GT 2 or 3 (n=45), no clinically significant DDI were expected in 67% and DDI requiring DAA dose adjustment were noted in 33% with sofosbuvir plus daclatasvir, allowing co-administration in 100%.

**Conclusions:** Potential DDIs will impact on DAA prescribing in HIV/HCV co-infection. HCV infection can be safely and successfully treated provided DDI are recognised and managed appropriately.

#### 452 Real-Life Renal Safety of Boosted TDF in HIV/HCV Patients on SOF/LDV

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**Background:** Ledipasvir increases tenofovir (TDF) exposures. In individuals taking EVG/COBI/TDF/FTC or TDF with a ritonavir (RTV)-boosted HIV PI, higher TDF exposures may increase the risk of renal toxicity.

**Methods:** To evaluate the renal safety of 12 or 24 weeks of Sofosbuvir/Ledipasvir (SOF/LDV), according to boosted TDF exposure, in a cohort of 159 HIV/HCV co-infected patients at a tertiary center in Madrid (Spain). We included all consecutive patients with complete renal function and overall safety follow-up data during therapy.

**Results:** From March 2015, 159 patients started SOF/LDV. HAART included TDF/FTC in 53 (33%); of them, 35 (66%) patients did not receive a boosted TDF regimen, whereas a "boosted TDF" regimen was used in 18 (34%): EVG/COBI/FTC/TDF in 10 (55%), FTC/TDF plus a RTV-boosted PI in 7 (39%), and simultaneous EVG/COBI/FTC/TDF and darunavir in one (5%).

At baseline there were no significant differences with respect to demographic data, genotype or HCV viral load, eGFR (CKD-EPI), or % of subjects with eGFR<70ml/min. Of note, more patients on boosted TDF received a 24w course of SOF/LDV (28% vs 0%, p=0.005).

During therapy we did not find significant differences in Creatinine or eGFR between both groups, nor in the rates of eGFR<70ml/min at the end of SOF/LDV: 2 patients (6%) in the non-boosted TDF group vs 3 patients (17%) in the TDF-boosted group (p=0.32). None of the 53 patients on TDF reached eGFR below 50ml/min, and only one patient on boosted-TDF (5%) stopped TDF due to renal impairment after 74 days of therapy (eGFR 56 ml/min).

**Conclusions:** In our "real life" population, SOF/LDV did not significantly worsen renal function in patients on "boosted TDF" (EVG/COBI/FTC/TDF or TDF with a RTV-boosted HIV PI).

Table 1. Baseline characteristics.

Baseline characteristics	TDF-No boosted N=35	TDF-Boosted N=18
Patients, men, n (%)	30 (85.7%)	17 (94.4%)
Age, years, mean±SD	50±7	51±3
HVC genotype 1, n (%)	27 (77.1%)	11 (61.1%)
Cirrhosis, n (%)	12 (34.3%)	9 (50%)
Fibroscan®, kPA±SD	13.47±9	13.98±10
HCV viral load at baseline Log <sub>10</sub> IU/L, mean±SD	5.94±1	6.27±1
CD4 cels/mm <sup>3</sup> , mean±SD	598±270	550±493
SOF/LDV 24 w, n (%)	0 (0%)	5 (28%)
TDF suspension, n (%)	0 (0%)	1 (8%)

Table 2. Glomerular filtration rate at baseline and at the end of treatment.

CKD-EPI eGFR (ml/min)	Baseline eGFR (ml/min) median (range)	Baseline <70 ml/min n (%)	End-treatment eGFR (ml/min) median (range)	End-treatment <70 ml/min n (%)
TDF-No boosted (n=35)	101 (63-114)	1 (3%)	97 (52-108)	2 (6%)
TDF-Boosted (n=18)	99 (71-113)	0 (0%)	101 (56-113)	3 (17%)

#### 453 Interactions Between ABT-493 Plus ABT-530 Combination and Rilpivirine or Raltegravir

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**Background:** ABT-493 (protease inhibitor discovered by AbbVie and Enanta) and ABT-530 (NS5A inhibitor) are direct acting antivirals developed as a combination regimen for treatment of chronic hepatitis C virus genotype 1-6 infection. Rilpivirine, a non-nucleoside reverse transcriptase inhibitor (NNRTI), and raltegravir, a HIV-1 integrase inhibitor are often used with other antiretroviral agents in the HIV-HCV co-infected population. Phase 1 drug-drug interaction (DDI) studies were conducted to evaluate pharmacokinetics, tolerability, and safety of ABT-493 + ABT-530 co-administered with rilpivirine or raltegravir.

**Methods:** Both rilpivirine and raltegravir DDI studies utilized open label, randomized, multiple-dose, non-fasting study designs. In each study, healthy adult subjects received ABT-493 300 mg QD + ABT-530 120 mg QD and rilpivirine 25 mg QD (N=24) or raltegravir 400 mg BID (N=12) alone or in combination.

Intensive pharmacokinetic assessments were performed for ABT-493, ABT-530, rilpivirine, and raltegravir on multiple days throughout the study. Effects of ABT-493 + ABT-530 on rilpivirine or raltegravir pharmacokinetics and vice versa were assessed by a repeated-measures analysis using SAS. Safety was evaluated via assessment of adverse events, vital signs, ECGs and clinical laboratory tests.

**Results:** Co-administration with multiple ABT-493 and ABT-530 doses increased rilpivirine C<sub>max</sub> and AUC<sub>inf</sub> by 105% and 84%, respectively, relative to rilpivirine alone; raltegravir C<sub>max</sub> and AUC<sub>12</sub> were increased by 34% and 47%, respectively, relative to raltegravir alone. ABT-493 and ABT-530 exposure following multiple QD doses, as determined by C<sub>max</sub> and AUC<sub>24</sub>, were similar when co-administered with rilpivirine or Raltegravir relative to administration of ABT-493 + ABT-530 alone (≤13% difference). All adverse events were mild in severity and assessed as not related to study drugs. No clinically significant vital signs, ECG or laboratory measurements were observed during the course of each study.

**Conclusions:** ABT-493 and ABT-530 exposure were not affected by rilpivirine or raltegravir. ABT-493 + ABT-530 increased rilpivirine and raltegravir exposures. Consistent with rilpivirine and raltegravir label recommendations for other drugs with similar magnitude of exposure increase, no dose adjustment is needed when ABT-493 and ABT-530 are coadministered with rilpivirine or raltegravir.

**Rilpivirine DDI Study Design:**

	Period 1 Days 1-7	Period 2 Days 1-14
Cohort I N=12	ABT-493 300 mg QD + ABT-530 120 mg QD	
	Rilpivirine 25 mg QD	
	Period 1 Days 1-14	Period 2 Days 1-7
Cohort II N=12	ABT-493 300 mg QD + ABT-530 120 mg QD	
	Rilpivirine 25 mg QD	

**Raltegravir DDI Study Design:**

	Period 1 Days 1-3	Day 1	Period 2 Days 2-7	Days 8-10
N=12	ABT-493 300 mg QD + ABT-530 120 mg QD			
	Raltegravir 400 mg BID			Raltegravir 400 mg BID

**454 Pharmacokinetics of Dolutegravir and Rilpivirine in Combination With SMV and SOF**

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**Background:** Anti-HCV treatment with direct-acting antivirals (DAAs)-based regimens is a priority in HIV/HCV co-infected patients (pts) with advanced liver disease. We evaluated plasma through concentration of dolutegravir and rilpivirine (DTG-RPV) used in combination with simeprevir plus sofosbuvir (SMV-SOF) in HIV/HCV co-infected pts with advanced liver disease.

**Methods:** Pts infected with HCV and HIV-1, with advanced liver disease (Metavir F3-F4), on antiretroviral therapy (ART), HIV-RNA <50 cps/mL at the start of anti-HCV treatment, treated for HCV with SMV plus SOF for 12 weeks and on treatment for HIV with DTG-RPV-including regimens were considered. Immuno-virological data were recorded at DAAs start (baseline, BL), week 4, week 12 and at post-treatment (PT) week 4, PT week 12. Plasma DTG, RPV, SOF, SMV trough concentrations were evaluated with a HPLC validated method at BL (only DTG, RPV were tested) and week 4. Results were reported as median (IQR) or frequency (%). Geometric means were also calculated to summarize through concentration values. Wilcoxon signed rank test applied to assess significant changes in plasma through concentration since BL.

**Results:** Eleven pts evaluated: 73% males, age 53 (52-54) yrs, duration of HIV infection 23 (22-25) yrs, nadir CD4+ 181 (109-326) cells/μL, CD4+ 515 (357-722) cells/μL, HCV-RNA 6.1 (5.6-6.4) log<sub>10</sub> cps/mL; HCV genotype (GT) was: 9 (82%) GT1a, 1 (9%) GT1b, 1 (9%) GT4a; 10 (91%) pts had Child-Pugh (CTP) A, 1 (9%) had CTP B; liver stiffness was 18.9 (12.2-33.8) KPa. Ribavirin was used in 8 (73%) pts. Main (>10%) ART regimens were: 5 (45%) DTG/RPV, 4 (36%) DTG/RPV/3TC. All pts completed anti-HCV treatment: 7 (64%) achieved HCV-RNA <12 UI/ml by week 4 and 11 (100%) at the end of treatment.

Geometric means (95%CI) of C<sub>trough</sub> at BL and w4 were: for DTG 1148 (550-2455) vs 1413 (776-2630) ng/mL (p=0.301), for RPV 115 (63-209) vs 141 (85-240) ng/mL (p=0.203).

Geometric means (95%CI) of SOF and SMV C<sub>trough</sub> at w4 were 380 (191-741) ng/ml and 2570 (1445-4571) ng/ml, respectively.

**Conclusions:** This is the first study evaluating pharmacokinetics of DTG and RPV-based antiretroviral regimen in patients treated with SOF plus SMV. DTG and RPV plasma exposure was not affected by concomitant DAAs and SOF and SMV plasma concentration were in the expected range. This antiretroviral regimen deserves further evaluations in larger samples of HIV/HCV co-infected subjects treated with DAAs.

**455 Effect of Direct Acting Antivirals on the Pharmacokinetics of Calcineurin Inhibitors**

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**Background:** Severe hepatitis C virus (HCV) recurrence affects post-transplant survival in HCV infected patients. We describe the effect of sofosbuvir-based anti-HCV therapy on disposition of anti-calcineurin immunosuppressive drugs.

**Methods:** Liver transplant patients (pts) with severe HCV recurrence who signed the informed consent were included in the ANRS C023 CUPILT cohort and their characteristics recorded. Immunosuppressive therapy backbone was either tacrolimus (TAC) or cyclosporine (CyA). They were treated according to HCV genotype with 2<sup>nd</sup> generation direct acting antivirals (DAA) including sofosbuvir (SOF) with either daclatasvir (DCV) +/- ribavirin (RBV) or simeprevir (SMV) at standard dosing. Predose blood samples were drawn before DAA initiation (D0) and at week4 (W4) after DAA initiation. Trough concentrations (C<sub>t</sub>) of TAC or CyA at steady state were measured by quality controls validated assays (immunoassay or LC-MS/MS). Apparent clearance (Cl/F) of TAC or CyA was estimated from the ratio of the dose per intake over the trough concentration (as a surrogate of average concentration at steady state) times the time interval between 2 doses Cl/F = D/(DDI \* C<sub>t</sub>). W4/D0 geometric mean ratio (GMR) and 2-sided 90% CIs (CI90) were calculated for Cl/F and compared to the 0.80-1.25 bioequivalence range. Unless otherwise indicated, results are medians and ranges.

**Results:** Twenty three pts were on TAC and 12 on CyA. Characteristics at inclusion were age 57years (43, 81), weight 72kg (45, 106) and MELD score 9 (0, 26). HCV genotypes were G1 (25 pts), G2 (2 pts) and G4 (8 pts). On the 3 pts on antiretrovirals, one was on efavirenz (EFV) and 2 on raltegravir-based regimen combined with 2 nucleoside analogs. Pt on EFV has the highest TAC Cl/F. Creatinine clearance (MDRD equation) remained unchanged at W4 compared to D0. Cl/F of TAC and CyA at D0 and W4 are shown in the table below.

**Conclusions:** Despite wide interindividual variability on TAC or CyA Cl/F, our data show that most liver transplant pts have an increased Cl/F on DAAs, statistically significant for TAC, leading to a decrease in concentrations and likely warranting an increased dosing. All these liver transplant pts should be monitored closely at the time of DAA initiation and during follow-up. These results need to be confirmed in a larger cohort of pts as well as the identification of factors explaining such drug-drug interaction.

	TAC					CyA				
	n pts	Median (range)		W4/D0		n pts	Median (range)		W4/D0	
		CI/F D0 (L/h)	CI/F W4 (L/h)	GMR	CI90		CI/F D0 (L/h)	CI/F W4 (L/h)	GMR	CI90
SOF/DCV/RBV	12	7 (3, 123)	11 (5, 116)	1.69	1.20, 2.38	7	44 (34, 91)	62 (30, 69)	1.05	0.84, 1.30
SOF/DCV	10	17 (5, 56)	20 (10, 75)	1.16	0.85, 1.60	1	63	91	-	-
Total SOF/DCV+/-RBV	22	9 (3, 123)	15 (5, 116)	1.43	1.12, 1.81	8	48 (34, 91)	64 (30, 91)	1.09	0.89, 1.33
SOF/SMV	1	5	9	-	-	4	69 (56, 97)	68 (37, 161)	1.01	0.68, 1.51

#### 456 Multiple-Dose Treatment With Rifabutin Reduces the Exposure of Doravirine

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**Background:** Doravirine is a novel, potent, HIV-1 non-nucleoside reverse transcriptase inhibitor that is primarily metabolized by oxidation via CYP3A4. A previous clinical trial demonstrated that co-administration of doravirine with multiple dose (MD) rifampin, a strong CYP3A4 inducer, resulted in decreased doravirine exposure. This study assessed the impact of MD rifabutin administration, a moderate CYP3A4 inducer, on the pharmacokinetics (PK) of doravirine.

**Methods:** This was an open-label, 2-period, fixed-sequence study in healthy adult subjects. In Period 1 (P1), a single dose (SD) of 100 mg doravirine was administered on Day 1. In Period 2 (P2), following a 7-day washout, rifabutin 300 mg was administered once daily for 16 days and co-administered with 100 mg doravirine on Day 14. Blood samples to measure doravirine concentrations were collected through 72 hours post-dose in P1 and P2.

**Results:** Eighteen subjects (15 male and 3 female) were enrolled. Following co administration with MD rifabutin, doravirine C<sub>max</sub> was not affected; however, exposure and C<sub>24</sub> values were reduced. The geometric mean ratios (90% confidence intervals) [doravirine + MD rifabutin/doravirine] for C<sub>max</sub>, AUC<sub>0-∞</sub>, and C<sub>24hr</sub> were 0.99 (0.85, 1.15), 0.50 (0.45, 0.55), and 0.32 (0.28, 0.35), respectively. There were no serious adverse experiences (AEs) and most AEs were mild in intensity. Three (16.7%) and 16 (94.1%) subjects reported at least 1 AE in P1 and P2, respectively. Most AEs in P2 were related to rifabutin treatment. The most common AEs were headache (1 subject in P1 and 8 subjects in P2) and fever (7 subjects in P2). Six subjects discontinued (DC'd) the study due to AEs: in P1, 1 subject was DC'd due to elevated GGT; in P2, prior to receiving doravirine, 5 subjects were DC'd due to fever with or without other flu-like signs and symptoms.

**Conclusions:** Doravirine was generally well tolerated when administered alone or with rifabutin. Multiple dosing of rifabutin significantly reduced doravirine AUC<sub>0-∞</sub> and C<sub>24</sub> via CYP3A4 induction. However, increasing the dose of doravirine above the clinical dose of 100 mg may mitigate the interaction such that rifabutin, and other moderate CYP3A4 inducers, may be co-administered with doravirine.

#### 457 Pharmacokinetics of Anti-TB Drugs in HIV-Infected and -Uninfected Children With TB

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**Background:** The diagnosis and treatment of children with tuberculosis (TB) is increasingly becoming a priority for national TB control programmes. Not many studies have investigated the impact of HIV infection on anti-TB drug concentrations in children. This study compares the pharmacokinetics of rifampicin (RMP), isoniazid (INH) and pyrazinamide (PZA) and nutritional status between HIV-infected and uninfected children with TB and correlate drug pharmacokinetics with TB treatment outcomes.

**Methods:** HIV-uninfected (n=84) and HIV-infected (n=77) children with TB receiving standard thrice weekly treatment, were recruited from six hospitals in India. All consecutive children attending the TB clinics of these hospitals during 2010 - 2013, meeting the study criteria and willing to participate in the study were recruited. Children had received anti-TB treatment (ATT) according to the Indian National Program guidelines for at least two weeks. Semi-intensive pharmacokinetic sampling was performed during intensive phase of ATT after directly observed administration of drugs. Drug concentrations were measured by high performance liquid chromatography. INH acetylase status was determined using saliva. Nutritional assessment was done by computing z scores based on the child's height, weight, age and gender. Children were followed up and treatment outcomes noted.

**Results:** Children with HIV & TB had significantly lower RMP peak concentration (C<sub>max</sub>) (2.6 vs. 5.1 ug/ml; p<0.001) and exposure (AUC<sub>0-8</sub>) (10.4 vs. 23.4 ug/ml.h; p<0.001) than those with TB. Among HIV-infected children, a significantly higher proportion had stunting (77% vs 29%; p<0.001) and underweight (73% vs 38%; p<0.001) compared to children with TB. Combining both groups, RMP C<sub>max</sub> (p=0.001; AOR=1.437; 95% CI:1.157 - 1.784) and PZA C<sub>max</sub> (p=0.027; AOR = 1.041; 95% CI: 1.005 - 1.079) significantly influenced treatment outcome.

**Conclusions:** HIV infection was associated with lower C<sub>max</sub> of RMP and INH and AUC<sub>0-8</sub> of RMP. Over 90% of children in both groups had sub-therapeutic RMP C<sub>max</sub>. C<sub>max</sub> of RMP and PZA significantly influenced TB treatment outcome in children with TB. This study, for the first time, has compared pharmacokinetic and nutritional data between HIV infected and uninfected children with TB. The findings have important clinical implications and suggest the need to increase anti-TB drug doses in children with HIV & TB.

#### 458 Optimal Use of Efavirenz in HIV+/TB+ Coinfected Children Aged 3 to 24 months

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**Background:** Poor tolerability and interactions between ARVs and rifampin-containing anti-TB therapy (ATT) limit treatment options for HIV+/TB+ children. EFV has minimal interactions with rifampin, making it a good option for HIV/TB co-infection but PK variability and formulation issues have precluded dosing guidelines for young children. We

sought to determine an EFV dose and study its safety and efficacy in HIV+ children from TB-endemic countries. Here we present the data for HIV+/TB+ infants 3 months to two years.

**Methods:** IMPAACT P1070 is a prospective, Phase I/II open-label 24 wk trial of EFV in HIV+ children 3–<36 months with or without TB. Using CYP2B6 genotype-directed, weight-band dosing subjects initiated EFV & 2 NRTIs at EFV doses of ~65mg/kg as opened capsules once-daily. These dosages are ~30% greater than those used in TB-uninfected infants (results not shown). CYP2B6 genotype was drawn at baseline and intensive PK was performed at wk 2. Doses were adjusted if outside the target AUC (35–180 mcg\*hr/mL). HIV-1 RNA and toxicity labs were drawn every 4–8 wks.

**Results:** 8 subjects 3 to <24 months receiving ATT (5 males, med age 13 months (10–17.5 IQR)) enrolled with median follow up of 24wk (18–25 IQR). All 8 were CYP2B6 GG/GT and initiated EFV at a median dose of 64.9 mg/kg (57.7–67.7 IQR). The median EFV AUC was 92.87 mcg\*h/mL (40.9–160.14 IQR). 6/8 subjects met the wk 2 AUC target, 1 was below (due to non-adherence) and 1 above (achieved the target range after dose reduction). Possibly treatment-related toxicities  $\geq$  gr 3 occurred in 1 subject (gr 4 SGPT/SGOT) at wk24. This resolved after discontinuing EFV, TMP/SMX and ATT and did not recur when EFV was restarted. 2 subjects discontinued EFV before wk24; 1 due to difficulty administering EFV and 1 for poor adherence. Baseline median RNA was 5.9 log<sub>10</sub> copies(cps) /ml (5.5–6.1 IQR) and 8/8 (100% ITT; 95%CI 63–100%) achieved either  $\geq$  1 log<sub>10</sub> drop from baseline RNA or <400 cps/ml by week 8; 5/6 subjects who completed 24 wks had RNA <400 cps/ml and 1/6 had 404 cps/ml.

**Conclusions:** Amplified dosing (~65.9 mg/kg) in co-infected HIV+/TB+ children with CYP2B6 GG/GT genotype produced therapeutic EFV concentrations in most children <2 yrs with excellent safety and virological outcomes. However, a lower dose is likely to be needed in this age group taking ATT with slow CYP2B6 metabolism, as is required in young children without ATT. Optimal exposure of EFV can be achieved in HIV+/TB+ children <2yrs but requires pretreatment genotyping.

#### 459 Interaction of Darunavir/Ritonavir and Darunavir/Cobicistat With Rifampicin In Vitro

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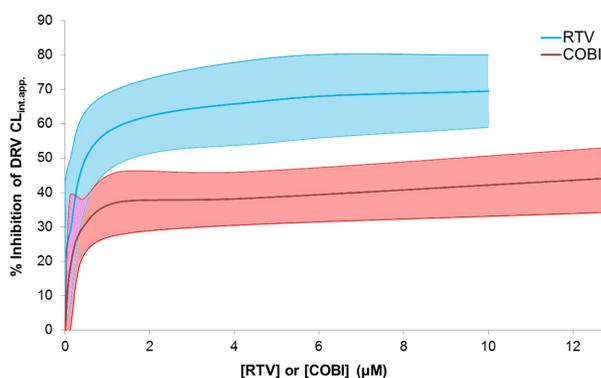
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**Background:** Clinical management of HIV patients co-infected with tuberculosis (TB) is hampered by drug-drug interactions (DDIs) that limit therapeutic options. Rifampicin (RIF), an important component of anti-TB treatment regimens, is a strong inducer of key metabolic enzymes, and thus can negatively affect antiretroviral bioavailability, clearance and efficacy. The aim of this study was to quantify DDIs between RIF and cobicistat (COBI)-boosted darunavir (DRV), and to compare this with DDIs between RIF and ritonavir (RTV)-boosted DRV (DRV/r) using an *in vitro* approach.

**Methods:** Cryopreserved primary human hepatocytes plated on collagen-coated cell culture plates were overlaid with Geltrex™ matrix and were treated with RIF (10  $\mu$ M) alone, or together with RTV (0.1–10  $\mu$ M) or COBI (0.13–12.76  $\mu$ M) in Williams' Medium E incubation medium, or were left untreated. Test compounds were replenished each day for a total of 72 hours, after which cells were treated with test compounds together with DRV (5  $\mu$ M) for one hour. Resultant DRV concentrations were quantified using HPLC-UV. Apparent intrinsic clearance ( $CL_{int,app}$ ) of DRV was calculated, and expressed as the mean  $\pm$  SD ( $\mu$ l/min/10<sup>6</sup> hepatocytes) of a total of three biological replicates, using cells obtained from three separate donors.

**Results:** Under control conditions where cells treated with DRV alone,  $DRV CL_{int,app}$  was  $13.2 \pm 1.5$   $\mu$ l/min, while following incubation with 10  $\mu$ M RIF,  $DRV CL_{int,app}$  increased to  $20.5 \pm 4.7$   $\mu$ l/min (+55% compared to control). Inclusion of 1  $\mu$ M RTV, or 1.28  $\mu$ M COBI, was sufficient to overcome the effect of 10  $\mu$ M RIF, reducing  $DRV CL_{int,app}$  by -15% and -3% compared to control, respectively. Using regression analysis, log<sub>10</sub> RTV and COBI concentrations were found to be associated with percentage inhibition of  $DRV CL_{int,app}$  ( $\beta = 20.7$ ,  $p = 0.001$  and  $\beta = 11.3$ ,  $p = 0.001$ , respectively; **Figure 1**).

**Conclusions:** DDIs between RIF and both DRV/r and DRV/COBI were quantified using an *in vitro* human hepatocyte model. RIF-induced elevations in  $DRV CL_{int,app}$  were overcome by co-incubation with RTV or COBI. RTV- and COBI-mediated attenuation of RIF-enhanced  $DRV CL_{int,app}$  occurred in a concentration-dependent manner, but RTV reversed RIF induction more strongly than COBI. These results provide an insight into the relative effect of RTV and COBI as pharmacoenhancers in the presence of RIF, and can be used to inform pharmacokinetic models for optimising regimens in patients receiving concurrent antiretroviral and anti-TB therapy.



**Figure 1:** Line graph showing percentage inhibition of the apparent intrinsic clearance of darunavir ( $DRV CL_{int,app}$ ) in primary human hepatocytes following incubation with ritonavir (RTV; 0.1–10  $\mu$ M), or cobicistat (COBI; 0.13–12.76  $\mu$ M) in combination with rifampicin (RIF; 10  $\mu$ M) for 72 hours, relative to the  $DRV CL_{int,app}$  observed when cells were incubated with 10  $\mu$ M RIF alone for 72 hours. Bold lines show mean and thin lines represent standard deviation.

#### 460 HIV-1 Attachment Inhibitor Prodrug BMS-663068: PK Assessment With Rosuvastatin

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**Background:** BMS-663068 is a prodrug of BMS-626529, a first-in-class attachment inhibitor that binds directly to HIV-1 gp120, preventing initial viral attachment and entry into host CD4+ T cells. In the HIV-infected population, hypercholesterolemia is a prevalent comorbidity and increases the risk of cardiovascular disease. Thus, BMS-663068 may be coadministered with statins. *In vitro*, BMS-626529 inhibits proteins that transport statins, specifically OATP1B1, OATP1B3, and BCRP ( $IC_{50}$  values of 32 $\mu$ M, 16.0 $\mu$ M, and 12.4 $\mu$ M, respectively), suggesting that BMS-626529 has the potential to affect the pharmacokinetics (PK) of statins. This study assessed the effect of multiple doses of BMS-663068 on the PK of rosuvastatin, a probe substrate for OATP and BCRP.

**Methods:** AI438048 was a Phase 1, open-label, single-sequence study performed in 18 healthy subjects, who received a single dose (SD) of rosuvastatin 10mg on Day 1 (Treatment A) followed by a 3-day washout, BMS-663068 600mg twice daily (BID) on Days 5–8 (Treatment B), rosuvastatin 10mg SD + BMS-663068 600mg BID on Day 9, and BMS-663068 600mg BID on Days 10–12 (Treatment C). Serial blood samples were collected for 96 hours post-dose on Days 1 and 9. Plasma concentrations were quantified by validated LC/MS/MS methods. Geometric mean ratios and 90% confidence intervals were derived for rosuvastatin PK parameters using linear mixed-effects models. Adverse events (AEs) were monitored throughout the study.

**Results:** After coadministration with BMS-663068, rosuvastatin peak ( $C_{max}$ ) and total (AUC[INF]) exposures increased by 78% and 69%, respectively, vs exposures observed with rosuvastatin alone (Table). Rosuvastatin terminal plasma half-life and time of peak exposure were unaffected by BMS-663068 coadministration. All treatments were generally well tolerated, with no deaths, serious AEs or discontinuations due to AEs. Overall, 6 subjects (33.3%) reported  $\geq 1$  AE, which were all mild to moderate, and the majority had resolved by study end.

**Conclusions:** BMS-663068 coadministration increased rosuvastatin exposure vs rosuvastatin administered alone ( $C_{max}$  and AUC[INF] increased by 78% and 69%, respectively). Multiple BMS-663068 doses were generally well tolerated when coadministered with rosuvastatin 10mg. *In vitro* and clinical data suggest that BMS-663068 can be coadministered with statins that are substrates of OATP or BCRP, but dose adjustment of certain statins may be required.

**Table: Statistical analysis results of rosuvastatin PK parameters with or without BMS-663068**

PK parameter	Treatment and comparison	N	Adjusted geometric mean	90% CI
$C_{max}$ (ng/mL)	A	18	2.43	1.92, 3.07
	C	18	4.33	3.58, 5.23
	<b>C vs A</b>	<b>18</b>	<b>1.784</b>	<b>1.524, 2.088</b>
AUC (0–T) (ng*h/mL)	A	18	28.2	23.3, 34.2
	C	18	48.3	41.3, 56.5
	<b>C vs A</b>	<b>18</b>	<b>1.713</b>	<b>1.476, 1.989</b>
AUC (INF) (ng*h/mL)	A	14	33.1	27.0, 40.5
	C	17	56.0	49.2, 63.8
	<b>C vs A</b>	<b>13</b>	<b>1.693</b>	<b>1.443, 1.985</b>

#### 461LB Early Evidence of Antiviral Activity and Safety of ABX464 in HIV Treatment-Naïve Patients

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**Background:** ABX464 is a first-in-class antiviral drug candidate for the treatment of patients with HIV-infection. It is an orally available small molecule that blocks HIV replication through an entirely novel mechanism, inhibition of Rev activity. Preclinical data in humanized mice showed that ABX 464 monotherapy had an antiviral effect which was sustained after treatment interruption ( Campos et al, Retrovirology 2015 12:30)

A prior food-effect study demonstrated a 3-fold increase in parent drug exposure when administered with food without a significant impact on the active glucuronide metabolite. **Methods:** The objective of this study was to evaluate the safety of ABX-464 at ascending doses versus placebo in HIV-infected treatment-naïve patients. Patients were randomized into successive cohorts of 8 patients where 6 received 14- or 21 days of ABX 464 and 2 placebo.

Patients from Mauritius and Thailand were included in the study after confirmation of HIV infection and no history of prior antiretroviral therapy.

At day 0, patients received the first dose of ABX-464/ placebo in a once daily schedule. Safety assessments and laboratory parameters were recorded throughout the study.

After completion of each cohort, a DSMB reviewed safety data and recommended whether the next cohort be initiated at a higher dose. Successive cohorts received 25, 50, 75, 100 and 150 mg QD. The 25, 50 and 100 mg.cohorts took drug fasting for 21 days, the 75 and 150 mg cohorts took drug with food for 14 days.

**Results:** Safety and Tolerability : The main adverse events noted were nausea, vomiting and headache. All adverse events were grade 1 or 2 and all patients completed at least 14 days of treatment.

Viral load reduction > 0.5 log was observed in 1/6 patients in the 75 mg cohort, 2/6 patients in the 100 mg cohort and 4/6 patients in the 150 mg cohort; there were no significant viral load changes in the 6 placebo patients from these cohorts.

**Conclusions:** ABX 464 was well tolerated in this first study in HIV infected patients.

ABX 464 monotherapy showed early antiviral activity in HIV-infected treatment naïve patients. These results warrant the further planned development of this novel acting antiretroviral drug.

#### 462 A Quantitative Model of ART Efficacy Explains the Clinical Success of Dolutegravir

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**Background:** The clinical success of antiretroviral drug combinations is highly correlated with the results of *in vitro* drug efficacy assays, except in the case of regimens containing first-generation integrase strand transfer inhibitors (INSTIs). The second-generation INSTI dolutegravir (DTG) has enjoyed enormous clinical success in the two years since its FDA approval, but its pharmacodynamic properties in combination with other antiretroviral drugs and against HIV-1 containing INSTI resistance mutations have not been thoroughly evaluated by quantitative *in vitro* models.

**Methods:** We used an *in vitro* infectivity assay to quantify the efficacy of DTG in combination with nucleoside analog reverse transcriptase inhibitor (NRTI) pairs abacavir/lamivudine (ABC/3TC) and tenofovir/emtricitabine (TDF/FTC). We compared empirical measurements of antiretroviral activity to the Bliss Independence and Loewe Additivity models of combined drug efficacy to calculate the degree of independence for the interactions between DTG and each NRTI or NRTI pair. From these results, we calculated the instantaneous inhibitory potential (IIP) of DTG-containing regimens at clinical concentrations. We also measured pharmacodynamic properties of DTG against HIV-1 containing INSTI resistance mutations.

**Results:** The combined efficacies of DTG and NRTIs follow the model of Bliss Independence for every combination tested except for DTG/TDF, which demonstrates synergy. The combination DTG/ABC/3TC follows the model of Bliss Independence, while DTG/TDF/FTC demonstrates synergy. Importantly, both three-drug combinations have average clinical IIP values of >6.5; at clinical concentrations, DTG-containing regimens reduce the number of productive infection events per dosing interval by an average of >6.5 logs.

The efficacy of DTG was quantified against a panel of seven viruses containing one or two InSTI resistance mutations. In contrast with other drug classes but consistent with first-generation InSTIs, mutations had less effect on the slope of the DTG dose-response curve than on the  $IC_{50}$ . This result was true even for viruses containing the DTG-specific R263K resistance mutation.

**Conclusions:** A quantitative *in vitro* efficacy model that correlates with clinical efficacy for all drug classes except first-generation InSTIs is consistent with the clinical efficacy of the second-generation InSTI DTG. In combination with previously published results, this model may be used to predict new DTG-containing regimens with high clinical efficacy.

#### 463 HIV-1 Transcription Inhibition by New p300 Inhibitors

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**Background:** Understanding the mechanism of HIV-1 transcription is key for developing a new class of antiviral drugs. HIV-1 Tat is an essential viral protein that transactivates HIV-1 transcription by binding to the TAR region of HIV-1 mRNA. p300-mediated acetylation of Tat is required for transactivation of the HIV-1 long terminal repeat (LTR). Full activation of NF- $\kappa$ B also requires p300-mediated acetylation. Since salicylate (SAL) is known to inhibit NF- $\kappa$ B and HIV-1 transcription, we hypothesized that SAL might inhibit p300 acetyltransferase activity.

**Methods:** First, we tested SAL in *in vitro* HAT assays and checked histone acetylation profile in HEK293T cells upon SAL treatment. Next, we did a secondary screen of FDA-approved chemical compounds that contain structures similar to SAL. Finally, we tested whether SAL and related FDA approved drugs inhibits HIV transcription and infection using HIV-1 LTR reporter assay and single round infection assays.

**Results:** We found that SAL inhibits p300 ( $IC_{50}$  of 10.2 mM) and other related acetyltransferase CBP ( $IC_{50}$  = 5.7 mM), but not PCAF and GCN5 in *in vitro* HAT assays. Kinetic analysis identified that SAL competes with acetyl CoA but not with histones. SAL suppressed the acetylation of histones residues H2A<sub>K5/R69</sub>, H2B<sub>K12/K15</sub>, and H3<sub>K56</sub> in HEK293T cells.  $IC_{50}$  for SAL-mediated inhibition of H2B<sub>K12/K15</sub> acetylation (4.8 mM) was close to drug plasma concentrations measured in humans following oral SAL administration (1-3 mM). A secondary screen of FDA-approved chemical compounds that contain structures similar to SAL identified a more potent p300 inhibitor, diflunisal (DFN,  $IC_{50}$  = 996  $\mu$ M), which is an old anti-inflammatory drug. We tested these drugs using HIV-1 LTR reporter assays. Co-transfection of both Tat and p300 synergistically activated the HIV-1 promoter, however, both SAL and DFN inhibited synergistic activation of HIV-1 transcription at lower  $IC_{50}$  than *in vitro* HAT inhibition ( $IC_{50}$  = 2.2 mM for SAL and 80  $\mu$ M for diflunisal). SAL and DFN inhibited HIV-1 infection at  $IC_{50}$  1.09 mM for SAL and 154  $\mu$ M for DFN measured by single round infection assays using vsv-g pseudotyped luciferase reporter HIV-1 and activated CD4 positive T-cells.

**Conclusions:** These results revealed novel epigenetic therapeutic targets for SAL, CBP and p300, and the precise mechanism how SAL and DFN inhibit HIV-1 transcription. These drugs could be used to suppress HIV-1 transcription and to treat other inflammatory pathologies including diabetes, cancer and neurodegenerative disorder.

#### 464 Maturation Inhibitor BMS-955176: Activity Against PI-Resistant Clinical Isolates

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**Background:** BMS-955176 is a second-generation HIV-1 maturation inhibitor (MI). HIV-1 isolates resistant to approved protease inhibitors (PI) harbor substitutions in both protease (Pr) (1<sup>o</sup>) and the substrate of Pr, Gag (2<sup>o</sup>). Such isolates reportedly have a higher frequency of Gag substitutions which could potentially exhibit cross-resistance to MIs. To assess the susceptibility of BMS-955176 to PI-resistant (PI<sup>R</sup>) viruses with Gag substitutions, we evaluated the virologic profile of a cohort of pre- and post-treatment (PT) clinical isolates from subjects who experienced PI therapy failure.

**Methods:** Longitudinal isolates (n=21) from 15 PI-treated subjects had a median (range) of 6 (2.3-11.7) years on PI therapy. All PT samples had major PI resistance-associated mutations (RAMs) in Pr and 17/21 had 2<sup>o</sup> changes in Gag associated with PI resistance (at positions 128,431,436,437,449,452,453). Phenotypic susceptibilities (fold-change- $IC_{50}$ , FC) were determined using either the PhenoSense Gag/Pr (PS) assay (Monogram Biosciences) or BMS gag/Pr pseudotype single (SC) and multiple cycle (MC) antiviral assays. Fold changes from baseline (FCFB = FC post/FC pre) < 3 were considered to be within the no-effect level based on current assay reproducibility data in control samples. Seven non-longitudinal highly PI<sup>R</sup> viruses containing multiple major and minor PI RAMs were also evaluated for BMS-955176 susceptibility.

**Results:** 19/21 PT samples from 15 PI-treated subjects had FCFBs within the no-effect level. The median (range) FCFB was 0.83 (0.05-27.4) [PS assay (11 subjects)] and 1.5 (1.0-2.2) [SC assay (4 subjects)]. The two PT samples with PS FCFB >3 were retested using the SC and MC assays. One of these two samples, with PS FCFB=27.4, was within the no-effect level of the SC assay with FCFB median (range) of 2.2 (1.9-2.5), while the other sample showed variable changes in susceptibility with SC FCFB median (range) =4.2 (2.3-6.1). Neither sample showed meaningful susceptibility changes in the MC assay (FCFB = 2.1 and 1.5, respectively). 2<sup>o</sup> Gag changes were not associated with a greater median BMS-955176 FCFB. A panel of 7 highly PI<sup>R</sup> non-longitudinal viruses were all susceptible (FC range 0.16-0.68) to BMS-955176.

**Conclusions:** BMS-955176 maintains antiviral activity against PI<sup>R</sup> isolates with emergent Pr and/or Gag mutations. This finding supports continued development of the second-generation MI BMS-955176 in treatment-experienced subjects with or without prior PI therapy.

#### 465 Resistance Pathways for Potent and Broadly Active HIV-1 Maturation Inhibitors

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**Background:** A betulinic acid-based compound, bevirimat (BVM), the first-in-class HIV-1 maturation inhibitor, acts by blocking a late step in protease-mediated Gag processing: the cleavage of the capsid-spacer peptide 1 (CA-SP1) intermediate to mature CA. BVM was shown to be safe and effective in reducing viral loads in HIV-1-infected patients. However, single-amino-acid polymorphisms in the SP1 region of Gag reduced HIV-1 susceptibility to BVM in patients.

**Methods:** We carried out an extensive medicinal chemistry campaign to identify BVM derivatives that demonstrate increased potency against consensus clade B strains of HIV-1 and are active against primary isolates with polymorphisms in SP1. Compound activity was tested in assays that measure CA-SP1 processing and virus replication kinetics. Selection experiments were performed to identify mutations that confer resistance to these novel compounds and a variety of virological, structural, and molecular approaches were applied to elucidate the mechanism of resistance for each mutant. To evaluate the effect of Gag polymorphisms and resistance mutations on the kinetics of Gag processing, pulse-chase radiolabeling assays were performed.

**Results:** We identified a set of BVM derivatives that are more potent than BVM against WT HIV-1 and show robust antiviral activity against SP1 polymorphic strains and clinical isolates. The best of these analogs retain significant activity against BVM-resistant mutants. Selection experiments identified an Ala-to-Val mutation at SP1 residue 1 (SP1-A1V). In addition, we also selected for the mutation CA-P157A, located in the major homology region (MHR) of CA. Remarkably, the P157A mutant was resistant to not only BVM and the second-generation BVM analogs but also to the structurally distinct maturation inhibitor PF-46396. Pulse-chase data demonstrate that CA-SP1 processing kinetics for P157A are similar to those of the WT. Analysis of the HIV-1 database reveals that Ala1 of SP1 and Pro157 of CA are conserved in ~99.95% of available sequences.

**Conclusions:** This study identifies a panel of BVM derivatives that display marked improvements relative to BVM in antiviral potency and breadth of activity. The characterization of resistant mutants provides novel insights into the structure of the maturation inhibitor-binding site and the role of SP1 and the CA MHR in virus assembly and maturation. This study will support ongoing clinical development of this class of inhibitors.

**466 Maturation Inhibitor BMS-955176: Integrated Model of Polymorphic Antiviral Responses**

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**Background:** HIV-1 maturation inhibitors (MIs) disrupt the final step in the HIV-1 protease-mediated cleavage of the Gag polyprotein, between capsid (CA) and spacer peptide 1 (SP1), leading to the production of non-infectious virus. BMS-955176 is a second generation MI with improved antiviral activity toward polymorphic Gag viruses. This study was conducted to understand the underlying mechanistic reasons for improvements to polymorphic coverage.

**Methods:** Antiviral activities of wild type (wt) and Gag polymorphic viruses were studied using single and multiple cycle replication formats. Gag CA/SP1 cleavage kinetics of wt and polymorphic virus-like particles (VLP) were determined by a novel LC/MS assay. A radiolabel binding assay was used to determine VLP/MI affinities and off rates. The cleavage, binding and antiviral data was used to create a integrated model for calculating rates of CA/SP1 cleavage.

**Results:** In antiviral assays, early generation MIs (EGMIs) do not achieve maximal percent inhibition (100% MPI) of certain Gag polymorphs, even at saturating concentrations, resulting in low levels of breakthrough virus (partial antagonism). BMS-955176 achieves higher MPIs of polymorphs, which is correlated with lower  $EC_{50}$  values. Mechanistic studies show that more rapid CA/SP1 cleavage of polymorphs (2-10-fold vs. wt) is responsible for reduced viral sensitivity to EGMIs. Enzymatically, *in vitro* inhibition of wt CA/SP1 cleavage by the EGMI BVM is lost at longer times, while inhibition by BMS-955176 persists. The origin of antiviral and enzymatic improvements by BMS-955176 lies in its higher affinity to Gag polymorphs. Biochemical (CA/SP1 cleavage rates, MI-specific Gag affinities) and virological (MPI) data were combined to create an integrated kinetic model to calculate rate reductions of CA/SP1 cleavage by MIs. These predictions are in accord with both preclinical antiviral potency and clinical viral load reductions as a function of MI and Gag polymorph.

**Conclusions:** The improved polymorphic antiviral activity and biochemical profile of BMS-955176 over EGMIs is a consequence of its higher affinity/slower rate of dissociation from its Gag target. Modeling calculates MI-induced reductions in the rates of CA/SP1 Gag cleavage that correlate well with preclinical antiviral potencies and MI monotherapy clinical response data. These findings offer new insights into MI activity and mechanism and may prove useful to a further understanding of clinical responses to MIs.

**467 Reduction of SIV-Mediated Immune Activation by p38 MAPK In Vivo Inhibition**

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**Background:** Differences in immune activation have been identified as the most significant difference between AIDS-susceptible and resistant species. p38 MAPK, activated in HIV and SIV infection, is key to induction of Interferon-stimulated genes (ISG) and cytokine-mediated inflammation and is associated with some of the pathology produced by HIV and SIV infection in AIDS susceptible primates. As small molecule p38 MAPK inhibitors are currently tested in human trials for other inflammatory diseases, we evaluated the effects of treating SIV-infected macaques with a p38 inhibitor in conjunction with ART.

**Methods:** Rhesus macaques were infected with SIV<sub>mac251</sub> and after 6 weeks ART and p38 MAPK inhibitor treatment with compound PH-797804 was initiated in one group of animals. Additional groups included ART treatment alone, p38 inhibitor alone, and naive controls. As primary end points we evaluated differences in expression of surface and intracellular molecules linked to immune activation and intracellular inflammatory cytokine expression in cell subpopulations of blood, lymph node and intestinal tissue, and plasma levels of inflammatory cytokines. As secondary end points, we evaluated the effects that treatments had on viral loads, preservation of central memory CD4+ T cells and anti-SIV immune responses. We also evaluate the treatment impact on protein levels of selected ISG.

**Results:** ART reduced viremia to 70-80 copies/ml. The p38 inhibitor did not further reduced the viremia, did not affect negatively anti-SIV immune responses and had no side effects. The p38 inhibitor treatment by itself had no significant effect on immune activation. When combined with ART, numerous immune activation markers were significantly reduced compared to the ART alone group. CD38/HLA-DR on Ki67 percentages in blood, lymph node and rectal CD4+ and CD8+ T cells and percentages of IL-6, IL-8, IFN- $\gamma$  and IFN- $\alpha$  producing cells were all significantly reduced. IRF7, pSTAT1 and IP-10 protein accumulation was also reduced in APC. Significant preservation of CD4+/IL22+ and CD4+/IL-17+ T-cells in PBMC, rectal and lymph node mononuclear cells and of central memory CD4+ T cells in blood was also observed in the ART+p38 inhibitor group compared to the ART group.

**Conclusions:** The p38 MAPK inhibitor used in this studies, already in clinical trials for other inflammatory diseases, significantly reduced immune activation during ART treatment and could be a desirable addition to antiretroviral therapy.

**468 PBMC From Patients on Chronic Treatment With Dasatinib Are Resistant to HIV Infection**

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**Background:** Inhibitors of tyrosin kinases (ITKs) such as imatinib, nilotinib, dasatinib and bosutinib are currently used for the treatment of chronic myeloid leukemia (CML). Imatinib, nilotinib and dasatinib have also been described to control HIV-1 replication *in vitro* through their negative effect on viral fusion. However, we determined that dasatinib is not a fusion inhibitor but it can actually interfere with SAMHD1 phosphorylation, preserving the activity of this antiviral factor and consequently, avoiding viral retrotranscription and proviral integration. Our aims were to evaluate the antiviral activity of dasatinib and other ITKs *in vitro* and to determine whether *in vivo* treatment with ITKs such as dasatinib may preserve SAMHD1 function and interfere with HIV-1 replication.

**Methods:** PBMCs from healthy donors were used to determine the effect of ITKs such as imatinib, nilotinib, dasatinib, bosutinib, saracatinib and KX2-391 on HIV-1 replication, SAMHD1 phosphorylation and HIV-1 fusion step. PBMCs from CML patients on treatment with ITKs such as Dasatinib were used to analyze SAMHD1 phosphorylation and susceptibility to HIV-1 infection *ex vivo*.

**Results:** 1) All assayed ITKs interfered with HIV-1 infection of PBMCs *in vitro*. Dasatinib, saracatinib, and KX2-391 were the most potent ( $IC_{50}$ =50.86nM,  $CC_{50}$ >10.0uM;  $IC_{50}$ =90.82nM,  $CC_{50}$ >3.0uM and  $IC_{50}$ =39.97nM,  $CC_{50}$ >4.0uM, respectively). 2) PBMCs from five CML patients on chronic treatment with Dasatinib for more than two years were resistant to HIV-1 infection *ex vivo*, showing 119-fold less proviral integration. No patient presented serious adverse events or infectious complications related to the use of dasatinib. An appropriate cytotoxic activity was preserved in these patients. 3) PBMCs from CML patients showed 68% less SAMHD1 phosphorylation in response to activating stimuli *ex vivo* than untreated controls. This is the main mechanism of action of dasatinib to interfere with HIV-1 infection because *in vitro* infection of PBMCs with virions containing Vpx, which degrades SAMHD1, overcame the inhibitory effect of Dasatinib. 4) Fusion of BlaM-Vpr-containing HIV-1 viruses with activated PBMCs treated with Dasatinib showed that Dasatinib was not a fusion inhibitor.

**Conclusions:** Dasatinib is the first compound currently used in clinic that was described to preserve the antiviral function of SAMHD1. ITKs such as Dasatinib in combination with antiretroviral therapy could make CD4 cells refractory to HIV-1 infection, thus reducing the size of the latent reservoir.

**469LB Efficacy of an Engineered Bispecific Anti-HIV Antibody in Humanized Mice**

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**Background:** While the search for an efficacious HIV vaccine remains elusive, the emergence of a new generation of virus-neutralizing monoclonal antibodies (Abs) has re-ignited the field of passive immunization as an alternative strategy for HIV prevention. However, the plasticity of HIV demands additional improvements to these Abs in order to better ensure their clinical utility. Here, we report the impressive *in vitro* and *in vivo* activity of an engineered bispecific Ab.

**Methods:** The CrossMab technology was used to construct a library of bispecific Abs. One potent and broad Ab, 10E8<sub>v2.0</sub>/iMab, was identified, with one arm targeting the human CD4 receptor using ibalizumab (iMab) and the other arm targeting gp41 using a modified 10E8 (10E8<sub>v2.0</sub>). We then evaluated its *in vivo* efficacy in NSG mice reconstituted with human hematopoietic cells. First, mice infected with HIV JR-CSF received weekly injections of 0.5 mg 10E8<sub>v2.0</sub>/iMab for 7 weeks, alone or in combination with 0.5 mg of an anti-gp120 engineered Ab. Second, uninfected mice receiving weekly injections of 0.2 mg 10E8<sub>v2.0</sub>/iMab were challenged 3 times intraperitoneally with JR-CSF to assess its protective efficacy. In both experiments, viral RNA in plasma was assessed weekly.

**Results:** 10E8<sub>v2.0</sub>/iMab showed breadth of 100% against a panel of 118 HIV strains, with mean IC<sub>50</sub> and IC<sub>80</sub> of 0.002 ug/mL and 0.006 ug/mL, respectively. *In vivo*, this Ab alone reduced the virus load by 1.7 log in infected mice after 2 weeks of treatment (Mann-Whitney test, p < 0.001 vs iMab + 10E8<sub>v2.0</sub> co-administration). Subsequent viral rebound was associated with mutations in the 10E8 epitope, implying that its antiviral activity was mainly mediated by the 10E8<sub>v2.0</sub> arm that is concentrated at the site of viral entry. The combination of 10E8<sub>v2.0</sub>/iMab and an anti-gp120 Ab led to a sustained viral load decrease of 2.3 log during the course of treatment. 10E8<sub>v2.0</sub>/iMab also provided complete protection in humanized mice against three systemic HIV challenges, whereas 16/19 saline-treated mice became infected after one challenge (log rank test, p = 0.0001).

**Conclusions:** 10E8<sub>v2.0</sub>/iMab appears to be the most potent HIV-neutralizing Ab described to date. Moreover, it has shown unprecedented activity for an Ab in both treating and preventing HIV in a humanized mouse model. 10E8<sub>v2.0</sub>/iMab thus holds promise as a novel prophylactic and therapeutic agent in the fight against HIV. 10E8<sub>v2.0</sub>/iMab could potentially serve as an anchor for a combination of antibodies to treat HIV on a monthly basis.

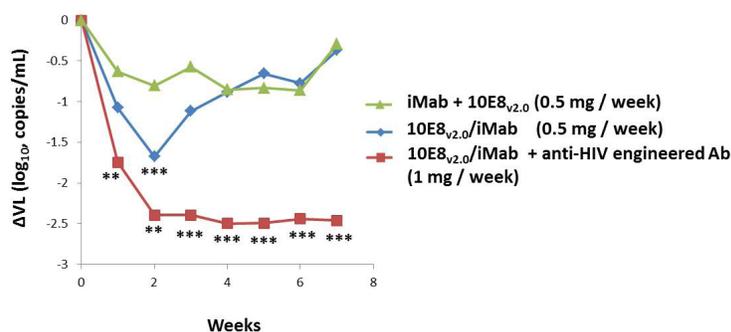


Figure 1: Therapeutic efficacy of HIV CrossMab 10E8<sub>v2.0</sub>/iMab *in vivo*. \*\* p < 0.01, \*\*\* p < 0.001 as determined by Mann-Whitney test.

**470 Doravirine 100mg QD vs Efavirenz +TDF/FTC in ART-Naive HIV+ Patients: Week 48 Results**

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**Background:** Doravirine (DOR), an investigational NNRTI with a novel resistance profile, was compared with efavirenz (EFV) in a randomized, double-blind, 2-part study in ART-naïve HIV-infected patients also receiving tenofovir/emtricitabine (TDF/FTC). Part 1 evaluated DOR 25, 50, 100, and 200 mg once daily and EFV 600 mg once daily; based on week 24 results, DOR 100 mg once daily was selected for ongoing evaluation. Part 2 enrolled additional patients to receive DOR 100 mg or EFV. At week 24 (Parts 1+2 combined), DOR 100 mg demonstrated antiretroviral activity and immunological effect similar to EFV (each with TDF/FTC) with significantly fewer CNS adverse events. Data through week 48 are now available from this ongoing study.

**Methods:** Week 48 data from patients who received DOR 100 mg or EFV in Part 1 (n=42 per group) and Part 2 (n=66 per group) were combined for this analysis. Patients were stratified at randomization by screening HIV RNA ≤ or >100,000 copies/mL. The primary efficacy endpoint is the proportion of patients with HIV RNA <40 copies/mL, using the non-completer=failure approach.

**Results:** 216 patients (93% male, 80% white, mean age 36 years) were randomized and treated. Mean baseline HIV RNA was 4.6 log<sub>10</sub> copies/mL in both the DOR and EFV groups, and mean CD4 counts were 432 and 448 cells/mm<sup>3</sup>, respectively. 88% of the DOR group and 85% of the EFV group completed 48 weeks of treatment. Reasons for discontinuation included adverse event (n=3 on DOR, 6 on EFV), lack of efficacy (0, 1), and other reasons (10, 9). Efficacy and safety results are shown in the table below. Drug-related AEs with incidence >5% in either treatment group were diarrhea (DOR 0.9%, EFV 6.5%), nausea (7.4%, 5.6%), dizziness (6.5%, 25.9%), headache (2.8%, 5.6%), abnormal dreams (5.6%, 14.8%), insomnia (6.5%, 2.8%), nightmares (5.6%, 8.3%), and sleep disorder (4.6%, 6.5%). There were no discontinuations due to drug-related AEs after week 24. Laboratory abnormalities ≥Grade 2 were uncommon in both groups.

**Conclusions:** DOR 100 mg once daily demonstrated antiretroviral activity and immunological effect similar to EFV (each with TDF/FTC) and was generally safe and well tolerated during 48 weeks of treatment in ART-naïve, HIV-1 infected patients. Drug-related AEs were significantly less frequent in the DOR group compared with the EFV group.

Week 48 Efficacy, including subgroup responses by screening HIV RNA			
Endpoint	DOR <sup>†</sup> (N=108)	EFV <sup>†</sup> (N=108)	Difference in % Response [DOR-EFV] (95% CI)
% with HIV RNA <40 copies/mL			
Overall ‡	77.8	78.7	-1.1 (-12.2, 10.0)
Screening HIV RNA ≤100K <sup>§</sup> (n=67, 62)	86.6	87.1	-0.5 (-12.7, 11.9)
Screening HIV RNA >100K <sup>§</sup> (n=35, 37)	74.3	83.8	-9.5 (-28.7, 9.7)
% with HIV RNA <200 copies/mL			
Overall ‡	85.2	84.3	0.9 (-8.9, 10.8)
Screening HIV RNA ≤100K <sup>§</sup> (n=67, 62)	89.6	91.9	-2.4 (-13.2, 8.6)
Screening HIV RNA >100K <sup>§</sup> (n=35, 37)	91.4	91.9	-0.5 (-15.6, 14.2)
Mean change in CD4 count (cells/mm <sup>3</sup> ) <sup>§</sup>	+192	+195	-3 (-47, 41)
Week 48 Clinical Adverse Event (AE) Summary			
% of Patients with:	DOR <sup>†</sup> (N=108)	EFV <sup>†</sup> (N=108)	Difference in % Response [DOR-EFV] (95% CI)
One or more AEs	87.0	88.9	-1.9 (-10.9, 7.1)
Drug-related AEs	31.5	56.5	-25.0 (-37.3, -11.8)
Serious AE	6.5	8.3	-1.9 (-9.5, 5.6)
Serious and drug-related AEs	0.0	1.9	-1.9 (-6.5, 1.6)
Discontinuation due to AEs	2.8	5.6	-2.8 (-9.2, 3.0)
<sup>†</sup> with TDF/FTC. <sup>‡</sup> Non-completer=Failure (NC=F) approach to missing data; 95% CI based on Miettinen and Nurminen's method with weights proportional to the size of each stratum. <sup>§</sup> Observed Failure (OF) approach to missing data.			

#### 471 Tolerability and Acceptability of Cabotegravir LA Injection: Results From ECLAIR Study

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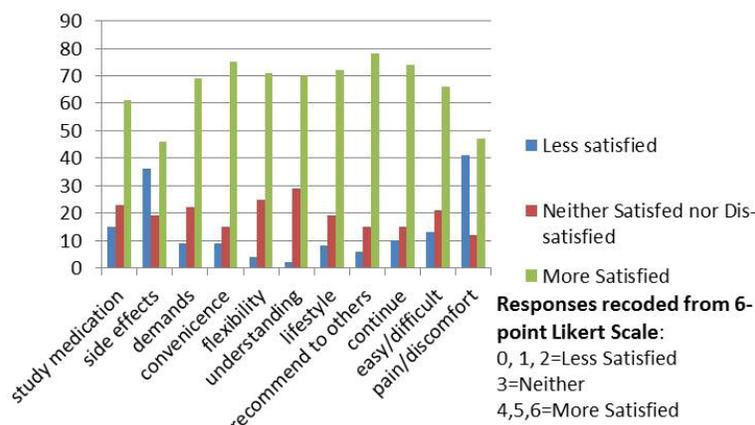
**Background:** Cabotegravir (CAB, GSK 1265744) is a Long-Acting (LA) injectable, in Phase 2 for treatment and prevention of HIV-1 infection. ÉCLAIR evaluated CAB LA injections for HIV PrEP. Secondary objectives include tolerability, satisfaction, and acceptability of CAB LA.

**Methods:** ÉCLAIR is a double-blind, randomised, multi-centre study in HIV-negative men, excluding males at high-risk of HIV-1. Participants were randomized (5:1) to QD oral CAB 30mg or placebo (PBO) for 4 weeks (W); thereafter IM injections (Inj) of 800mg CAB LA or PBO (saline) q12W x 3 cycles. Satisfaction, tolerability, and acceptability were self-assessed with Study Medication Satisfaction Questionnaire (SMSQ) with 11 items adapted from the HIV-Treatment Satisfaction Questionnaire and Study Medication Questionnaire (SMQ), which consists of 4 items evaluating adherence with study medication. SMSQ and SMQ were administered at W 6, 18, and 30 (1W after CAB LA inj).

**Results:** ECLAIR randomized/treated 126 participants (CAB: 105; PBO 21). Eighteen withdrew from CAB: five during oral phase, six after oral but prior to inj, and seven during the inj phase. Of those receiving inj, 87/94 (93%) of CAB LA, and 20/21 (95%) PBO completed inj phase. AEs due to CAB included inj site pain (82%), headache (23%), and inj site swelling (20%). 1W following second inj, the majority of participants were more satisfied with IM CAB compared with once daily oral CAB, on all 11 items included in the SMSQ [figure below]. At 1W post third CAB LA inj, 64/86 (75%) of participants were satisfied with CAB IM, 21% neither satisfied nor dissatisfied and, 4% dissatisfied. Overall, 75/86 (87%) of participants reported as willing to recommend medication and 68/86 (79%) a willingness to continue with study medication. Tolerability of CAB LA inj showed 57/86 (66%) of participants were satisfied with side effects with 13% neither satisfied or dissatisfied and 55/86 (64%) were satisfied with the amount of pain/discomfort with 14% neither satisfied or dissatisfied. SMQ results show 73/86 (85%) of participants almost never found it difficult to comply with the q12W schedule.

**Conclusions:** Secondary endpoints from ECLAIR help interpretation of safety data and provide a robust participant-centred perspective. While CAB LA inj site pain was common, discontinuation was infrequent. Results suggest participants experienced a high level of overall satisfaction and cited preference for a LA inj based on dimensions such as convenience, flexibility and ease of use.

#### WK18: Comparing LA CAB with Oral CAB



#### 472 Attachment Inhibitor Prodrug BMS-663068 in ARV-Experienced Subjects: Week 96 Analysis

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**Background:** BMS-663068 is a prodrug of BMS-626529, an attachment inhibitor that binds directly to HIV-1 gp120, preventing initial viral attachment and entry into host CD4+ T-cells. AI438011 is an ongoing Phase 2b, randomized, controlled trial investigating the safety, efficacy and dose-response of four doses of BMS-663068 vs atazanavir/ritonavir (ATV/r) in treatment-experienced (TE) HIV-1-infected subjects. At Week 24/48 BMS-663068 had similar response rates across all doses and to ATV/r and was generally well tolerated. We report results through Week 96.

**Methods:** TE adults ( $\geq 1$  week exposure to  $\geq 1$  ARV) with viral loads (VL)  $\geq 1,000$  c/mL and susceptibility to BMS-663068 (BMS-626529  $IC_{50} < 100$  nM), raltegravir (RAL), tenofovir disoproxil fumarate (TDF), and ATV were randomized equally to four BMS-663068 arms (400 or 800 mg BID; 600 or 1200 mg QD) and a control (ATV/r 300/100 mg QD), each with a backbone of RAL 400 mg BID+TDF 300 mg QD. BMS-663068 1200 mg QD was selected as the open-label continuation dose after Week 48 and pooled efficacy and safety results for BMS-663068 are included.

**Results:** 254 subjects were randomized and 251 treated across all arms. Median age was 39 years, 60% were male, 62% were non-white. Median baseline (BL) VL was 4.85  $\log_{10}$  c/mL (43%  $\geq 100,000$  c/mL); median CD4+ T-cell count was 230 cells/ $\mu$ L (38%  $< 200$  cells/ $\mu$ L). In total, 67% (167/251) subjects completed 96 weeks of therapy. At Week 96, 61% of BMS-663068- and 53% of ATV/r-treated subjects had HIV-1 RNA  $< 50$  c/mL (mITT; Table). In the observed analysis, 90% of BMS-663068- and 90% of ATV/r-treated subjects had HIV-1 RNA  $< 50$  c/mL (Table). Observed virologic response rates for BMS-663068 vs ATV/r by BL VL  $< 100,000$  c/mL were 87% vs 95%, respectively, and by BL VL  $\geq 100,000$  c/mL were 94% vs 80%, respectively. Mean increase in CD4+ T-cell count from BL through Week 96 was 219 cells/ $\mu$ L for BMS-663068 and 250 cells/ $\mu$ L for ATV/r. BMS-663068 was generally well tolerated and no BMS-663068-related AEs led to discontinuation (D/C).

**Conclusions:** At Week 96, BMS-663068 continued to show similar virologic response rates (mITT and observed) and immunologic reconstitution to ATV/r in TE subjects. BMS-663068 was generally well tolerated and no BMS-663068-related AEs led to D/C. These results support the ongoing Phase III trial evaluating BMS-663068 in heavily TE adults with limited therapeutic options ( $\leq 2$  classes of active antiretrovirals remaining) due to resistance, tolerability issues and contraindications (NCT02362503).

**Table: Week 96 pooled efficacy results**

Parameter	BMS-663068 + TDF (300 mg QD) + RAL (400 mg BID)	ATV/r (300/100 mg QD) + TDF (300 mg QD) + RAL (400 mg BID)
<b>Week 96 mITT analysis (FDA SnapShot algorithm)</b>		
HIV RNA <50 c/mL, n/N (%)	122/200 (61)	27/51 (53)
<b>Week 96 observed analysis (subjects with data within Week 96 window)</b>		
HIV RNA <50 c/mL, n/N (%)	123/137 (90)	28/31 (90)

**473 Predictors of CD4 Count Recovery in the CIPRA HT-001 Trial of Early ART**

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**Background:** Early antiretroviral therapy (ART) in the CIPRA HT-001 study improved survival and decreased tuberculosis (TB) risk. After an interim analysis, all participants were offered ART regardless of CD4 count, thus the impact of ART timing on CD4 recovery is underestimated by intention to treat analyses.

**Methods:** From August 2005 to July 2008, 816 treatment-naïve HIV-infected Haitians with a CD4 count between 200 - 350 cells/mm<sup>3</sup> were randomized to immediate ART or to defer ART until CD4 <200 or AIDS illness occurred. In June 2009, all participants were offered ART regardless of CD4. Data were collected until August 2014. We examined median CD4 counts from the time of ART initiation and used generalized estimating equations (GEE) to assess independent predictors of CD4 recovery. An on-ART model incorporated viral suppression, TB co-infection and adherence. We estimated the time to a CD4 increase of 100, 200 and 300 and the time to an absolute CD4 >350 or >500 using Kaplan-Meier analysis stratified by gender and by CD4 at ART start.

**Results:** 763 of 816 participants (94%) started ART and are included in the analysis, 408 in the immediate treatment arm and 355/408 (87%) in the deferred arm. In the deferred arm 185/355 (52%) started ART with a CD4 <200. Median CD4 counts over time were higher in those starting ART with CD4 ≥200 compared to <200, but with overlapping interquartile ranges. In GEE models, a higher CD4 at ART start, female gender and time on ART were independent predictors of CD4 recovery (p<.001). When we controlled for CD4 at ART start, the randomization arm was not an independent predictor of CD4 recovery or the rate of increase. In the on-ART model, a pre-ART viral load >100,000 copies/mL and viral suppression after 1 year of ART were associated with a greater CD4 recovery (p=.006 and p<.001), and active TB while on ART with a lower CD4 (p=.03). Controlling for adherence to clinic visits and drugs did not alter these associations. Participants with CD4 <200 had a faster rise in CD4 by 100 and 200 cells/mm<sup>3</sup> compared to those who started with CD4 ≥200 (log-rank p=0.02 and .01) but fewer reached an absolute CD4 count >500 (80% vs 90% p<.001). Men were slower than women to increase by 200 or 300 CD4 cells/mm<sup>3</sup> (p=.03 and .003) and fewer men reached an absolute CD4 count >500 (83% vs 91% p=.01).

**Conclusions:** A higher CD4 count at ART start, female gender, viral suppression and time on ART are independent predictors of CD4 recovery. Active TB is associated with impaired CD4 recovery on ART.

Variable	Model at ART Start			Model On-ART		
	Estimate	SE	p-value	Estimate	SE	p-value
Randomization arm	-0.2	9.5	0.98	-11.1	10.7	0.30
Time since ART start	17.8	1.1	<0.001	20.8	1.2	<0.001
Randomization arm*Time since ART start	1.3	1.5	0.39	0.4	1.7	0.80
Age at ART start	0.0	0.6	1.00	-1.3	0.6	0.03
Male gender	-55.0	10.0	<0.001	-55.0	9.6	<0.001
Underweight (BMI <= 18)	-15.4	17.3	0.37	12.8	16.2	0.43
CD4 count at ART start	0.8	0.1	<0.001	0.8	0.1	<0.001
WHO HIV stage at ART start	-4.5	8.2	0.58	3.8	7.9	0.63
Poverty	-11.9	10.4	0.25	-10.1	10.1	0.32
History of TB prior to ART start	-21.5	16.5	0.19	-29.6	16.4	0.07
Hemoglobin at ART start	2.7	1.7	0.10	2.2	1.6	0.16
Viral load >100,000 at ART start	13.7	10.6	0.20	27.7	10.2	0.01
PPD status	-4.7	10.1	0.64	2.6	10.5	0.81
Creatinine Clearance < 60 mL/min	-21.8	14.5	0.13	-18.1	13.8	0.19
TB while on ART				-34.2	15.7	0.03
Viral load < 50 c/mL at 1 year				115.4	12.2	<0.001
Adherence with clinic visits				13.0	7.4	0.08
Suboptimal ART adherence				-10.9	10.0	0.28

Underweight defined per WHO criteria for body mass index, lowest poverty category <\$125/year, hemoglobin values normalized for sex specific deviation from WHO normal, creatinine clearance calculated by Cockcroft-Gault [adjusted], adherence to study visits assessed numerically, suboptimal ART adherence defined as <100% on 3 day recall.

**474 Effect of Immediate ART on Risk of Severe Bacterial Infections: The START Trial**

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**Background:** The START trial conducted amongst HIV+ people with CD4 count >500/mm<sup>3</sup> showed that risk of bacterial infectious disorder (BID) was reduced in those randomized to immediate ART compared with those randomized to defer ART until CD4 count <350/mm<sup>3</sup>. We expand on this finding by including additional bacterial infection events and we investigate whether the effect is due to the increase in CD4 and neutrophil counts that occurred in the immediate ART arm.

**Methods:** All START participants were included in analyses. Cox proportional hazards regression was used to model the time to serious bacterial infection (SBI). SBI was defined as a composite of bacterial pneumonia (BP) (confirmed by the endpoint review committee) or any BID (as reported in the START paper) which satisfied at least one of the following three criteria: grade 4 severity, requiring unscheduled hospitalisations or causing death. We estimated the effect of randomized assignment, on the risk of SBI. We considered two models: model (i) single factors adjusted for age and sex; model (ii) multivariable adjustment for all factors (See Table 1). Models were stratified by geographic region.

**Results:** Of 4,685 participants included in analyses 94 experienced at least one SBI during a median follow-up of 2.8 years. The median baseline neutrophil count (interquartile range) was 2810.0/mm<sup>3</sup> (2176.2, 3678.2). Latest average neutrophil count over follow-up was 231/mm<sup>3</sup> higher, and latest average CD4 count was 194/mm<sup>3</sup> higher, in the immediate ART arm (p<.0001). Immediate ART was associated with a reduced risk of SBI [Hazard Ratio (HR), 0.42; 95% CI, 0.27-0.65, p=0.0001; consistent for two main components of SBI (BID HR=0.38, BP HR=0.42)] in model (i), but not after adjustment for time-updated factors in model (ii) [HR, 0.72; 95% CI, 0.41-1.27, p=0.25]. Higher latest BMI [HR per 5kg/m<sup>2</sup>, 1.33; 95% CI, 1.11-1.59, p=0.0017] was associated with an increased risk of SBI. Higher latest CD4 count [HR per 100 cells/mm<sup>3</sup>, 0.83; 95% CI, 0.74-0.92, p=0.0006] was associated with a reduced risk of SBI. Latest neutrophil count was not associated with SBI [HR per 100 cells/mm<sup>3</sup>, 0.90; 95% CI, 0.19-4.16, p=0.89].

**Conclusions:** Immediate ART reduces the risk of SBI among HIV+ persons with high CD4 count and normal neutrophil count. This benefit is partly explained by ART-induced increases in CD4 count but not by increases in neutrophil count. Our study demonstrates the protective effect of immediate ART in reducing the risk of a broad spectrum of serious infections.

**Table 1 Factors associated with severe bacterial infections - Cox proportional hazards regression**

Covariate	Model (i) <sup>a</sup> Hazard Ratio (95% C.I.)	P-value	Model (ii) <sup>b</sup> Hazard Ratio (95% C.I.)	P-value
<b>Treatment arm (ref deferred ART)</b>				
Immediate ART	0.42 (0.27 - 0.65)	0.0001	0.72 (0.41 - 1.27)	0.2541
<b>Age (per 10 years)</b>	1.23 (1.02 - 1.50) <sup>c</sup>	0.0334	1.14 (0.92 - 1.42)	0.2204
<b>Female sex (ref male)</b>	1.58 (0.93 - 2.70) <sup>c</sup>	0.0917	1.68 (0.72 - 3.88)	0.2276
<b>Race (ref White)</b>				
Asian	1.76 (0.43 - 7.17)	0.4293	2.12 (0.51 - 8.90)	0.3045
Black	1.21 (0.62 - 2.34)	0.5734	1.21 (0.58 - 2.51)	0.6119
Latino/Hispanic	0.29 (0.08 - 0.98)	0.0455	0.30 (0.09 - 1.03)	0.0562
Other	1.26 (0.38 - 4.18)	0.7010	1.40 (0.42 - 4.71)	0.5839
<b>Latest Body Mass Index (per 5 kg/m<sup>2</sup>)</b>	1.25 (1.05 - 1.49)	0.0115	1.33 (1.11 - 1.59)	0.0017
<b>Baseline smoking status</b>	1.18 (0.77 - 1.81)	0.4520	1.25 (0.79 - 1.98)	0.3415
<b>Mode of infection with HIV (ref MSM)</b>				
Sex with person of opposite sex	0.84 (0.41 - 1.72)	0.6292	0.63 (0.29 - 1.37)	0.2457
Injection-drug use	2.29 (0.87 - 6.03)	0.0941	1.72 (0.52 - 5.74)	0.3773
Blood products, other or unknown	0.50 (0.15 - 1.68)	0.2632	0.27 (0.06 - 1.18)	0.0826
<b>Time since HIV diagnosis at baseline (years)</b>	1.01 (0.95 - 1.07)	0.7456	1.00 (0.94 - 1.06)	0.9327
<b>Hepatitis B status (ref negative)</b>				
Positive	1.04 (0.25 - 4.26)	0.9573	1.14 (0.27 - 4.74)	0.8585
Unknown	1.05 (0.38 - 2.90)	0.9290	0.78 (0.23 - 2.64)	0.6930
<b>Hepatitis C status (ref negative)</b>				
Positive	1.92 (0.92 - 3.99)	0.0817	1.22 (0.47 - 3.15)	0.6886
Unknown	1.14 (0.36 - 3.62)	0.8292	1.30 (0.32 - 5.25)	0.7087
<b>Latest CD4 count (per 100 cells/mm<sup>3</sup>)</b>	0.79 (0.72 - 0.87)	<.0001	0.83 (0.74 - 0.92)	0.0006
<b>Latest HIV RNA (log<sub>10</sub> copies/mL)</b>	1.34 (1.16 - 1.54)	<.0001	1.07 (0.89 - 1.30)	0.4674
<b>Latest Hemoglobin (per 10 g/dL)</b>	0.39 (0.08 - 1.91)	0.2471	0.63 (0.11 - 3.74)	0.6148
<b>Latest Platelet count (per 100,000 cells/mm<sup>3</sup>)</b>	0.79 (0.56 - 1.11)	0.1725	0.91 (0.64 - 1.30)	0.6063
<b>Latest Neutrophil count (per 10,000 cells/mm<sup>3</sup>)</b>	0.64 (0.14 - 2.89)	0.5655	0.90 (0.19 - 4.16)	0.8917
<b>Latest Total Lymphocyte count (per 100 cells/mm<sup>3</sup>)</b>	1.00 (0.99 - 1.01)	0.8872	1.02 (1.00 - 1.05)	0.0520
<b>Latest Albumin (g/dL)</b>	0.58 (0.34 - 0.96)	0.0360	0.77 (0.43 - 1.37)	0.3766

MSM: men-who-have-sex-with-men.

<sup>a</sup>Model (i) covariates fitted singly with adjustment for age and sex; stratified by geographic region of residence<sup>d</sup>.

<sup>b</sup>Model (ii) multivariable effects of covariates; stratified by geographic region of residence<sup>d</sup>.

<sup>c</sup>Estimates from a model including age and sex only.

<sup>d</sup>Geographic region of residence: Africa, Asia, Oceania, Europe & Israel, North America, South America.

**475 Increased Quality of Life With Immediate ART Initiation: Results From the START Trial**

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**Background:** With HIV managed as a chronic illness, quality of life (QOL) is one important outcome in assessing HIV treatment strategies. Development of HIV-related illnesses or medication side effects can both affect QOL. The Strategic Timing of Antiretroviral Therapy (START) study randomized antiretroviral therapy (ART) naive participants with CD4 counts >500 cells/mm<sup>3</sup> to starting ART immediately vs. deferring ART until CD4 counts declined to 350 cells or clinical disease required ART. We compared immediate vs. deferred ART groups for changes in QOL.

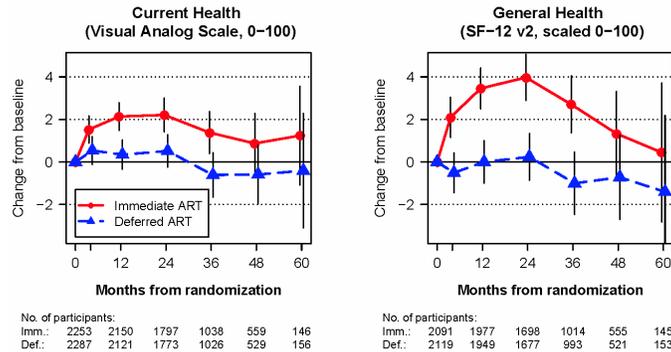
**Methods:** At baseline, Month 4, 12 and then annually, participants completed a visual analogue scale (VAS) for self-assessment of overall current health and the Short-Form 12-Item Health Survey version 2 (SF-12v2) with 4 week recall. We computed three QOL outcomes from SF-12v2: (1) General health perception (GHP); (2) Physical component summary (PCS), and (3) Mental component summary (MCS). All QOL outcomes are scaled 0-100 (higher score=better QOL). PCS and MCS scores are standardized to a mean=50 in a U.S. reference population. We compared immediate and deferred ART groups for QOL changes from baseline using longitudinal mixed models adjusted for visit and baseline QOL.

**Results:** Of 4684 START participants, 4561 had both baseline and follow-up QOL data: median baseline CD4=651 cells, median age=36 years, 27% were female, and 46% from high-income countries. Mean QOL baseline scores (with standard deviation) were VAS=80.9 (15.7), GHP=72.5 (21.5), PCS=53.7 (7.2), MCS=48.2 (10.5). Mean follow-up time was 2.6 years. The immediate group spent 95% of follow-up time on ART vs. 28% for the deferred group. Throughout follow-up, all changes in QOL favored the immediate group;

modest but significant differences were seen as early as 4 months with increases through 12 months (Fig. 1). Estimated treatment differences during follow-up were: VAS=1.9 (95% CI 1.2-2.5); GHP=3.6 (2.8-4.5); PCS=0.8 (0.5-1.1); MCS=0.9 (0.4-1.3) ( $p < 0.001$  for each QOL measure).

**Conclusions:** In this HIV-positive population with CD4 >500 cells/mm<sup>3</sup> that was generally in good health, all QOL measures improved in the immediate compared to the deferred ART group. These findings provide further support to the superiority of early ART as reported for major clinical outcomes in the START study.

**Figure 1. Mean Change in Quality of Life (95% CI)**



**476 Gender and Racial Disparities in Initial Antiretroviral Treatment Outcome: ACTG A5257**

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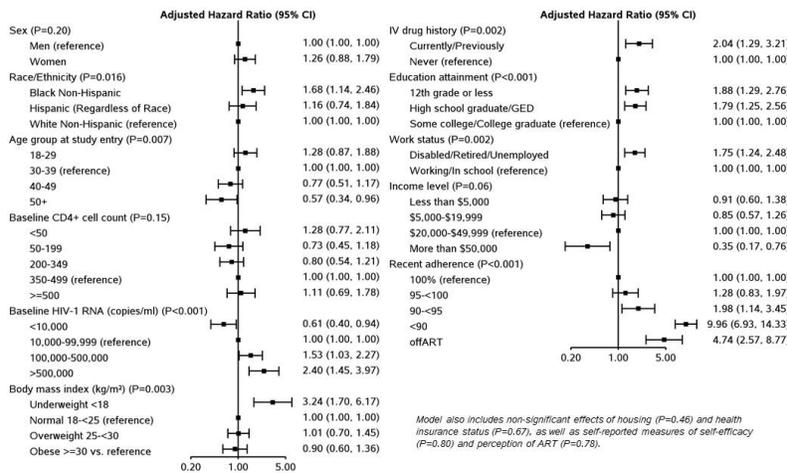
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**Background:** Inferior virologic outcomes of initial antiretroviral treatment (ART) for women and ethnic/racial minority groups have been reported. We examined this finding in a US-based setting with modern ART regimens and determined whether socio-demographics or non-adherence explain these differences.

**Methods:** ACTG A5257 randomized 1809 participants to ART with emtricitabine/tenofovir disoproxil fumarate plus atazanavir/ritonavir (/r), raltegravir, or darunavir/r. Study follow-up ended in 2013. This planned secondary analysis included 1762 participants categorized as non-Hispanic white, non-Hispanic black, and Hispanic based on self-report. Cox proportional hazards models examined the association of sex and race/ethnicity on the hazard of virologic failure (VF) adjusting for demographic and baseline factors including age, HIV-1 disease status and socio-demographic variables; adherence by self-report was included as a time-updated covariate. Analyses were intent to treat.

**Results:** The study sample was 34% white, 43% black, and 22% Hispanic. Median age was 37 years; 24% were women. Probability of VF by 96 weeks [95%CI] was 11% [10, 13] in men and 16% [12, 19] in women. VF probability was 7% [5, 9] in whites, 17% [14, 20] in blacks and 13% [10, 17] in Hispanics. Differential effects of sex or race/ethnicity by treatment arm were not apparent ( $P > 0.40$ ). A greater VF risk for women compared to men was apparent in unadjusted analysis ( $P = 0.005$ ) but not after adjustment for race/ethnicity ( $P = 0.29$ ). Compared to whites, blacks and Hispanics had a greater hazard of VF (unadjusted HR=2.8 [2.0, 3.8] and 2.0 [1.4, 2.8], respectively). While adjustment for socio-demographic factors appeared to account for the excess VF risk for Hispanics (1.2 [0.7, 1.8]), an excess risk remained for blacks after adjustment for socio-demographics and adherence (1.7 [1.1, 2.5]). Other factors associated with higher VF risk included non-adherence, younger age, high pre-ART viral load, low income, less education, IV drug history, and not working or in school (Figure).

**Conclusions:** Women and ethnic/racial minority groups in the US remain at greater risk of VF of initial ART with modern ART regimens. For women and Hispanics, this excess risk appears explained by race/ethnicity and socio-demographics, respectively. In contrast, an excess risk of VF for blacks remains after adjustment for non-adherence and socio-demographics. This work helps define populations at high VF risk who may benefit from early targeted interventions.



Model also includes non-significant effects of housing (P=0.46) and health insurance status (P=0.67), as well as self-reported measures of self-efficacy (P=0.80) and perception of ART (P=0.78).

**477 HIV Pretreatment Drug Resistance in Mexico: A Nationally Representative WHO Survey**

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**Background:** To ensure sustainability of antiretroviral treatment (ART) programmes after ART scale up, the WHO has proposed a global standardized HIV drug resistance (DR) monitoring and surveillance strategy, including the assessment of pre-treatment HIVDR (PDR).

**Methods:** We present the first nationally representative WHO HIV PDR survey performed in Mexico from February to July 2015. Twenty-five clinics were selected to contribute with participants from 136 Ministry of Health facilities by probability-proportional-to-size sampling, according to the number of ART initiators observed in each clinic during 2013. PDR was assessed from plasma virus, based on the WHO surveillance HIVDR mutation list, using the Stanford CPR tool. All samples were processed in a WHO-accredited lab by Sanger sequencing using the software RECall and by next generation sequencing (NGS) using the software HyDRA.

**Results:** A total of 274 participants were included in the study; 84% were men. The median CD4+ T cell count was 257 cells/uL reflecting the known late presentation to clinical care in the country. PDR to any antiretroviral (ARV) drug was 12.0% (95% CI: 8.4-16.5%). NNRTI PDR was highest (6.9%), followed by NRTI PDR (5.1%) and PI PDR (2.6%). The most frequent PDR mutations were RT K103N (4.0%), M41L (1.1%) and PR L90M (1.8%). The prevalence of PDR to any ARV drug estimated with NGS at a 20% DR mutation frequency threshold was 12.2%, but increased considering lower thresholds: 14.2% at 10%; 17.3% at 5%; 30.3% at 2%. The most frequent minority variants (<5% of the viral population) included PR M46I, N88D and RT D67G, K70E. At the 2% DR mutation frequency threshold, intermediate levels of NNRTI (7.8%), NRTI (10.9%) and PI (10.1%) PDR were observed. Three putative transmission clusters were found, one including a male and a female from Baja California with M41L, one with two MSM from Sonora with Y181C and one with a female and an MSM from Veracruz with DR to two drug classes.

**Conclusions:** PDR in Mexico remains at the intermediate level, but individual PDR level to NNRTI has also reached intermediate level with high frequency of K103N, consistent with the wide use of efavirenz-containing first line regimens in the country. Low frequency DR mutations mainly to NRTI and PI were observed. Evidence of DR mutation transmission was found in specific geographic areas involving both heterosexuals and MSM. These observations warrant continuous PDR surveillance in the country.

**478 The Dynamics of Drug Resistance Detected During Acute HIV Infection**

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**Background:** HIV drug resistance detected during acute infection may be a result of transmission from an infected partner, selection by pre-exposure prophylaxis (PrEP), or error prone replication. Here we describe the long-term dynamics of resistance detected during early acute HIV infection.

**Methods:** The Partners PrEP Study was a randomized trial in which serodiscordant partners were assigned to emtricitabine co-formulated with tenofovir (FTC/TDF) or TDF alone compared to placebo, and monitored for HIV infection monthly. In a previous study, baseline samples from 137 HIV seroconverters were tested for drug resistance using 454 ultra deep sequencing at the time seroconversion was first detected. Here, we conducted resistance testing by 454 on plasma samples at 6, 12 and 24 months following seroconversion from individuals with resistance mutations detected during acute infection at frequencies  $\geq 1\%$  that had a Stanford score  $\geq 30$  and cause resistance to non-nucleoside or nucleoside reverse-transcriptase inhibitors (NNRTIs or NRTIs), including both PrEP and non-PrEP antiretrovirals.

**Results:** There were 35/137 (26%) individuals with resistance detected at frequencies  $\geq 1\%$  at the time seroconversion was first identified: 30 had resistance to non-PrEP antiretrovirals (not TDF or FTC) likely due to transmitted resistance or error-prone replication and 11 individuals had PrEP-related mutations (K65R, K70E and M184I); of whom 6 had both PrEP-related and non-PrEP mutations. Of the 35, 31 individuals had resistance results available through 12-24 months. Resistance faded to frequencies  $< 1\%$  in 16/31 (52%) individuals by 6 months after seroconversion and in 21/31 (68%) by 24 months. PrEP-selected mutations did not persist. In 5 individuals, resistance persisted at low frequencies between 1-10% of the viral population and in 3 individuals, resistance mutations K103N (n=2) or Y181C (n=1) persisted at frequencies  $\geq 99\%$  throughout follow-up.

**Conclusions:** Among individuals who acquired HIV in a PrEP trial, non-PrEP (non TDF or FTC) mutations accounted for the majority of resistance detected during acute infection. Persistence of high frequency resistance was limited to 2 NNRTI mutations that were most likely transmitted. Most of the resistance present during acute infection faded below detection by 24 months regardless of whether it was selected or transmitted. These data suggest that in the absence of antiretroviral treatment, onward transmission of resistance is most likely to occur early after HIV infection.

**479LB WITHDRAWN****480 Large Cluster Clinical Isolates Show Facilitated Escape From Integrase Inhibitors**

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**Background:** Integrase inhibitors (INIs), including raltegravir (RGV), elvitegravir (EVG) and dolutegravir (DTG) are a drug class of choice in first-line therapy. Emergent resistance to EVG and RGV include N155H and G140A/G148RHQ pathways, conferring RGV/EVG cross-resistance, and Y143RHC (RGV) and T661/E92Q/G (EVG) pathways. To date, DTG shows a higher barrier to resistance with no reported mutations in first-line therapy at 96 or 148 weeks. Phylogenetics shows a metamorphosis of the Quebec HIV epidemic towards large cluster outbreaks, averaging 43 linked transmissions/cluster. "Super-transmissible" viral lineages showed facilitated development of resistance in cell culture, revealing pathways to DTG resistance that could not be otherwise ascertained.

**Methods:** Phylogenetic analysis identified cluster group association of newly diagnosed subjects recruited into the Montreal PHI cohort. Viral stocks from representative large cluster (cluster size 44, 44, 40, 24, 23, 71) and solitary transmissions (n=6) were amplified through co-culture of patient CD8-depleted peripheral blood mononuclear cells with human cord blood mononuclear cells (CBMCs). Selections of resistance to DTG, EVG, and RGV, were performed by repeat serial passage in CBMCs in the presence of increasing drug concentrations of drugs, based on weekly RT assays. Genotyping was performed at select passages to evaluate time to the development of drug resistance.

**Results:** Whereas viruses belonging to solitary transmissions showed no emergent drug resistance to DTG at week 30, super-cluster lineages acquired DTG resistance within 6-12 weeks. Superclusters developed DTG resistance along R263K (2), S153Y, or H51Y pathways. Parallel EVG selections led to more complex resistance pathways. Dual selections with DTG and 3TC showed R263K emergence prior to M184I/V. Whereas DTG selections failed to accumulate mutations, R263K acquired with EVG selections led to further acquisition of INI mutations. Phenotypic assays monitored relative drug susceptibility to DTG, EVG, and RGV in the context of resistance mutations and natural polymorphisms.

**Conclusions:** Although DTG confers a high barrier to resistance, large cluster viral lineages fast forward escape from drug pressure, providing unique insights on emergent resistance to INIs.

**481 An Informatics Approach to Predicting Rates of Transmitted HIV-1 Drug Resistance**

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**Background:** National transmitted HIV-1 drug resistance (TDR) rates are typically monitored through HIV surveillance data and this represents the gold-standard for determining TDR rates in various populations. However, it often requires several years to prepare data for presentation and publication resulting in long lag times between data accumulation and result dissemination. Here we present a computational method using de-identified clinical test results from a large commercial database to infer TDR rates in near real-time.

**Methods:** A HIPAA-compliant unique Patient ID was assigned to each test result in the LabCorp Clinical Database. New cases of HIV diagnosis were inferred using the linked results from HIV diagnosis, viral load and drug resistance tests. We developed a novel inference model which employs a successive series of filters to derive a subset of resistance assays that were likely to have been performed at the time of initial HIV diagnosis. We used the 2009 surveillance drug resistance mutations (SDRM) list from the Stanford University HIV Drug Resistance Database to determine mutational frequencies. We validated the model by comparing the predicted 2008-2011 NRTI, NNRTI, and PI frequencies to ranges established from prior TDR surveillance studies. We then used our model to calculate rates of TDR for 2012-2014 per drug class, including INIs. This model allows us to assess prevalence rates according to age group, gender, and geography.

**Results:** We identified 8612 putative baseline resistance tests from 2008-2011. The rates for NRTI (7.6%), NNRTI (11.2%), and PI (2.9%) SDRMs compared favorably with previously reported TDR rates, as did the overall rates for any SDRM (17%). For 2012-2014, we analyzed 14,325 putative baseline resistance tests and observed an overall rate for TDR mutations of 17.2%. We observed prevalence rates of 6.3%, 12.3%, 2.7%, and 0.6% for the NRTIs, NNRTIs, PIs, and INIs, respectively.

**Conclusions:** Our model demonstrates a novel real-time method for inferring the prevalence of TDR by drug class. This model suggests that national TDR rates for NRTI, NNRTI, and PIs from 2012-2014 have not decreased relative to prior years and that INI TDR rates were surprisingly low, possibly reflective of the efficacy of current antiretroviral regimens which incorporate this class. Near real-time TDR data can facilitate early detection of new drug resistance strains should they arise, such as in response to PrEP. Analysis of 2015 rates will be included for presentation.

**482 Impact of Transmitted Thymidine Analogue Mutations on Responses to First-Line ART**

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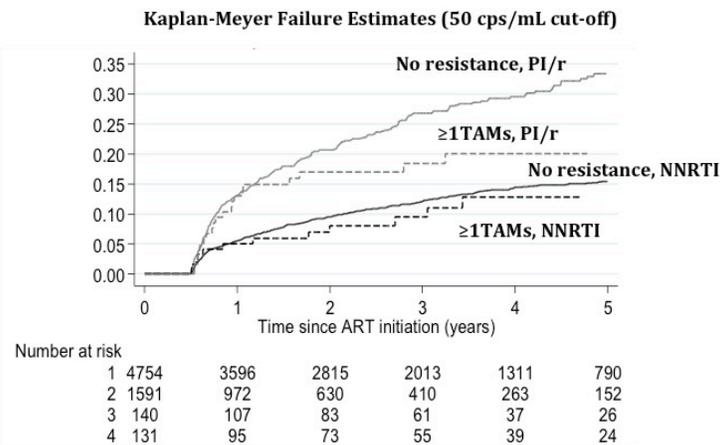
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**Background:** Thymidine analogue mutations (TAMs; RT codons 41, 67, 70, 210, 215, 219) are a prevalent form of transmitted drug resistance (TDR) in Europe and North America, commonly occurring as singleton revertants of T215Y/F (e.g., T215E), and thought to often represent onward transmission from ART-naïve subjects. Although PI/r-based therapy is recommended for patients with transmitted TAMs, it is not known whether alternative regimens carry an increased risk of virologic failure (VF). The study aim was to analyze ART outcomes in subjects with ≥1 TAM (and no other resistance) vs. subjects without evidence of resistance.

**Methods:** Subjects underwent genotypic resistance testing in 1998-2012 prior to starting TDF or ABC + 3TC or FTC + PI/r (ATV, DRV, FPV, LPV) or NNRTI (EFV, NVP, RPV). VF definition: confirmed viral load >50 (or 200) cps/mL after ≥6 months of ART, or one viral load >50 (or 200) cps/mL followed by a treatment change. Time to VF was analyzed using Kaplan Meier plots (figure) and Cox models adjusted for age, ethnicity, risk group, pre-ART viral load and CD4 count, and ABC use.

**Results:** Of 6926 patients evaluated before ART initiation, 6345 (92%) had no resistance; 271 (4%) had ≥1 TAM, including 204/271 (75%) with singleton TAMs, most commonly T215 revertants (112/271, 41%). VF risks at the 50 cps cut-off were 808/6345 (13%) in subjects with no resistance vs. 33/271 (12%) in subjects with ≥1TAM (P=0.53, log rank test). VF risks in subjects with no resistance were 304/1591 (19%) for PI/r use vs. 504/4754 (11%) for NNRTI use (HR=2.2; 95% CI 1.9-2.5; P<0.001). The same direction of effect was observed with ≥1TAM: 16% (21/131) for PI/r vs. 9% (12/140) for NNRTI (HR=1.7; 0.8-3.4, P=0.15). At the 200 cps cut-off, VF risks were 401/6345 (6%) in subjects with no resistance vs. 12/271 (4%) in subjects with ≥1TAM (P=0.14, log rank test). VF risks in subjects with no resistance were 149/1591 (9%) for PI/r use vs. 252/4754 (5%) for NNRTI use (HR=1.9; 1.6-2.4, P<0.001). With ≥1TAM, VF risks were 6/131 (5%) for PI/r vs. 6/140 (4%) for NNRTI (HR=0.9; 0.3-2.8, P=0.87).

**Conclusions:** This cohort analysis supports the hypothesis that in patients with ≥1 TAM as the sole form of TDR (predominantly singleton T215 revertants), there was no apparent virologic advantage of starting ART with a PI/r-based regimen. As the influence of confounding factors cannot be excluded, the data should be regarded as providing a framework for designing a controlled trial.



**483 Increasing Prevalence of Silent Mutations in HIV-1 Subtype B RT Which Alter Fitness**

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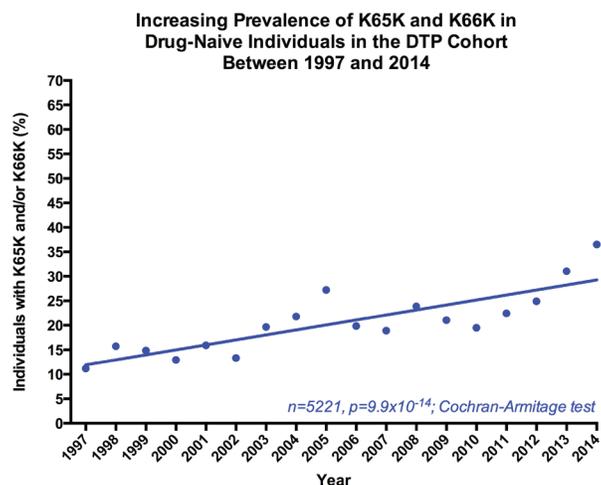
**Background:** Resistance to combination antiretroviral therapy (cART) in HIV-1 infected individuals is typically due to non-synonymous mutations that change the protein sequence; yet, the selection of synonymous or 'silent' mutations in HIV-1 has been reported<sup>1</sup>. Silent K65K and K66K mutations in HIV-1 reverse transcriptase (RT) are associated with thymidine analog mutations (TAMs) D67N and K70R, which confer decreased susceptibility to most HIV-1 nucleoside RT inhibitors. We previously showed that D67N/K70R in HIV-1 RT increase indel frequency by 100-fold, contributing to impaired viral fitness, and that either K65K or K66K reversed these defects<sup>2</sup>. However, the impact of the silent mutations in clinically-relevant virus backbones and their prevalence in a population over time are unknown.

**Methods:** A retrospective analysis of all drug-naïve HIV-infected individuals enrolled in the Drug Treatment Program (DTP) at the British Columbia Centre for Excellence in HIV/AIDS between 1997 and 2014 examined the prevalence of K65K/K66K in this population. A longitudinal analysis of HIV-1 RT sequences from 2131 individuals from the same cohort assessed the temporal appearance of K65K/K66K relative to TAMs. Growth competition assays evaluated the fitness of multidrug-resistant (MDR) HIV-1 variants ± K65K or K66K derived from a patient isolate.

**Results:** A retrospective analysis of all drug-naïve HIV-infected individuals in the DTP cohort revealed that K65K and K66K increased in prevalence in drug-naïve individuals from 11% in 1997 to 36% in 2014 [p=9.9x10<sup>-14</sup> (Cochran-Armitage test), n=5221] (Fig.1). K65K and K66K were selected in 316 individuals (15%) followed longitudinally. In 95% of

cases, these mutations occurred in the absence of TAMs, suggesting that there may be a role for these silent mutations independent of TAMs. K65K and K66K conferred a fitness advantage of  $2.01 \pm 0.11\%$  and  $2.41 \pm 0.41\%$  respectively to MDR HIV-1 variants in the absence of drug pressure ( $n=3$  for each).

**Conclusions:** K65K and K66K confer a fitness advantage in the context of MDR virus, even in the absence of drug. An unexpected tripling in prevalence of these mutations in drug-naïve individuals over the past 20 years was observed. Approximately one in three individuals with subtype B virus now harbor these mutations, suggesting that their potential impact should not be ignored.



**484 No Effect of HIV-1 Subtype C on Virological Failure Rate With First-Line TDF Regimens**

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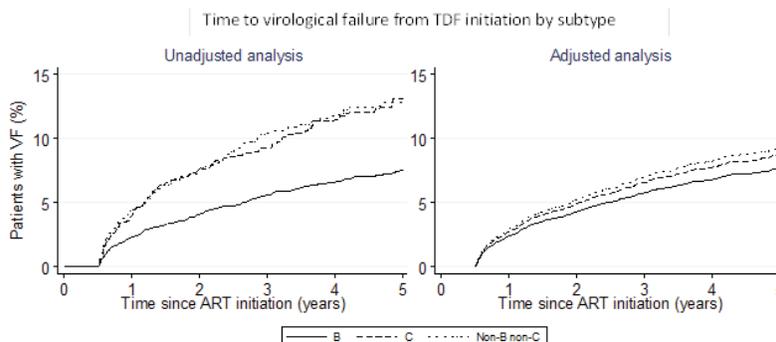
<sup>1</sup>Med Rsr Council Clinical Trials Unit at Univ Coll London, London, UK; <sup>2</sup>Birmingham Heartlands Hosp, Birmingham, UK; <sup>3</sup>Brighton and Sussex Hosps NHS Trust, Brighton, UK; <sup>4</sup>HIV i-Base, London, UK; <sup>5</sup>Royal Free London NHS Fndn Trust, London, UK; <sup>6</sup>Univ Coll London, London, UK; <sup>7</sup>Africa Cntr for Hlth and Pop Studies, Mtubatuba, South Africa

**Background:** In vitro and clinical studies have shown that subtype C viruses have a greater propensity to develop a K65R mutation due to polymorphisms at codons 64–66. This has potentially important public health implications given that subtype C infection accounts for around 50% of HIV infections worldwide and with the expanded use of tenofovir (TDF) as per WHO 2013 recommendations. We have exploited the wide diversity of viral subtypes within the UK to examine whether viral subtype influences the rate of virological failure (VF) on first-line TDF-containing regimens.

**Methods:** Patients were included if HIV care was received at a participating clinic in the UK CHIC study; their first-line regimen was TDF+(XTC)+(EFV, NVP, LPV/r, DRV/r or ATV/r); and  $\geq 2$  viral loads (VLs) measured after 6 months following ART initiation. Subtypes were defined according to Rega-3, based on resistance tests conducted pre-therapy or at treatment failure. Time to VF (2 consecutive VLs  $>200$  copies/ml after 6 months of ART) was analysed using Cox models, adjusting for demographic factors, baseline CD4 and VL, ART regimen, and year of initiation. Follow-up was censored at last VL or discontinuation of TDF. Multiple imputation was used to include patients with missing subtypes, taking advantage of the strong association with demographic factors.

**Results:** 8746 patients were included and followed for a median of 3.3 years; 5465 (4123 observed, 1342 average of imputed) were subtype B, 1455 (823, 632) subtype C, and 1826 (1203, 623) non-B/non-C. Subtype B patients were mostly white (83%) and MSM (85%) while subtype C mostly black (70%) and heterosexual (79%). Subtype non-B/non-C patients were demographically more mixed (35% white, 53% black; 26% MSM, 63% heterosexual). Risk of VF for subtype non-B/non-C (173, 9.5%) was similar to subtype C (142, 9.8%) (aHR=1.1, 95% CI 0.8-1.4). In unadjusted analyses, patients with subtype B infection had a much lower risk of VF (309, 5.7%) than subtype C (HR=0.5, 95% CI 0.4-0.7). However this difference was markedly reduced in adjusted analyses (aHR=0.9, 95% CI 0.6-1.2, P=0.41), largely mediated by the effects of exposure group and ethnicity.

**Conclusions:** Patients infected with subtype C virus on a first-line TDF containing regimen appear not to experience a higher rate of VF with differences observed explained by demographic factors rather than a subtype effect. This is a reassuring finding for expanded use of TDF in southern Africa, India, and other areas where subtype C virus predominates.



**485 National Molecular Surveillance of Recently Acquired HIV Infections, Germany 2013-14**

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**Background:** Continuous molecular HIV surveillance provides valuable public health information concerning the transmission of drug resistant viruses and the dynamics of currently circulating variants. To enable an up-to-date molecular analysis of HIV-genotypes circulating in Germany, the Robert Koch Institute (RKI) has established a surveillance

system based on recently acquired HIV infections. The aim is to assess the current prevalence of transmitted drug resistant (TDR) variants and HIV-1 subtypes in the main HIV-transmission groups: men who have sex with men (MSM), women/men with heterosexual contacts (HET) and persons with intravenous drug use (PWIDs) with respect to their origin and place of infection.

**Methods:** Newly diagnosed cases are reported to the RKI as a statutory duty for anonymous notification. Diagnostic laboratories provide dried serum spot (DSS) of ~60% of all newly diagnosed HIV infections reported. DSS serologically classified as “recently acquired infections” (<140 days; BED-CEIA, Sedia) were genotyped in the HIV-*pol*-region to identify TDR and to determine the HIV-1 subtype. The results are linked to notification data from the report.

**Results:** In 2013 and 2014 a total of 1,963/6,371/DSS originated from a recent infection. Of these, 881 were successfully sequenced and analysed. Total TDR was 10.7%, comprising 4.3% with mono resistance to nucleotide reverse transcriptase inhibitors (NRTIs), 2.7% to non-NRTIs, 2.7% to protease inhibitors and 0.6% and 0.3% with dual and triple class resistances, respectively. HIV-subtype B was most prevalent with 76.2%. Non-B infections were identified more often in HET compared to PWIDs or MSM (79%; 39%; 12%, all  $p < 0.05$ ). Non-B subtypes were also more frequently found in patients originating in countries other than Germany (49% vs. 15%;  $p < 0.05$ ) and in patients infected outside of Germany (65% vs. 14%;  $p < 0.05$ ).

**Conclusions:** TDR prevalence in recent HIV infections among notified newly diagnosed HIV patients in Germany remained high (>10%) in 2013/2014 and is comparable to other European countries, including with regard to the proportions of resistance classes. Therefore, genotypic resistance testing of HIV prior to first-line treatment should be continued. Our data also demonstrate that subtype B infections remains the most frequently transmitted subtype in the country based on its high prevalence in MSM.

#### 486 Influence of Transmitted Drug Resistance on CD4 Decline Among ART-Naïve HIV Patients

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**Background:** The presence of transmitted drug resistance mutations (TDRM) may influence the natural history of HIV infection. We evaluated the effect of TDRM on CD4 count decline in a large European cohort collaboration.

**Methods:** Data from several European HIV clinics (ViroLAB, EuResist and EuroSIDA contributing clinics; Royal Free and St Mary's Hospital, London; University of Bari) were merged. Individuals were included if they were aged  $\geq 18$  and had  $\geq 1$  CD4 count and  $\geq 1$  genotypic resistance test before starting antiretroviral therapy (ART). Baseline was defined as the date of the first available CD4 count. TDRM were identified using the WHO 2009 surveillance list, and were presumed to have been present since the time of infection. Linear mixed models with a random intercept and slope were used to estimate the effect of TDRM on CD4 slopes.

**Results:** 6326 individuals were included: 74% were male and 65% infected with a subtype B virus. The median follow-up was 1.2 (IQR=0.07-3.4) years. Overall, 623 individuals (9.9%) had at least 1 TDRM (NRTI:7.0%, NNRTI:3.0% and PI:2.5%). The most common mutations were thymidine analogue mutations for the NRTIs (5.9%), K103N for the NNRTIs (1.8%) and L90M for the PIs (0.9%). The median baseline CD4 count was 418 (IQR=284-580) cells/mm<sup>3</sup>, and there was no evidence that this differed according to the detection of TDRM (426 in those with TDRM v. 417 in those without,  $p=0.14$ ). The viral set point (median of the mean distribution of all pre-ART RNA measurements) was 4.4 log<sub>10</sub> cp/ml among individuals with TDRM and 4.5 among those without ( $p=0.07$ ). In unadjusted models, the overall estimate of CD4 decline was 54 cells/year in the whole population; 56 cells/year among those with TDRM and 54 cells/year among those without (difference=-2.30 cells/year, 95%CI=-9.67; +5.03,  $p=0.54$ ). After adjustment for potential confounders (Table 1), there was no evidence to suggest that the rate of CD4 decline differed according to TDRM presence ( $p=0.29$ ). There was also no evidence to suggest that CD4 slopes differed according to the class of resistance present (Table 1). 71 (1.1%) individuals had M184V, and we could not find any evidence that this was associated with baseline CD4 counts ( $p=0.15$ ) or CD4 slopes ( $p=0.68$ ).

**Conclusions:** In one of the largest European datasets of resistance tests results from ART-naïve individuals, we were not able to find any evidence supporting the hypothesis that the rate of CD4 decline in the absence of ART is different between patients with and without TDRM.

**Table 1. Annual CD4 decline (cells/mm<sup>3</sup>) according to presence of TDRM**

		Unadjusted	Adjusted <sup>1</sup>		
		Slope (95% CI)	Slope (95% CI)	Difference (95% CI)	P-value
Any TDRM	Wild-type (N=5703)	-53.96 (-56.30; -51.63)	-57.39 (-60.20; -54.57)		
	Any TDRM (N=623)	-56.27 (-63.25; -49.29)	-62.16 (-70.55; -53.77)	-4.78 (-13.63; +4.07)	0.29
NRTI <sup>2</sup>	Wild-type (N=5703)	-54.02 (-56.37; -51.68)	-57.41 (-60.23; -54.59)		
	$\geq 1$ NRTI (N=442)	-55.32 (-63.34; -47.31)	-61.09 (-70.74; -51.44)	-3.68 (-13.73; +6.37)	0.47
NNRTI <sup>2</sup>	Wild-type (N=5703)	-53.87 (-56.17; -51.58)	-57.45 (-60.25; -54.65)		
	$\geq 1$ NNRTI (N=189)	-65.58 (-78.89; -52.27)	-72.25 (-88.02; -56.48)	-14.80 (-30.81; +1.22)	0.07
PI <sup>2</sup>	Wild-type (N=5703)	-53.87 (-56.17; -51.58)	-57.34 (-60.12; -54.57)		
	$\geq 1$ PI (N=158)	-45.29 (-58.72; -31.86)	-50.39 (-66.56; -34.22)	6.95 (-9.46; +23.36)	0.41

1. Adjusted for baseline age, gender, mode of infection, subtype, cohort, viral set point, calendar year of the resistance test and baseline CD4 counts. Individuals with missing covariate values (N=749) were excluded from multivariable analyses.

2. Individuals with dual and triple-class resistance could contribute data to all the relevant analyses.

#### 487 No Evidence of Sexual Transmission of Minority HIV Drug Resistance Mutations in MSM

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**Background:** During primary HIV infection, the detection of minority drug resistant mutations (DRM), defined as <20% of sampled population, may be a consequence of (i) sexual transmission, (ii) *de novo* mutations, and/or (iii) technical errors.

**Methods:** Baseline blood samples were collected from 32 HIV+ antiretroviral-naïve, phylogenetically- and epidemiologically-linked male source and recipient partners, within a mean of 64 days (range: 11-170) after the recipient's estimated date of infection; 23 longitudinal samples were available for 11 recipients over a mean of 267 days (range: 11-1,520). Next generation sequencing (NGS) of HIV reverse transcriptase (RT) was performed (Roche 454); filtered reads were screened for nucleoside and non-nucleoside RT inhibitor DRM (Stanford >35). NGS error was estimated using confidence limits for %DRM and a binomial model was used to determine background error rate for each site. The likelihood of sexual transmission of minority DRM was assessed using Bonferroni-adjusted paired t-tests. We estimated the site-wise mutation rate, selection (dN/dS ratio), and linkage between minority DRM and accessory mutations (AM). Longitudinal persistence of minority DRM was assessed by mixed-effects regression analysis adjusting for HIV RNA levels.

**Results:** NGS identified minority DRM in baseline samples from all sources and recipients (mean: 3, range: 1-13), with a total of 139 DRM from 22 sites (9 NNRTI, 13 NRTI) and an average frequency of 4.07% (range: 0.02-15% with average NGS error of 0.0017 mutations/site). In samples evaluated shortly after infection, we found: 1) no association between the presence of minority DRM in the source and recipients (all  $p > 0.1$ ), 2) no increased mutation rates (permutation test  $p > 0.05$ ), 3) no enrichment for diversifying or purifying

selection at DRM sites compared to other sites (Exact  $p > 0.05$ ), and 3) no association between AM and DRM. Longitudinal analyses within recipients revealed a significant decrease in the frequency of minority DRM over time, while overall viral diversity increased ( $p < 0.05$ ).

**Conclusions:** Using data from transmission pairs, we found no evidence of sexual transmission of minority DRM. The presence of minority DRM only during early infection, when effective population size is low, is consistent with the mutation-selection balance hypothesis, in which deleterious mutations (i.e. DRMs) are more efficiently purged from the population later in infection when the larger effective population size allows more efficient selection.

#### 488 Transmitted Drug Resistance in HIV-1 Subtype C Hyperacute Infection

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**Background:** Transmitted Drug Resistance (TDR) in HIV-1 presents a risk for the future success and longevity of Highly Active Antiretroviral Therapy (HAART) especially in resource limited settings in which no genotypic testing is conducted prior to treatment initiation and options for treatment and salvage therapy is limited. For HIV-1 subtype C, the most prevalent subtype in sub-Saharan Africa, there is limited information available on the prevalence of TDR and the persistence of TDR associated mutations, aspects which are integral in informing decisions on future treatment strategies for HIV-1. We investigated the prevalence and persistence of low frequency TDR associated mutations in HIV-1 subtype C hyperacute infection.

**Methods:** HIV uninfected women at high risk for HIV infection were followed and sampled twice weekly as part of a comprehensive prevention and empowerment programme. Plasma samples from 14 participants, who were infected with HIV-1 during study follow-up, were obtained within one week of onset of plasma viremia (OPV) and at regular intervals thereafter and subject to ultra-deep pyrosequencing (454 Life Sciences, Roche Diagnostics). Data was analysed using the amplicon variant analyser where the cut-off for low frequency drug resistance mutations (DRMs) was 1%.

**Results:** UDPS identified low frequency DRMs in 8 out of 14 participants (57%). The K65R nucleotide reverse transcriptase inhibitor (NRTI) associated DRM was the most prevalent low frequency DRM detected (6 out of 14 participants, 47%). Other DRMs detected included the D67N (3.88%) NRTI-associated DRM, the F53L (17.6%) and M46L (6.3%) protease inhibitor (PI) associated DRMs and the T97A integrase strand transfer inhibitor (InSti) associated DRM. The K103N NNRTI-associated DRM was detected in high frequency. Whilst the K103N DRM persisted at 1 year after OPV, the F53L and M46L DRMs reverted as early as 7 days after OPV.

**Conclusions:** Our results showed that low frequency mutations are common in HIV-1 subtype C hyperacute infection. Most of these mutations reverted rapidly and thus would be undetectable by routine methods used in the surveillance of TDR. The possible storage of TDR associated mutations in latent reservoirs raises concern for their reemergence under drug selection pressure and their subsequent impact on treatment outcomes. Further work to identify viral species in latent reservoirs is suggested for this cohort.

#### 489 Integrase Inhibitors-Transmitted Drug Resistance Detected by UltraDeep Sequencing

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**Background:** Transmitted Drug Resistance (TDR) can impair first-line antiretroviral therapy response. Moreover, HIV-1 minority resistant variants (MRV) can be a source of virological failure if they are present before antiretroviral treatment: it was mainly shown for non nucleoside reverse transcriptase inhibitors first line based regimens. Few data are available for TDR Integrase Strand Transfer Inhibitors (ISTIs). In this work, we have studied resistance mutations in integrase gene by Sanger sequencing and UltraDeep Sequencing (UDS) in ISTI-naïve patients.

**Methods:** Integrase genotypic analysis was performed by Sanger sequencing and by UDS. Plasma samples of 65 treatment-naïve Men having Sex with Men (MSM) patients were analyzed from the amino acid 53 to 281. GS Amplicon Variant Analyzer was used to analyze the UDS data, with a detection threshold of MRV of 1% (forward and reverse). Resistance was interpreted according to the last version of ANRS algorithm ([www.hivfrenchresistance.org](http://www.hivfrenchresistance.org)).

**Results:** Among the 65 patients, 60% of them were infected by B subtype. Viruses of six patients harbored majority resistant mutations by Sanger sequencing (four L74I and two E157Q mutations). Three viruses harbored MRV detected by UDS only: two R263K (at a rate of 9.7%, mutational load: 7099 copies/mL; and 13.5%, 8345 copies/mL) and one E138K mutations (at 4.8%, 111 copies/mL). All these mutations were retrieved among B subtype viruses.

**Conclusions:** None of the three classical ISTIs signature resistance mutations (at positions 143, 148 and 155) were retrieved. However, in this population of MSM naïve-treatment patients, the prevalence of ISTI-resistance mutations, mainly related to polymorphisms, seems to be relatively high (9.2% by Sanger and 13.8% by UDS). In conclusion, with the increase use of ISTIs in clinical practice, TDR for this therapeutic class should be carefully monitored in the future, as well as the impact of these MRV on the virological response.

#### 490 Linkage of Rare Drug Resistance Mutations Detected by New Ultrasensitive SGS

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**Background:** Targeted next generation sequencing (NGS) is a powerful tool for detecting low frequency, HIV-1 drug-resistant mutations but frequent *in vitro* recombination prevents accurate detection of linked mutations and assessment of phylogenetic relationships. We developed a new NGS-based ultrasensitive single-genome sequencing (uSGS) assay that eliminates *in vitro* recombinants and reduces PCR errors to investigate linkage of resistance mutations and the phylogenetics of variants in plasma samples.

**Methods:** NGS uSGS libraries were generated by tagging cDNA molecules from plasma RNA with primer IDs, optimizing PCR, and generating overhangs on amplicons for efficient ligation of Illumina adaptors. Libraries were sequenced with paired-end Illumina MiSeq technology. A modified version of the algorithm of Zhou, *et al.* was used to remove sequences whose primer IDs contained PCR/sequencing errors. The uSGS pipeline further eliminated PCR recombinants and PCR/sequencing errors by applying a “>80% majority rule” to each site in alignments of reads with the same primer ID. uSGS data were analyzed for unique linkage patterns and for phylogenetics using neighbor-joining (NJ) analyses.

**Results:** Using the uSGS assay, a median of 1227 SGS were obtained from each of 3 plasma samples from 2 ART-experienced donors. The presence of clusters of resistant variants on independent nodes of NJ trees implied that resistant variants emerged independently and diversified primarily due to stochastic changes rather than from *in vivo* recombination with other variants. Within clusters of variants, rare, linked resistance mutations were detected in each sample. In the 1st sample from one donor, a single variant was detected with linked mutations in RT at codons 106, 108, and 101 (0.06% frequency) on a background of 67N (98.9% aac). In a 2nd sample from the same donor obtained 2 weeks later, a different 67N mutation (aat, 1.1%) was linked to the previously detected rare mutations at codons 106 and 101. Similarly, in plasma from the second donor, rare (0.08% frequency) linked mutations were detected at codons 70, 108, and 184.

**Conclusions:** The new ultrasensitive SGS assay described here can detect rare, linked mutations at drug resistance sites and permits accurate phylogenetic analyses of HIV variants. This capability will improve the understanding of resistance evolution and could help identify individuals at risk of treatment failure because of linked resistance mutations.

**491LB Large-Scale Transmission and Clustering of HIV Protease Resistance in Ontario, Canada**

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**Background:** Transmitted HIV drug resistance has important potential clinical and public health implications. Transmission of significant resistance to HIV protease inhibitors is particularly uncommon. Pre-therapy HIV drug resistance genotyping has been recommended as standard of care in Ontario, Canada since 2005, and subsequent to 2014, performed automatically on "new" viral load requests. Since the implementation of "reflex" genotyping, an unusual pattern of HIV drug resistance (very high PI resistance without substantial nRTI or NNRTI resistance) was observed in multiple patients sent for routine pre-treatment testing.

**Methods:** We reviewed the epidemiologic and clinical characteristics of all HIV-positive individuals in care in the province of Ontario, Canada. Phylogenetic trees were inferred for HIV pol sequences from the first sample for each patient for whom testing was available (N=11,550 patients). Tip-to-tip distances (patristic distance < 0.02) between sequences from different individuals on the phylogeny were used to define clusters. Resistance data and patient characteristics were super-imposed on phylogenetic trees to identify clusters of transmitted protease resistance.

**Results:** There were 49 untreated patients with PI resistance identified in a single large cluster. Typically each patient had at least seven PI mutations detected at the first pre-treatment genotype (all of 10I,33F,48V,54T,71V,74S and 82A), conferring significant levels of inferred resistance to most PIs except darunavir. These mutations were usually observed in combination with nRTI "revertant" mutations (41L and 215L or S).

Resistant patients were observed from 2005 (N=4) to present, with the majority observed in recent years (N=9 in 2014 and 17 in 2015). All sequences clustered closely together in a phylogenetic tree. All patients were male, and the median age was 29 years at the time of sampling. The majority of cases (80%) were observed in Toronto. Isolated cases with a similar resistance profile have also been observed in other provinces; however the vast majority have been in Ontario.

**Conclusions:** High level protease inhibitor resistance can occur with sufficient replicative fitness to circulate for more than a decade in the community, suggesting the potential for transmission of extensively drug resistant HIV, which could threaten our treatment paradigms. Systematic surveillance of HIV resistance in untreated individuals remains important even as the incidence of resistance in treated populations declines overall.

**492LB Prevalence and Incidence of Integrase Drug Resistance in BC, Canada, 2009–2015**

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**Background:** In British Columbia (BC), use of the integrase inhibitors (INI) raltegravir (RAL), elvitegravir (EVG) and dolutegravir (DTG) in antiretroviral (ART) regimens has increased from 10% of ART-treated persons in 2009 (540 RAL-treated) to 32% in 2015 (978 RAL, 500 EVG and 1011 DTG). This study characterizes the evolution of incident and prevalent INI resistance from 2009 to Oct. 2015 and tests the hypothesis that prevalence of INI drug resistance mutations is increasing over time.

**Methods:** HIV-1-infected persons age ≥19 years were included if they received ART and drug resistance testing through BC Centre for Excellence in HIV/AIDS programs between Jan-2009 and Oct-2015.

Persons with INI or other (reverse transcriptase, protease) resistance were defined as those having at least one sample with a score ≥30 (intermediate or high level resistance) by the Stanford HIV drug resistance algorithm v7.0.1.

Incident cases of INI resistance were counted in the first year they appeared and were categorized by the INI temporally associated with new resistance: RAL, EVG, DTG, or Unclassifiable (resistance predated INI use in BC).

Prevalent cases of INI and other resistance and INI mutations were counted in each year they appeared. Annual prevalence/1000 ART-treated persons was calculated at year end (31-Oct in 2015). Changes in prevalent resistance over time were tested for trend (generalized additive model, R© v3.2.2).

**Results:** A total of 57 persons had intermediate or high level INI resistance. Prevalence of INI resistance/1000 ART-treated persons increased from 1.07 in 2009 to 6.8 in 2015, see Figure 1a (trend p<0.001, R<sup>2</sup> 0.99). During this period, other ART resistance declined from 331 to 285/1000 (trend p<0.001, R<sup>2</sup> 0.98). Figure 1a depicts evolution of prevalent INI resistance mutations.

Until 2013, most new cases of INI resistance were associated with RAL use (Figure 1b). In 2014 and 2015, 8/19 (42%) of new INI resistance followed EVG or DTG use: Five cases were associated with EVG (mutations: two 66A/I and one each 92Q, 145S, 147G) and three emerged during DTG therapy (mutations: 66I and two 263K). Seven persons were ART experienced and one (DTG, 66I) was ART naïve.

**Conclusions:** The prevalence of INI resistance remains low compared to other ART resistance, but is increasing with expanded INI use. Emergent INI resistance has been observed during treatment with RAL, EVG and DTG in ART naïve and experienced patients.

Evolution of Integrase (INI) Drug Resistance Mutations Among Antiretroviral (ART)-Treated Persons in British Columbia

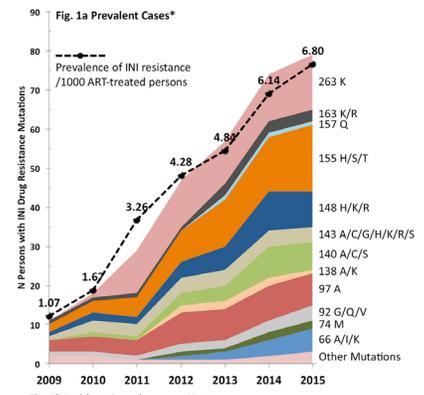


Fig. 1a Prevalent Cases\*

Fig. 1b Incident Cases\*

Year	2009	2010	2011	2012	2013	2014	2015
N	6	4	12	8	8	11	8
n	n	n	n	n	n	n	n
INI drug exposure	0	0	0	0	0	0	3
Dolutegravir	0	0	0	0	0	2	3
Elvitegravir	6	3	9	8	7	7	2
Raltegravir	0	1	3	0	1	2	0
Unclassifiable	0	0	0	0	0	0	0

\*Persons with Integrase score ≥30, Stanford HIV drug resistance algorithm v7.0.1

**493LB Divergent ARV Resistance at Screening for ACTG A5288 Study of 3rd-Line ART in RLS**

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**Background:** HIV-1 drug resistance remains an important cause of failure of 2nd-line antiretroviral therapy (ART) but limited resistance data exist from resource-limited settings (RLS). We analyzed the frequency and patterns of resistance at screening for ACTG A5288: a study of 3<sup>rd</sup>-line ART after prior exposure to NRTI, NNRTI and PI in RLS.

**Methods:** Plasma samples from a screening visit for enrollment into A5288 had protease and reverse transcriptase genotyped in real time by DAIDS-certified laboratories in RLS. Resistance mutations and scores were determined using the Stanford algorithm (v6.2). Subtype was determined by phylogenetic analysis. Associations of drug class resistance with HIV-1 subtype, screening HIV RNA, and nadir CD4 were evaluated.

**Results:** 665 plasma samples were available for analysis from Asia (171, 26%), South America (138; 21%), Eastern Africa (180; 27%), Southern Africa (170, 26%) and other locations (6; <1%). Median age was 41, 48% female, median HIV RNA 4.5 log<sub>10</sub> cps/ml and median nadir CD4 65 cells/mm<sup>3</sup>. HIV subtype C (48%), B (20%) and A1 (18%) were most common. Prior exposure to NVP (63%) was more common than EFV (56%). At time of screening, TDF (67%) and 3TC (90%) were the most commonly prescribed NRTIs with either LPV/r (55%) or ATV/r (43%); 6% had RAL exposure. High-level or intermediate resistance was common (Table): 519 candidates (78%) had resistance to one or more drugs. 137 samples (21%) had resistance to 1 drug class, 207 (31%) to 2 drug classes, and 175 (26%) to all 3 drug classes (NRTI, NNRTI, PI). However, 461 (69%) showed susceptibility to 2<sup>nd</sup>-line regimens (1

NRTI + ATV/r or LPV/r) and the majority were susceptible or had low-level resistant to DRV/r (97%) and ETR (79%). Nadir CD4 count but not screening HIV RNA was significantly associated with the number of resistant drug classes but quantitative differences were small and unlikely to be helpful clinically (Table).

**Conclusions:** Highly divergent resistance profiles were observed among study candidates being evaluated for 3<sup>rd</sup>-line ART in RLS. The majority remained susceptible to 2<sup>nd</sup>-line regimens (69%) but others had high level resistance to 2 (31%) and 3 drug classes (26%). Routine clinical parameters were not discriminatory for the extent of resistance. These results indicate that objective measures of ART adherence and access to both resistance testing and newer ARVs are needed to guide 3<sup>rd</sup>-line ART in RLS.

**Table: High-level or Intermediate Resistance to Antiretroviral Drug Classes defined by the Stanford Algorithm version 6.2 and Association with Nadir CD4 count.**

Number of classes with resistance	Total	Nadir CD4 count (cells/mm <sup>3</sup> )			P-value**
	N=665	N=56*	<200 N=564	>200 N=92	
Susceptible/Low resistance to all 3 drug classes	146 (22%)	142 (22%)	114 (20%)	28 (30%)	0.006
Resistance to 1 drug class	137 (21%)	137 (21%)	118 (21%)	19 (21%)	NS
Resistance to 2 drug classes	207 (31%)	206 (31%)	176 (31%)	30 (33%)	NS
Resistance to 3 drug classes	175 (26%)	171 (26%)	156 (26%)	15 (16%)	NS

\* 9 participants had missing nadir CD4 count  
 \*\* P-value adjusted for screening log<sub>10</sub> HIV-1 RNA and HIV-1 subtype  
 NS-not significant

**494LB Prospective Randomized HIV Drug Resistance Testing of Kenyans Before First-Line ART**

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**Background:** Testing for HIV drug resistance prior to initiation of ART has been found to be cost effective in high-resource communities and is recommended by advisory panels, but due to costs is not used in resource-limited communities. The prevalence of transmitted HIV drug resistance has increased in Kenya during the past ten years. We hypothesized that a relatively simple and inexpensive point mutation assay could identify HIV drug resistance in individuals qualifying for 1<sup>st</sup>-line ART in Kenya, and that use of the test results to choose the ART regimen would improve virologic suppression after 12 months of treatment.

**Methods:** A randomized clinical trial tested Kenyans qualifying for 1<sup>st</sup>-line ART using a quantitative oligonucleotide ligation assay (OLA). Each subject randomized to OLA was tested at codons 103, 181, 184 and 190 in *pol* reverse transcriptase to detect and quantify mutations as <10% (not resistant) or ≥10% (resistant) within their quasispecies. Subjects randomized to receive pre-ART OLA with ≥1 resistance mutations were given 2<sup>nd</sup>-line ART with lopinavir/rt, whereas each individual randomized to "no OLA" received standard of care (SOC) non-nucleoside reverse transcriptase based ART. Plasma HIV RNA was measured pre-ART, at 12 months of ART, and if >40c/mL, again at 15 months.

**Results:** OLA was implemented at Coptic Hope Center in Nairobi. A total of 991 subjects enrolled into the study in 2013/14. The overall prevalence of resistance was 8.3%. The 12-month study was completed in November 2015.

Testing for HIV drug resistance using a relatively simple and inexpensive point mutation OLA was feasible in Kenya. The OLA testing and initiation of 2<sup>nd</sup>-line lopinavir/rt-based ART did not significantly impact the overall rate of virologic suppression (1<sup>o</sup> outcome). Among individuals with resistance detected, rates of virologic suppression were improved by pre-ART OLA testing (2<sup>o</sup> outcome).

**Conclusions:** OLA testing for resistance did not improve rates of ART suppression at the population level. Because OLA testing improved outcomes of those with resistance, this study suggests that virologic failure was principally due to other factors. If rates of transmitted resistance continue to increase to increase in Kenya, OLA testing may positively affect overall rates of HIV suppression, and currently, identifying individuals with resistance and starting them on 2<sup>nd</sup>-line lopinavir/rt-based ART may reduce transmission of drug resistance.

Table: HIV drug resistance, deaths, loss-to-follow-up & HIV RNA outcome			
	OLA	SOC	P value
Individuals enrolled >2 years of age	494	497	
HIV resistance ≥10% at entry	46 (9.3%)	36 (7.3%)	0.250
HIV resistance <10% at entry	16 (3.3%)	17 (3.4%)	1.000
Deaths	30 (6.1%)	39 (7.8%)	0.318
Deaths with ≥10% resistance	5 (1.0%)	2 (0.4%)	0.286
Deaths with 2-9% resistance	1 (0.2%)	1 (0.2%)	1.000
Lost-to-follow-up (LTFU)	54 (10.9%)	45 (9.1%)	0.342
Prior to ART	7 (1.4%)	3 (0.6%)	0.223
LTFU with ≥10% resistance	5 (1.0%)	6 (1.2%)	1.000
LTFU with 2-9% resistance	4 (0.8%)	2 (0.4%)	0.451
HIV RNA >400c/mL at 12 ART	38/409 (9.3%)	47/408 (11.5%)	0.305
HIV resistance ≥10% at entry	5/36 (13.9%)	14/28 (50.0%)	0.002
HIV resistance 2-9% at entry	3/11 (27%)	5/15 (33%)	1.000

**495 More Efficacious Drugs Lead to Hard Selective Sweeps in HIV Drug Resistance Evolution**

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**Background:** In the early days of HIV treatment, drug resistance occurred rapidly and predictably in all patients, but under modern treatments, resistance arises slowly, if at all. The probability of resistance should be controlled by the rate of generation of resistance mutations. If many resistance mutations arise simultaneously, evolution of resistance proceeds by soft selective sweeps in which multiple adaptive mutations spread concomitantly, but if resistance mutations occur rarely in the population, then a single mutation can spread alone in a hard selective sweep. We look for genetic signatures to test the hypothesis that the transition from fast to slow evolution of drug resistance was accompanied by a transition from soft to hard selective sweeps.

**Methods:** We examine 6,717 HIV-1 direct PCR sequences from patients treated with first-line therapies between 1989 and 2013 from the Stanford HIV Drug Resistance Database and determine genetic diversity and the number of drug resistance mutations for each sequence. We fit generalized linear models for each type of treatment to measure how a drug resistance mutations taking over the virus population in a patient affects viral population diversity. This effect should be small if resistance establishes via soft sweeps, but large and negative if hard sweeps predominate.

**Results:** We find that resistance to treatments with low clinical efficacy generates patterns consistent with soft sweeps, as marked by the relatively small decrease in diversity associated with resistance. In contrast, populations receiving treatments with high clinical efficacy showed large decreases in diversity associated with a drug resistance mutation taking over the population within a patient, a pattern more consistent with hard sweeps. Among patients given treatments with 30% efficacy, sequences with 3 DRMs are predicted to have marginally fewer ambiguous calls as those with 0 DRMs (0.5 fewer ambiguous reads over 1000 bases), but among those patients given treatments with 80% efficacy, sequences with 3 DRMs are predicted to have 10 fewer ambiguous calls than those with 0 DRMs over 1000 bases, a substantial decrease in genetic diversity.

**Conclusions:** We confirm that the transition from fast to slow evolution of drug resistance was indeed accompanied with the expected transition from soft to hard selective sweeps. These results suggest that effective drugs may push HIV-1 populations into a hard sweep regime in which populations must wait long periods of time for the correct mutation.

#### 496 Pooled Week 48 Analysis of HIV-1 Drug Resistance in E/C/F/TAF Phase 3 Studies

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**Background:** Seven ongoing Phase 3 studies are evaluating the efficacy and safety of the elvitegravir (E)/cobicistat (C)/emtricitabine (F)/tenofovir alafenamide (TAF) fixed dose combination (E/C/F/TAF) in ART-naïve adult (GS-US-292-0104 and GS-US-292-0111) and adolescent (GS-US-292-0106) subjects, virologically suppressed subjects with (GS-US-292-0119) or without (GS-US-292-0109)  $\geq 2$  class resistance, subjects with mild to moderate renal impairment (GS-US-292-0112), and subjects with HIV/HBV co-infection (GS-US-292-1249). Virologic success rates of E/C/F/TAF at Week 48 using FDA snapshot analysis and HIV-1 RNA  $< 50$  copies/mL was high and similar among all studies (86.6–97.2%) and showed non-inferiority to comparator arms. Here we present a pooled Week 48 resistance analysis for these Phase 3 studies across the different treatment populations.

**Methods:** Genotypic analyses were performed at screening to assess HIV-1 protease (PR), reverse transcriptase (RT) and integrase (IN) susceptibility to study drugs. Confirmed virologic failure visits through Week 48 or at discontinuation with  $\geq 400$  copies/mL HIV-1 RNA were analyzed for emergent genotypic and phenotypic resistance.

**Results:** A total of 2308 subjects were enrolled in these E/C/F/TAF studies. Among ART-naïve adults, 16 of 866 were analyzed; 7 (0.8%) developed NRTI RAMs (M184V/I, n=7; K65R, n=1) and also primary INSTI RAMs (T66I/A, n=2; E92Q, n=2; Q148R, n=1; N155H, n=1). Among ART-naïve adolescents, 2 of 50 subjects were analyzed and did not develop RAMs. Among virologically suppressed subjects, 4 of 959 were analyzed; 1 developed resistance (M184M/I) and resuppressed to  $< 50$  copies/mL before treatment discontinuation. Among virologically suppressed subjects with prior  $\geq 2$  class resistance, none of the 110 subjects met the analysis criteria. Among renally impaired subjects, 2 of 248 were analyzed; both subjects had multi-class resistance detected: 1 pre-existing and 1 due to possible re-infection followed by resuppression to  $< 50$  copies/mL. Among HBV co-infected subjects, 0 of 75 subjects met the analysis criteria.

**Conclusions:** In these 7 Phase 3 studies, E/C/F/TAF achieved high rates of virologic suppression through 48 weeks of treatment. The presence of PI, NRTI, or NNRTI RAMs at baseline did not affect treatment response. Resistance development to  $\geq 1$  components of E/C/F/TAF was rare in all studied populations, even in highly treatment-experienced subjects switching to E/C/F/TAF.

#### 497 HIV Drug Resistance Testing Among Patients New to HIV Care in the United States

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**Background:** To guide antiretroviral therapy (ART), the U.S. Department of Health and Human Services and the International Antiviral Society—USA recommend baseline genotypic HIV drug resistance testing for HIV-infected persons at entry into care or as soon as possible after diagnosis. We assessed reported HIV genotype testing of patients new to HIV care among HIV care providers in the United States.

**Methods:** We used data collected during 06/2013–01/2014 through the Medical Monitoring Project HIV Provider Survey, which was administered to a nationally representative sample of HIV care providers in the United States. Providers were asked for what proportion of patients new to HIV care they order HIV genotype testing as part of the initial evaluation; we included responses from 1,193 providers who answered this question. We weighted the data to account for unequal selection probabilities and non-response. We performed bivariate analyses and calculated prevalence ratios (PR) and 95% confidence intervals (CI) to examine differences in genotype testing for all patients by provider, practice, and medical care characteristics, including qualifications as an HIV specialist as defined by the HIV Medicine Association or the American Academy of HIV Medicine.

**Results:** In all, 84.5% (CI=80.4%–88.5%) of providers reported ordering genotype testing for all patients; 8.8% (CI=5.9%–11.7%) for more than half, but not all patients; and 6.7% (CI=2.8–10.6%) for half of patients or fewer. Ordering genotype testing for all patients was significantly less common among: non-specialists (PR=0.89, CI=0.80–0.99) compared with HIV specialists; providers in private practices (PR=0.91, CI=0.82–1.00) compared with those at other facility types; and providers who first prescribe ART based on CD4 count (PR=0.83, CI=0.75–0.91) compared with providers who prescribe ART regardless of CD4 count.

**Conclusions:** Most providers in the United States reported ordering genotype testing for all patients new to HIV care. Providers in private practice and non-specialists were less likely to order genotype testing for all patients and may benefit from additional support to implement drug resistance testing guidelines. As providers move toward adopting guidelines for universal ART prescription, we may also see increasing adoption of baseline genotype testing recommendations.

#### 498 Protease Inhibitor Resistance at 2nd-line HIV Treatment Failure in Sub-Saharan Africa

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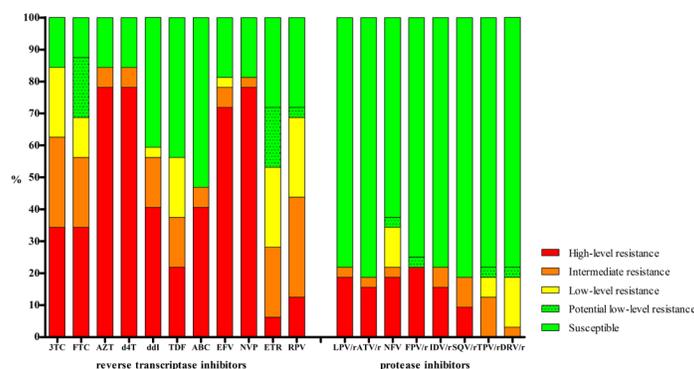
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**Background:** As antiretroviral therapy (ART) programs in sub-Saharan Africa mature, increasing numbers of HIV-positive people will experience treatment failure, and require second- or third-line ART. It is yet unclear how many patients will develop protease inhibitor (PI) resistance and need third-line ART regimens.

**Methods:** HIV-1 positive adults were enrolled in the PanAfrican Studies to Evaluate Resistance Monitoring (PASER-M) cohort, at the time of switch to second-line PI-based ART, and included in the analysis if they received  $>180$  days of second-line ART. We assessed risk factors for virological failure (viral load  $>400$  cps/ml) after up to 3 years of second-line PI-based ART using Cox models. If viral load was  $\geq 1,000$  cps/ml, *pol* genotyping was performed. Drug resistance mutations were scored using the 2014 IAS-USA drug mutation list and genotype susceptibility was calculated using the Stanford algorithm Version 7.0.

**Results:** Of 227 included participants, 25.0% (n=54/216) experienced virological failure at some point during follow-up at a rate of 138.9 failures (95%CI 106.4–181.3) per 1,000 person-years. In multivariable analysis the risk factors for virological failure were: failing a non-standard non-nucleoside reverse transcriptase inhibitor (NNRTI)-based first-line regimen (hazard ratio [HR] 7.10; 95%CI 3.40–14.83;  $p<0.001$ ) or PI-based first-line regimen (HR 7.59; 95%CI 3.02–19.07;  $p=0.001$ ) compared to ZDV/3TC/NNRTI, PI-resistance at switch (HR 6.69; 95%CI 2.49–17.98;  $p<0.001$ ) and  $<95\%$  adherence (HR 3.05; 95%CI 1.71–5.42;  $p=0.025$ ). For 32/43 (74%) participants with VL  $\geq 1,000$  cps/ml during follow-up, genotypic data was available. At least one drug resistance mutation was found among 22/32 (69%) participants. Major PI mutations were detected in 7 (21.9%). The acquired mutations conveyed reduced susceptibility to all PIs (**figure**).

**Conclusions:** While over 85% of participants on 2<sup>nd</sup>-line ART had viral suppression after up to 36 months, major PI resistance was detected in 22% of those failing second-line ART. This represents approximately 3% of people initiating 2<sup>nd</sup>-line ART. Future treatment of these individuals require third-line drugs (i.e. darunavir/ritonavir, etravirine and raltegravir), which are currently unavailable in sub-Saharan Africa. To ensure long-term ART success, availability of third-line drug options, preferably guided by HIV drug resistance testing, is urgently needed.



**Figure. Antiretroviral drug resistance at second-line failure.**  
Among 32 participants with plasma HIV viral load >1,000 RNA copies/ml and genotype available during follow up, genotype susceptibility of the reverse transcriptase inhibitors and protease inhibitors were calculated using the Stanford algorithm version 7.0.2. When multiple genotypes were available for the same participant, the most conservative susceptibility score measured was used (i.e. the highest level of resistance), including genotypes at switch to second-line.

3TC: lamivudine, FTC: emtricitabine, AZT: zidovudine, d4T: stavudine, ddI: didanosine, TDF: tenofovir, ABC: abacavir, EFV: efavirenz, NVP: nevirapine, ETR: etravirine, RPV: rilpivirine, LPV: lopinavir, ATV: atazanavir, NFV: nelfinavir, FPV: fosamprenavir, IDV: indinavir, SQV: saquinavir, TPV: tipranavir, DRV: darunavir, r: ritonavir (booster).

**499 Genome-Wide Association of HIV Whole Genomes Provides Insights Into Drug Resistance**

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**Background:** Genome-wide association studies (GWAS) have considerably advanced our understanding of human traits and diseases. With the increasing availability of whole genome sequences (WGS) for pathogens, it is important to establish whether GWAS of viral genomes could reveal important biological insights. Here we perform the first proof of concept analysis examining the selection of antiretroviral therapy (ART) associated variants.

**Methods:** We performed a GWAS of drug resistance (DR) in a sample of 343 HIV subtype C patients failing 1<sup>st</sup> line treatment in rural KwaZulu-Natal, South Africa. The majority and minority variants within each sequence were called using GATK and PILON, and GWAS was performed within PLINK. HIV WGS from patients exposed to different antiretroviral drugs (zidovudine, stavudine, tenofovir, efavirenz, nevirapine and lopinavir) were compared to sequences derived from individuals naïve to the respective treatment.

**Results:** GWAS methodology was validated by identifying five associations on a genetic level that led to amino acid changes known to cause DR. Further, we identified two variants within amino acid 68 of the reverse transcriptase protein associated with tenofovir exposure (p-value=5.38E-06 & 1.45E-05; Odds Ratio=11.9 & 2.89) previously described as potential fitness compensatory mutations. We replicated these associations in the Stanford University HIV Drug Resistance Database (488 exposed vs. 9,357 unexposed, p<0.001). We also identified a possible additional DR variant for tenofovir within amino acid 91 of the matrix region of the Gag protein. Replication in publicly available datasets was not possible here due to the lack of Gag sequences.

**Conclusions:** These results validate the applicability of GWAS to HIV WGS data with respect to phenotypes with large genetic effects such as DR. The sample size required was also relatively small. The data also highlight how GWAS can provide novel and possibly clinically relevant insights into pathogen genomes in an era of high throughput sequencing.

**500 Receipt and Timing of Genotypic HIV Drug Resistance Testing in the United States**

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**Background:** The U.S. Department of Health and Human Services recommends genotypic HIV drug resistance (DR) testing upon entry to care; however, receipt and timing of DR testing has not been well characterized. We examined DR testing at and after initiation of HIV care in the United States.

**Methods:** We analyzed data from the U.S. National HIV Surveillance System (NHSS) for persons aged ≥ 13 years with HIV infection diagnosed in 2013 who were linked to care (i.e., had a CD4 count or viral load test) within 3 months of diagnosis and resided in a jurisdiction with complete laboratory reporting and high reporting of nucleotide sequence data from DR testing (Los Angeles County, Michigan, New York, South Carolina, Texas, and Washington). We assessed the proportion of individuals who received DR testing at or after linkage and the distribution of time between linkage to care and DR testing. Among those who received DR testing, we conducted Mantel-Haenszel chi-square tests to identify factors associated with testing at the same time (i.e., in the same month) as linkage to care.

**Results:** Of 11,351 persons in these jurisdictions who received a diagnosis of HIV infection during 2013, 9,435 (83%) were linked to care within 3 months of diagnosis. Among those linked to care, 6,106 (65%) ever received DR testing and 5,996 (64%) received DR testing within 12 months of linkage to care. Of those tested within 12 months of linkage, 4,195 (70%) received DR testing in the same month as linkage and an additional 1,153 (19%) within 1 month of linkage. The proportion of individuals who received testing at the time of linkage differed significantly across racial/ethnic groups (p = .01) and age groups (p = 0.03). The proportion receiving DR testing at linkage was lower among blacks/African Americans (66%) compared to whites (71%) and Hispanics/Latinos (70%), and among those aged < 35 years (67%) compared to older individuals (70-71%).

**Conclusions:** NHSS data indicate that almost two-thirds of HIV-infected persons linked to HIV care received DR testing within 12 months of initiation of care, which may be an underestimate if not all DR tests were reported to surveillance. The timing of DR testing suggests that most providers order DR testing at entry to care as recommended.

**501 HIV Integrase Genotypic Resistance Testing Among HIV-Infected Persons in New York**

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**Background:** Data on integrase (IN) inhibitor resistance come mainly from clinical trials and in vitro studies and there are few population-based IN resistance studies. Since IN inhibitors-based regimens are now commonly recommended as initial regimens but routine screening for IN resistance is not recommended, it is important to monitor population-based information on IN resistance among the newly diagnosed HIV patients.

**Methods:** Laboratories conducting genotypic resistance testing for residents of New York State (NYS) are required to report the nucleic acids sequences to NYS Department of Health (DOH). HIV integrase genotypic resistance tests (IN-GRTs) received 12/09 through 7/15 were paired with cases' PR-RT tests for the same date. Sequences were analyzed by NYSDOH's in-house Resistance Analysis System. The first IN test result for each individual was linked to case in NYS' HIV surveillance registry. IN tests within 3 months of HIV diagnosis were classified as "initial". Time from diagnosis to IN test, case demographics, IN resistance, and the relation of IN and PR-RT resistance were examined.

**Results:** 5,627 IN tests were linked to NYS HIV registry; 4,208 (75%) had paired PR-RT tests. 3,533 (63%) cases were stage 3 HIV infection at time of IN testing. Among the 4,626 cases where the IN test was not an initial test, 63% of first IN tests occurred  $\geq 11$  years after HIV diagnosis. Resistance to  $\geq 1$  IN drug was seen in  $< 1\%$  of 1,001 initial tests and in 8.6% of 4,626 non-initial tests. Among 3,415 non-initial tests for which paired PR/RT sequences were obtained, 400 (11.7%) showed IN resistance, 231 (6.8%) showed resistance to  $> 1$  drug class and 55 (1.6%) showed resistant to all drug classes. Of 3,515 newly diagnosed HIV cases in NYS in 2014, 16% had an initial IN test, compared to 55% who were tested for PR-RT. Higher rates of initial IN testing were seen among males (18%), Whites and Hispanics (19%), MSM and MSM/IDU risk (21%), and New York City residents (18%).

**Conclusions:** Our data shows the rate of IN testing at time of diagnosis is rising but is lower than PR-RT testing, which is consistent with the recommendations. Infrequent resistance seen in the initial-test group suggests transmitted IN inhibitor resistance is not a major concern at present. Initial IN testing varies across population subgroups. Time from HIV diagnosis to IN test and percent stage 3 HIV infection in our study suggests that in this period the majority of IN-GRTs were ordered for long-standing cases at advanced stage of disease.

## 502 Immunologic Criteria Are Poor Predictors of Virologic Outcomes in Nigeria

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**Background:** Nigeria has made significant gains in scaling-up access to HIV prevention, treatment and support services and by the end of 2014, provided antiretroviral therapy (ART) to over 747,382 individuals. The main objective of this study was to determine predictors of immunologic failure in absence of routine viral load (VL) monitoring.

**Methods:** This was a retrospective cohort study of 12,456 HIV-infected patients enrolled into HIV care at the University of Abuja Teaching Hospital between February, 2005 and December, 2014. Immunologic failure defined as having a decrease in CD4 cell count to pretherapy baseline level (or below) or persistent CD4 levels  $< 100$  cells/mm<sup>3</sup> after 6 months on ART. HIV genotyping was performed on a subset of patients with 2 consecutive VL measurements  $> 1,000$  copies/ml. To identify predictors of immunologic failure, univariate and multivariate analyses were performed using log binomial models to estimate relative risks (RR) and confidence intervals. All statistical analyses were performed with the statistical software package SAS release 9.1 (SAS Institute Inc, Cary, North Carolina).

**Results:** A total of 5,928 patients who initiated ART were included in the analysis. The entry point for 3,924 (66.2%) was through VCT, 3,468 (58.5%) were initiated on NVP containing regimen and 2,140 (36.1%) initiated on TDF, baseline CD4 was  $268 \pm 23.7$  cells/ul, and mean VL was  $3.3 \pm 1.3 \log_{10}$  copies/ml. Among 2,602 patients with immunologic failure, 868 (33.3%) had VL measurements and 381 (43.9%) of these had a detectable VL. Fifty six samples (56/198; 28.3%) had no resistance; 160 (80.1%) harbored NRTI resistance; 151 (76.3%) M184I/V; 29 (14.6%) had  $\geq 3$  TAMs, and 37 (18.7%) had K65R, of which all were on TDF. One hundred and sixty-two samples (81.8%) harbored NNRTI resistance; 72 (36.4%) Y181C and 68 (34.3%) K103N with 53 % having  $\geq 2$  efavirenz associated mutations. Service entry point [RR (95%CI): 0.79 (0.64–0.91);  $p < 0.001$ ]; being on NVP containing regimen [RR (95%CI): 1.21 (0.99 – 1.45);  $p = 0.023$ ]; WHO stage III or IV [0.76 (0.60 – 0.96);  $p = 0.013$ ]; baseline CD4 cell count  $< 200$  cells/ul [0.19 (0.16 – 0.22);  $p < 0.001$ ]; male gender [1 (1.07–1.40);  $p = 0.005$ ] were associated with immunologic failure.

**Conclusions:** Immunologic criteria for failure erroneously classified patients without virological replication as failing therapy in our program. Clinico-immunological monitoring without viral testing resulted in frequent unnecessary ART regimen switches and accumulation of HIV drug resistance mutations.

## 503 Global Tenofovir Resistance Following 1st-Line Regimens for Adult HIV-1 Infection

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**Background:** Antiretroviral therapy (ART) is pivotal for controlling HIV-1 infection through widescale treatment as prevention (TasP) and pre exposure prophylaxis (PrEP).

Tenofovir (TDF) is a key component of both approaches, though few data exist on regional burden of TDF resistance and the risk factors for its emergence. Tenofovir (TDF) is a key component of both approaches, though few data exist on regional burden of TDF resistance and the risk factors for its emergence.

**Methods:** We conducted an international multi-centre retrospective study of individuals undergoing genotyping following virological failure with 1<sup>st</sup>-line TDF-containing ART (with a 3TC or FTC plus either efavirenz (EFV) or nevirapine (NVP)). Meta-analysis and multiple logistic regression were used to identify covariates associated with emergence of TDF resistance (defined as presence of K65R/N or K70E/G/Q mutations).

**Results:** Prevalence of TDF resistance amongst 1926 patients in 36 countries with treatment failure (as locally defined) was highest in low and middle income regions: 59.8% West/Central, 55.9% in Eastern and 55.2% in Southern Africa; 39% in Asia; 35.3% in Latin America, and lowest in high income regions: 18.8% in Western Europe and 22.6% in North America. Pre-ART CD4 cell count was associated with TDF resistance across regions (OR 1.49 (1.26–1.77) for CD4 count  $< 100$  cells/mm<sup>3</sup> versus  $\geq 100$  cells/mm<sup>3</sup>). Use of 3TC versus FTC and NVP versus EFV increased the risk of tenofovir resistance [OR 1.49 (1.20 – 1.84)] and [OR 1.46 (1.28–1.67)] respectively across regions. The mean plasma viral load at virological failure was not different in the presence or absence of TDF associated mutations [145,700 copies/ml (SE 12,480) versus 133,900 copies/ml (SE 16650),  $p = 0.626$ ].

**Conclusions:** TDF resistance emerges in a high proportion of patients who develop virological failure on a TDF-containing first line regimen in low-middle income regions. The risk of TDF resistance was also independently associated with a pre-ART CD4 count  $< 100$ , the use of 3TC compared with FTC, and the use of EFV compared with NVP. Based on viral loads at failure, TDF-resistant viruses have the potential to be as transmissible as viruses without TDF resistance.

## 504 Incompatibility of HIV-1 Resistance to Both Cenicriviroc and Neutralizing Antibodies

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**Background:** Cenicriviroc is a CCR5 antagonist, which prevents human immunodeficiency virus type 1 (HIV-1) from cellular entry. The CCR5-binding regions of the HIV-1 envelope glycoprotein are important targets for neutralizing antibodies (NAb), and cenicriviroc-resistant mutations may therefore affect sensitivity to NABs.

**Methods:** We used *in vitro* induction of HIV-1 variants resistant to cenicriviroc or NABs. Sensitivity of resistant variants and their recombinants to cenicriviroc and to NAB was determined to examine the relationship between resistances to cenicriviroc and to NAB.

**Results:** The cenicriviroc-resistant variant KK652-67, which was isolated *in vitro* in the presence of an increasing concentration of cenicriviroc, was sensitive to neutralization by NABs against the V3 loop, the CD4-induced (CD4i) region, and the CD4-binding site (CD4bs), compared with the parental HIV-1 strain KKWT from which cenicriviroc-resistant KK652-67 was obtained. The V3 region of KK652-67 was important for cenicriviroc resistance, similar to other CCR5 antagonists, and critical to the high sensitivity of NABs to the V3, CD4i, and CD4bs epitopes. Moreover, variants resistant to anti-V3 NAB 0.5y and anti-CD4i NAB 4E9C were induced from cenicriviroc-resistant KK652-67 by *in vitro* passages in the presence of each NAB. Acquisition of resistance to both 0.5y and 4E9C resulted in reversion to the cenicriviroc-sensitive phenotype comparable to the parental KKWT. Resistance to 0.5y and 4E9C was caused by novel mutations, R315K, G324R, and E381K, in the V3 and C3 regions near the cenicriviroc-resistant mutations. Importantly, these amino acid changes in the CCR5-binding region were also responsible for reversion to the cenicriviroc-sensitive phenotype.

**Conclusions:** These results suggest the presence of key amino acid residues where resistance to cenicriviroc is incompatible with resistance to NABs. This implies that cenicriviroc and neutralizing antibodies may restrict the emergence of resistant variants each other.

**505 Molecular Dynamics of the CD4-Mimetic Resistant HIV-1 Gp120 by MD Simulation**Shigeyoshi Harada<sup>1</sup>; Masaru Yokoyama<sup>2</sup>; Shuzo Matsushita<sup>3</sup>; Hironori Sato<sup>2</sup>; Tetsuro Matano<sup>1</sup>; Kazuhisa Yoshimura<sup>1</sup><sup>1</sup>Natl Inst of Infectious Diseases, Shinjuku, Japan; <sup>2</sup>Natl Inst of Infectious Diseases, Musashimurayama, Japan; <sup>3</sup>Kumamoto Univ, Kumamoto, Japan

**Background:** CD4 mimetic small compounds (CD4MCs), such as NBD-556, inhibit the gp120-CD4 interaction and can also induce conformational changes in gp120 by exposing masked epitopes of neutralizing antibodies on the Env protein. Recently, we have reported the resistance induction of the primary KP-5P virus (subtype B, R5) against the three CD4MCs (NBD-556, YYA-021 or JRC-II-191). Resistance against CD4MCs was associated with V255M, T375N/I or M426I substitutions. In this study, we investigated how the mutated positions affect CD4MCs recognition and sensitivity to other CD4MCs.

**Methods:** Infectious KP-5P clones with CD4MC-resistant mutation were constructed. The susceptibility of the infectious clones to the CD4MCs, was determined using the T2M-bl assay. We also simulated the gp120 3D structures by MD simulation model.

**Results:** Two of three mutated residues, V255M and T375I, are located at the bottom of the Phe43 cavity, while M426I is at the edge of the cavity. Clones with V255M or T375I were highly resistant against the CD4MCs (NBD-556, YYA-021 or JRC-II-191), but the M426I single mutated clone had moderately resistance to those, except for YYA-021. On the other hand, the clone with M426I was more resistant to sCD4 than those with V255M and T375I mutations, because the Phe43 residue of sCD4 is located at a shallow position in the cavity compared to the CD4MCs. We also simulated the binding form between the KP-5P gp120 and NBD-556, YYA-021 or JRC-II-191 using an MD simulation method. The results showed that (i) V255M mutation abolished the interaction of gp120 and CD4MCs except for JRC-II-191, and (ii) M426I mutation disconnected a hydrogen bond between Lys130 and Glu429, thus the NBD-556 or JRC-II-191 binding site shifted different from the usual, but they were still hold in the cavity, while YYA-021 binding was abolished by the M426I mutation.

**Conclusions:** These data gave elucidation of the molecular details governing the interactions between gp120 and CD4MCs, and will assist in synthesizing novel CD4MCs to search for drugs with more potent power to change the tertiary structure of Env for opening the neutralizing epitopes.

**506 Suppress NNRTI-Resistant Mutants by Doravirine at Clinically Relevant Concentrations**

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**Background:** Doravirine (DOR), currently in Phase 3 clinical trials, is a novel and potent human immunodeficiency type 1 virus (HIV-1) non-nucleoside reverse transcriptase inhibitor (NNRTI). To assess potential clinical efficacy of DOR against prevalent NNRTI-resistant mutants including rilpivirine (RPV)-associated mutants, the inhibitory quotient (IQ) was calculated based on clinical trough concentration at a dose of 100 mg (selected for Phase 3 trial) and the  $IC_{50}$  determined in the presence of 100% normal human serum (NHS). To evaluate the capability of DOR, efavirenz (EFV), and RPV in suppressing K103N, Y181C, and K103N/Y181C mutants, in vitro resistance selection was performed with the mutant viruses at clinically relevant concentrations of the NNRTIs.

**Methods:** Antiviral assays were performed using laboratory HIV-1 isolates (WT and respective mutants) and MT4-GFP cells. Cell infection was carried out in RPMI 1640 medium in 10% fetal bovine serum and incubated for 20 hours. The infected cells were washed and resuspended in 100% NHS and then added to plates containing the compounds at various concentrations. The inhibitory potency was determined based on the ratio of green cells determined at 48h and 72h. In vitro resistance selection was performed in a 96-well format with prevalent NNRTI-associated mutants using MT4-GFP cells at clinically relevant concentrations of NNRTIs in the presence of 10% NHS. For every passage, viral breakthrough was monitored for GFP positive cells.

**Results:** DOR displayed IQs of 39, 26, and 21 against the K103N, Y181C, and K103N/Y181C mutants, respectively. In contrast, RPV exhibited IQs of 4.6, 1.4, and 0.8, respectively against the same mutant viruses, while EFV showed IQs of 2.5, 60, and 1.9, respectively. In addition, IQs were also determined with RPV-associated mutants such as E138K/M184V, E138K/M184I, E138K and other K101E mutants. The IQs of DOR with the panel of mutants were significantly higher than those of RPV and comparable to that observed for EFV. Results from in vitro resistance selection conducted with K103N, Y181C, and K103N/Y181C mutants indicated that no viral breakthrough was observed in the selection with DOR, whereas viral breakthrough was detected in the selection with EFV against K103N and K103N/Y181C mutants and with RPV against Y181C and K103N/Y181C mutants.

**Conclusions:** DOR may present a higher barrier for viruses to develop resistance compared to EFV and RPV, which should be a valuable addition to current available antivirals for HIV treatment.

**507 The Effect of E157Q in HIV-1 Integrase on R263K-Mediated Dolutegravir Resistance**Kaitlin Anstett<sup>1</sup>; Vincent Cutillas<sup>2</sup>; Robert Fusco<sup>1</sup>; Thibault Mesplede<sup>2</sup>; Bonnie A. Spira<sup>2</sup>; Mark A. Wainberg<sup>2</sup><sup>1</sup>McGill Univ, Montreal, QC, Canada; <sup>2</sup>McGill Univ AIDS Cntr, Montreal, QC, Canada

**Background:** The integrase strand transfer inhibitor (INSTI) dolutegravir (DTG) has a high genetic barrier to resistance, which has only been selected thus far in treatment-experienced patients and in tissue culture. The novel R263K substitution in integrase (IN) is the predominant mechanism of DTG resistance in INSTI-naïve patients. However in INSTI-experienced patients, resistance emerges through the accumulation of resistance substitutions for other drugs of this class. E157Q can be selected after treatment with raltegravir (RAL), and is a polymorphism present in the circulating virus as well. We have also previously identified E157 as an interactor with the IN DNA binding inhibitor FZ41. As it was recently reported that a patient failed RAL and subsequently DTG with the E157Q substitution, we investigated the effects of this substitution on the emergence of R263K, its effects on enzyme biochemical function, and viral infectivity and drug resistance.

**Methods:** E157Q and R263K were introduced into the pET15b IN protein expression vector and pNL4.3 viral vector by site-directed mutagenesis. Strand transfer and DNA binding activities were measured with fluorescence-based biochemical assays using purified recombinant IN proteins. Viral infectivity and drug resistance were measured through the infection of T2M-bl cells and observation of luciferase production.

**Results:** R263K decreased IN strand transfer, DNA binding activities, and NL4.3 infectivity by ~20% when compared to WT. Neither biochemical function nor infectivity showed a significant decrease from WT when the E157Q mutant was evaluated, and the presence of this substitution in the R263K background partially restored the enzymatic and infectious defects conferred by the latter mutation. Despite this restoration, neither virus with E157Q displayed increased resistance to FZ41, although susceptibility to DTG was modestly decreased.

**Conclusions:** DTG is arguably one of the best current therapies for HIV infection, displaying a high genetic barrier for resistance and very few treatment failures to date. However, we show that the E157Q substitution is able to restore the defects in enzyme function and viral infectivity that are conferred by the DTG resistance mutation R263K. As position 157 in IN is polymorphic, its presence at the initiation of DTG therapy is possible, which could lead to the selection of a replicatively competent, DTG-resistant virus. This could have important consequences for the clinical care of HIV-positive individuals.

**508 Drug Resistance Mutations in HIV-2 Patients Failing Raltegravir and Dolutegravir**Silvia Requena<sup>1</sup>; Carmen de Mendoza<sup>2</sup>; Teresa Cabezas<sup>3</sup>; Rosa García<sup>4</sup>; Maria Jose Amengual<sup>5</sup>; Ana Belen Lozano<sup>3</sup>; Juan Manuel Fernandez<sup>3</sup>; Vicente Soriano<sup>6</sup>; Ana Treviño<sup>7</sup>; for the Spanish HIV-2 Study Group<sup>1</sup>Puerta de Hierro Rsr Inst, Majadahonda, Spain; <sup>2</sup>La Paz Univ Hosp, Madrid, Spain; <sup>3</sup>Hosp de Poniente, Almeria, Spain; <sup>4</sup>Hosp Universitario Fundación Jiménez Díaz, Madrid, Spain;<sup>5</sup>Corporación Sanitaria Parc Taulí, Barcelona, Spain; <sup>6</sup>Hosp La Paz, Madrid, Spain; <sup>7</sup>Hosp Universitario Puerta de Hierro de Madrid, Madrid, Spain

**Background:** A broader extent of replacements at the integrase of HIV-2 compared to HIV-1 might enable greater cross-resistance between raltegravir (RAL) and dolutegravir (DGV) in HIV-2 patients (Smith et al. *Retrovirology* 2015;12:10). Studies assessing drug resistance mutations in HIV-2 patients that fail on RAL are scarce. No studies have tested in HIV-2 the virological response prior HIV-2 RAL failures.

**Methods:** The integrase coding region was sequenced using an in-house nested-PCR protocol in both integrase inhibitor (INI) naïve and experienced HIV-2 viremic patients. Mutations associated to RAL and DGV resistance in HIV-1 were characterized. Plasma HIV-2 RNA changes were measured following DGV introduction in a subset of patients with RAL failure.

**Results:** From a total of 319 HIV-2 patients recorded at the HIV-2 Spanish cohort up to September 2015, a total of 52 integrase sequences from 30 patients had been obtained (21 RAL-naïve and 9 RAL-experienced). Only two secondary mutations (E138A and Q148E) were found in two out of 21 RAL-naïve patients. The resistance mutation profile in 8 out of 9 viremic patients with RAL failure was: A153G+N155H (3); E92Q+T97A+N155H (1); G140S+Q148R (1); G140A+Q148R (1); T97A+A119T+Y143G+A153S (2) and T97A+Y143C (1).

Five of these 8 patients were subsequently rescued with DGV. One patient harbouring N155H+E92Q+T97A regained and has kept on sustained viral suppression for 34 months of DGV, accompanied with large CD4 gains (from 124 to 270 cells/μl). Another patient harbouring T97A+Y143G+A153S achieved viral suppression along with CD4 gains from 86 to 308 cells/μl. However, one patient with multidrug resistance harbouring N155H+A153G experienced 1 log drop in HIV-2 RNA along with CD4 gains from 16 to 147 cells/μl, but subsequently experienced virological failure and CD4 drop to 54 cells/μl selecting V151I. This is the first report of DGV resistance in HIV-2. Data on the remaining two patients (both subtype B clades) and N155H+A153G and Y143C+T97A will be presented since they are still on too early follow-up.

**Conclusions:** There is a wide repertoire of resistance mutations at the integrase in HIV-2 patients failing on RAL. Although DGV may allow successful rescue in most HIV-2 RAL failures, we report and characterize the first case of resistance to DGV in one HIV-2 patient.

### 509 Prevalence of Minority Resistant Variants in HIV-2 Naïve Patients: ANRS C05 Cohort

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**Background:** To assess the prevalence of minority resistant variants (MRV) and X4-minority variants in antiretroviral-naïve HIV-2-infected patients included in the French HIV-2 Cohort (ANRS C05).

**Methods:** Antiretroviral-naïve HIV-2-infected patients with detectable plasma viral load (VL) (>100 c/mL) were assessed. We performed UltraDeep Sequencing (UDS) (Roche 454<sup>+</sup> Life Sciences) in protease (PR) and reverse transcriptase (RT) regions issued from plasma viruses. Only mutations >1% were considered and interpreted with HIV-2 ANRS list (Charpentier et al., 2015). HIV-2 tropism was assessed by UDS of V3 loop region. Tropism of each sequence read was interpreted with HIV-2 major determinants of CXCR4 co-receptor use (L18X, V19K/R, V3 global net charge, insertions at position 24).

**Results:** 47 patients were assessed (median age: 48 years [IQR=36-57], 61% women, 68% originating from West Africa, **12% at CDC-C stage**). At time of sampling, median CD4 cell count was 326/mm<sup>3</sup> (IQR=215-438) and median VL was 2130 c/mL (IQR=816-4495). 67% of patients were infected with HIV-2 group A and 33% with group B. Protease and RT UDS was successful in 41 (87%) and 38 (81%) samples, respectively. Prevalence of virus with PR or RT drug resistance mutations (DRM) using 1% and 20% detection threshold was 17.1% (95%CI=5.5-28.7) and 7.3% (95%CI=0.0-15.4), respectively. DRM detected at the 20% detection threshold were M184V in one case and N69S in two cases. MRV exhibiting at least 1 NRTI DRM were detected in 1 patient (2.6%, 95%CI=0.0-6.8), showing the mutation N69S in a proportion of 1.4%. MRV exhibiting at least 1 PI DRM were detected in 4 patients (9.8%, 95%CI=0.7-18.9): (i) two I50V-mutated MRV (1.6% and 1.0%); (ii) one V47A (1.0%); and (iii) one with both I50V and I54L (1.2% and 1.1%, respectively). Tropism was assessed in 19 samples (mean number of reads=7591) showing 2 samples (11%) exhibiting X4-tropic viruses in more than 50% of the reads. Among the 17 remaining samples, X4-minority variants were detected in 11 (65%) in a median proportion of 0.41% (IQR=0.33-0.76).

**Conclusions:** In this first study assessing the prevalence of MRV in HIV-2 infection, we observed a two to three-fold higher prevalence of DRM in antiretroviral-naïve patients when 1% detection threshold of mutations was used compared to 20% threshold. In addition, X4-minority variants were detected in the majority of patients.

### 510 Evaluation of an EIA-Based Testing Algorithm Using Dried Blood Spots From South Sudan

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**Background:** Due to high sensitivity of enzyme immunoassays (EIA) some level of false positivity is expected when used alone or in an EIA-based testing algorithm for HIV diagnostic testing. This is problematic for antenatal clinic (ANC) surveys whose data are used for surveillance purposes as it can lead to inflated prevalence estimates. DBS are preferred specimen for HIV surveillance surveys as they are simple, inexpensive, and can be prepared in non-laboratory settings. However, use of DBS specimens requires modification and optimization of EIA procedures. We present results from an EIA testing algorithm compared to the US Centers for Disease Control (CDC) external quality assurance (EQA) testing algorithm applied to specimens from the South Sudan 2012 ANC survey.

**Methods:** DBS specimens collected for the South Sudan 2012 HIV ANC Survey were tested at the Juba Teaching Hospital Laboratory. Of the 11,155 specimens collected in the ANC survey, 1059 HIV-negative and 285 HIV-positive specimens were sent to CDC Atlanta for EQA testing. As part of the South Sudan testing algorithm, the specimens were screened on Vironostika Uni-form II plus EIA (Biomérieux, France) and reactivities were confirmed by Murex HIV-1.2.0 EIA (Abbott, Germany). Retesting at CDC was performed using an optimized DBS elution protocol on Genetic Systems HIV-1/2 plus O (GS 1-2-0, Bio-Rad, California) followed by confirmation of positives on Cambridge Biotech HIV-1 Western Blot (WB) (Maxim Biomedical, Maryland). Specimens with discrepant final classification between the two algorithms were further confirmed by WB.

**Results:** Of the 1344 of specimens tested, the overall agreement of the South Sudan and CDC algorithms was 97.5%; however, 97.2% and 89.1% agreements were observed amongst the negatives and positives, respectively. A total of 33 specimens (2.5%) were discrepant between the algorithms and of those, 29 (88%) specimens were negative and 4 (12%) specimens were indeterminate according to WB results. The optical density values of most false positive (FP) specimens were low on EIAs indicating inherent false positivity of EIAs that is magnified when DBS specimens are used.

**Conclusions:** Our result suggests a high FP rate with EIA-testing algorithm alone. These results highlight the need to incorporate a more specific assay such as WB or similar test in the testing algorithm to confirm the HIV status and ensure the accuracy of HIV prevalence data. Use of DBS specimens requires additional optimization steps to ensure accurate results.

### 511 Comparison of Cross-Sectional Incidence Assay Results From DBS and Plasma

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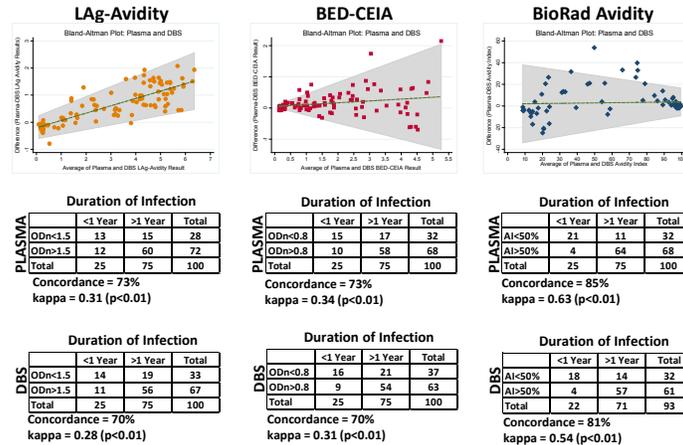
**Background:** Assays have been developed for cross-sectional HIV incidence estimation using plasma samples. Large scale surveillance programs are planned using dried blood spot (DBS) specimens for incidence assessment. However, limited information exists on the performance of HIV cross-sectional incidence assays using DBS. This study compared results obtained from three incidence assays using plasma and DBS samples.

**Methods:** The assays evaluated were: Maxim HIV-1 Limiting Antigen Avidity EIA (LAg-Avidity), Sedia HIV-1 BED-Capture EIA (BED-CEIA), and CDC optimized BioRad HIV-1/2 Plus O Avidity-based Assay (BioRad Avidity) using pre-determined cutoff values. 100 matched HIV-1 positive plasma and DBS samples, with known duration of infection, from the

CEPHIA repository were tested. All assays were run in duplicate for each sample type. To examine the degree of variability within and between results for each sample type, both categorical and continuous results were analyzed. Bland Altman,  $R^2$  values and Cohen's kappa coefficient ( $\kappa$ ) were used to assess correlation.

**Results:** Intra-assay variability using the same sample type was high for all assays  $R^2$  0.96 to 1.00. The  $R^2$  values comparing DBS and plasma results for LAg-Avidity, BED-CEIA, and BioRad Avidity were 0.96, 0.93, and 0.84, respectively. The concordance and  $\kappa$  values between DBS and plasma for all three assays was  $>92\%$  and  $>0.82$ , respectively. The Bland-Altman analysis showed significant differences between plasma and DBS samples, see figure. For all three assays, a higher number of samples were classified as recent using DBS samples, see figure. Seven DBS samples were excluded from the BioRad Avidity analysis due to protocol guidelines.

**Conclusions:** Data generated using DBS and plasma samples was highly correlated. However, there is a scalar problem as the assays perform differently across their dynamic range. DBS samples were more likely to be classified as recent by all three assays, which may lead to over estimation of incidence in surveys using performance criteria derived for plasma samples. These results suggest that the performance characteristics of these incidence assays may be different than those calculated using plasma samples.



512 **Laboratory Evaluation of the GeneXpert® HIV-1 Viral Load Assay in Zimbabwe**

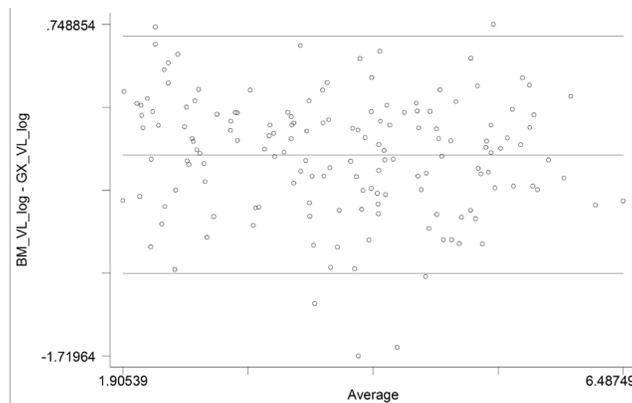
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**Background:** In most resource limited countries with a high HIV burden, current Viral Load (VL) testing technologies are based at the centralized laboratory levels. New HIV VL point-of-care (POC) tests hold the potential to simplify ART monitoring and bring diagnostics closer to the patient. The objective of the study was to perform a laboratory validation of the diagnostic accuracy of the GeneXpert® HIV-1 Quant assay compared to the predicate laboratory reference standard BioMérieux NucliSens Version 2.0 Easy Q/Easy Mag (NucliSens v2.0) for VL testing

**Methods:** EDTA anticoagulated plasma samples from 392 consenting patients on ART were tested for HIV-1 VL at the National Microbiology Reference Laboratory (NMRL) in Harare, Zimbabwe from June-September 2015. Paired samples were tested with each reference and index test following the manufacturer's instructions and laboratory SOPs. The technician performing the Xpert VL test was blinded to the result of the reference test. Statistical analysis was conducted in STATA 13.0 and included sensitivity, specificity, positive predictive value (PPV), linear regression and Bland-Altman (BA) analysis.

**Results:** Of the 392 samples were tested in the Xpert® HIV-1 Quant assay, 375 produced a VL result (3.4% true error rate). The sensitivity of the Xpert® HIV-1 Quant assay in detecting HIV VL as at 1000copies/ml threshold compared to the NucliSens v2.0 assay was 98.2% [95% CI: 93.8-99.8], specificity 97.7% [95%CI: 95.1-99.2] and PPV 94.8% while NPV 99.7%. Bland-Altman mean bias between the two methods was -0.22 log<sub>10</sub> copies/ml [95% CI: -0.292 to -1.52] and the LOA for the bias were -1.11 to 0.66; p-value=0.125. Spearman's correlation coefficient,  $R^2=0.952$ . Concordance between the two assays at 1000copies/ml threshold was 97.8%. Only 1.6% of patients (6 of 375) and 0.5% (2 of 375) were misclassified above and below this threshold respectively by the index assay compared to predicate assay. The system proved easy-to-use with minimum sample preparation and handling time, with an average daily capacity of 16 samples per 8hour day

**Conclusions:** Xpert® HIV-1 Quant gives HIV VL results comparable to NucliSens v2.0 assay. Findings from this study support the consideration of Xpert® HIV-1 Quant testing for the roll-out of HIV VL monitoring and may contribute towards reaching the ambitious UNAIDS target of 90% ART patients with undetectable VL



**513 Weakly Reactive HIV Rapid Diagnostic Test Kits Should Not Be Reported As Positive**

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**Background:** HIV rapid diagnostic test (RDT) kit manufacturers require that reactive bands observed in the test area of the kit are reported as positive regardless of intensity of coloration. Some studies have suggested that weakly reactive RDT results should not be considered positive except in blood donor screening. However, a reliable estimate of the proportion of weakly reactive bands in 3<sup>rd</sup> generation HIV RDT's that would actually be false positive has not been published. We assessed the percentage of weakly reactive bands that were subsequently confirmed negative by HIV DNA-PCR.

**Methods:** Over an 11 year period (2002 to 2013) we determined the proportion of inconclusive results that were later confirmed negative by DNA PCR. Inconclusive results were based on the observation of a weakly reactive band on at least one of three 3<sup>rd</sup> generation HIV RDT kits used within a sequential testing algorithm. The three kits were Alere Determine, Chembio Stat-Pak, and Uni-Gold. The algorithm was as follows; all tests were initially run on Determine. If negative, reported as negative; if positive they were confirmed with Stat Pak. If discrepant, Uni-Gold was used as a tie breaker. HIV-1 DNA PCR Roche Amplicor Version 1.5 was used as the confirmatory test method.

**Results:** 39,294(12.9%) of 303,010 individuals screened for HIV were positive. 468 (0.15%) of the same initially had inconclusive HIV RDT results. The HIV status of the 468 individuals was resolved by HIV-1 Western Blot (17), HIV-RNA PCR (14), or HIV-DNA PCR (437) confirmatory tests. 224 of the 437 cases confirmed by DNA-PCR RDT's were independently repeated by another tech to control for individual variability. Only these 224 cases were included in this analysis. Of these 224 cases, 148 (66.1%) were negative by HIV DNA PCR. Of note, repeating the 224 tests gave a total of 448 instances of testing with both Determine and Sat-Pak RDT kits, and 441 testing instances with Uni-Gold kit. (Detailed results Table attached).

**Conclusions:** The majority of weakly reactive RDT results from all three kits used turned out to be negative on confirmatory HIV DNA PCR testing. This is contrary to manufacturers' instructions to interpret all such tests as positive. Our results also lend credence to previously published calls for testing algorithms to refer weakly reactive RDT test results for confirmatory testing. This is especially pertinent in the era of ART initiation based solely on HIV RDT results and scaled-up HIV surveillance programs in resource limited settings.

Kit	Kit Result	HIV-DNA Result				Total result type	Total kit tests
		POS		NEG			
		No.	%	No.	%		
Determine	W.POS	60	25	180	75	240	448
	POS	90	48.1	97	51.9	187	
	NEG		0.0	16	100.0	16	
	IND	2	40.0	3	60.0	5	
StatPak	W.POS	69	47.9	75	52.1	144	448
	POS	9	40.9	13	59.1	22	
	NEG	74	26.2	208	73.8	282	
	IND	0		0		0	
Unigold	W.POS	72	32.0	153	68.0	225	441
	POS	53	57.0	40	43.0	93	
	NEG	21	17.1	102	82.9	123	
	IND	0				0	

**514 Extending HIV Avidity Recency Detection by Addition of p24Ag Detection**

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**Background:** Increasing the period in which HIV recency assays can identify recent infection without increasing misclassification of specimens as recent will allow studies to use smaller sample sizes. We describe an HIV avidity assay that identifies recent HIV infection by detection of low avidity antibodies along with detection of HIV p24 Ag in anti-HIV negative specimens thus extending the duration that recent HIV infection can be determined.

**Methods:** Specimens were tested for using the BioPlex 2200 HIV Ag-Ab assay. This '5<sup>th</sup> generation' assay allows separate detection and identification of anti-HIV 1, anti-HIV-2 and HIV p24 Ag unlike 4<sup>th</sup> generation which give a single signal. An antibody avidity modification using 1M guanidine allowed determination of an avidity index. Presence of p24 Ag was determined on each sample and a subset of specimens diluted with Guanidine and PBS. Results were compared with an existing HIV incidence assay (HIV Limiting Antigen). 150 specimens were tested comprising HIV seroconversion panels, known anti-HIV positive specimens and acute p24 Ag only positive specimens were used.

**Results:** Antibody avidity increased over time. Where p24 Ag was detected prior to seroconversion addition of guanidine and/or dilution of specimen had only a minimal effect on level of p24 detection but it remained detectable. Using a cut-off of <80% AI for recent infection concordance with LAg assay result was over 90%. Of four mismatched specimens two were close to the recency cut-off for either LAg or BioPlex avidity.

**Conclusions:** This assay has a sensitive and specific HIV diagnostic capability and potential to allow HIV recency discrimination on the same platform. Initial results show a comparable performance to the market leading incidence assay in those anti-HIV positive. However, the ability to identify Ab negative/Ag positive specimens increases the duration in which 'true' recent infection can be determined. This increases duration of detectable recent infection by up to two weeks but importantly identifies 'true' recent infections that may otherwise be discarded. Our data demonstrate that assays containing an antigen detection component are compatible with use in HIV incidence methodologies. The potential of this assay to be able to provide specific diagnostic differentiation of HIV-1 from HIV-2 and p24 Ag with modification to allow identification of recent HIV infection may change HIV diagnostic algorithms to allow the incorporation of recency testing into routine practice.

**515 Western Blot Index for Estimating Recency of HIV Infection**

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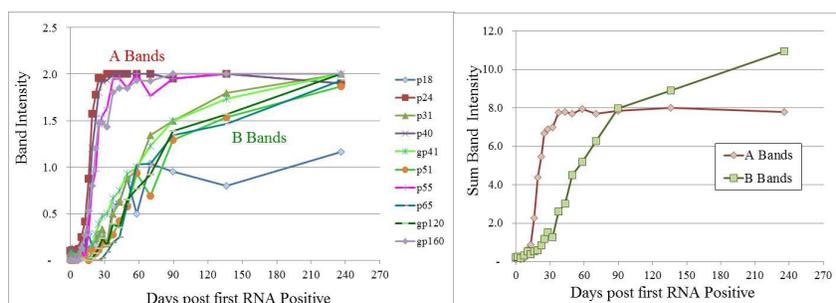
**Background:** Estimation of time of HIV infection can be useful in epidemiological studies of incidence of infection, in establishing a link to probable risk behavior, and in narrowing the search for likely partners to whom infection could have been passed. Current HIV Recency assays such as the BED CEIA and HIV-1 Limiting Antigen (LAg)-Avidity EIA have major limitations due to variable immune response among individuals, variability in different HIV-1 subtypes, and false recency rates in some individuals. Sequential bleeds from recently HIV infected individuals exhibits progressive appearance of specific Western blot (WB) bands which can be utilized to estimate time of infection. We examined WB profiles of closely spaced bleeds from newly infected individuals from the RV217 ECHO trial to generate a WB Index (WBI) which can be useful as a measure of recency of infection.

**Methods:** Individuals at high risk of HIV infection in Kenya, Uganda, Tanzania and Thailand were monitored twice weekly by HIV RNA Aptima assay (Hologic, LaJolla, CA), the earliest available indicator of infection. Blood from first time RNA positive individuals was collected at frequent intervals and tested by Western blot (Bio-Rad, Hercules, CA). Bands

were grouped according to timing of appearance, the intensities of each band was scored from 0-2+, and individual intensities or sums of bands plotted as time after first detection of infection.

**Results:** Figure 1 shows the relative intensity of bands from 20 seroconverting individuals post first Aptima RNA positivity. Antibodies to p24, p40, gp160 and p55 (A Bands) develop quickly after infection, with maximum reactivity by 25-40 days post infection. A more slowly evolving response is seen for p31, p65, gp41, gp120 and p51, (B Bands). A WBI of A and B bands can be used to estimate time post infection between 15 days to up to 270 days with some consistency. The subtypes included in this analysis include A, B, C, D, and CRF\_01AE, all of which show similar evolution profiles.

- I. The WBI accurately distinguishes between new, well established, or advanced infection and provides a useful estimate of the time of HIV infection.
- II. Is independent of virus subtype and applicable to different populations;
- III. Uses results from an assay already used for confirmation of HIV infection, allowing for greater flexibility in application (including developing countries)
- IV. Demonstrates a low False Recency Rate, thereby offering a more accurate measurement of incidence.



### 516 Low Prevalence of False Prior HIV Diagnoses in Chokwe District, Mozambique

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**Background:** Recent reports suggest that many clients may be falsely HIV diagnosed using rapid HIV testing algorithms in some resource-limited settings. Population-based prevalence of false prior diagnosis (dx), however, has not been reported. As part of the Chokwè Mozambique Health Demographic Surveillance System (CHDSS), we evaluate the prevalence of false prior dx in a high HIV-prevalence district.

**Methods:** CHDSS conducts annual demographic surveillance of approximately 95,000 residents. In 2014-15, all available household residents 15-59 years of age were offered home-based HIV Testing and Counseling and asked about prior dx, use of HIV care, and ART. The national rapid testing algorithm (RT) was conducted at the CHDSS laboratory on whole blood of prior dx clients; dried blood spots were sent to CDC for confirmatory testing (CT) of those who tested RT negative (neg) or indeterminate (ind). CT included 3<sup>rd</sup>-generation EIA for all specimens, Multispot or Western blot (if EIA positive, pos), and gp41 total nucleic acid PCR (if EIA neg). CT-neg clients were contacted by district medical staff to offer RT re-testing, verify prior dx, and help clients understand that they are not HIV-infected.

**Results:** The HIV prevalence among 25,344 residents tested was 20.2%. Prior dx was reported by 3170 (12.5%) clients. RT results of prior dx clients: 3132 pos, 5 ind, 33 neg. CT was conducted on 36 prior dx specimens (5 ind, 31 neg); one RT-neg and three RT-ind specimens were CT-pos. Of 32 CT-neg clients, 23 were re-contacted, and all re-tested RT-neg. When asked to verify their prior dx, ten (43%) clients reported *never* previously testing HIV-pos. Reasons for misclassification included misunderstanding the question, hope of receiving services, and mental illness. Assuming two RT-neg specimens not available for CT are true HIV-neg, and that all non-contacted clients are true prior dx (n=11), the maximum unweighted prevalence of false prior dx is 0.76% (24/3160). Of the 24 false prior dx clients, 21 (91.7%) showed their care and treatment client card, and 13 (54%) were confirmed by medical record or pill bottle to be on ART.

**Conclusions:** In over 25,000 residents tested in a high HIV-prevalence district of Southern Mozambique, at least 99.2% of residents who reported a prior HIV diagnosis were confirmed to be HIV infected, suggesting self-report of prior dx is valid and that RT misdiagnoses are rare. Although reassuring, the few confirmed false prior dx underscores the need to confirm infection before ART initiation.

### 517 HIV Incidence Assay Performance Required to Monitor Prevention Intervention Impact

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**Background:** Cross sectional, 'Recent Infection Testing Algorithm' (RITA)-based estimation of national HIV incidence is being implemented in several African countries in 2015-17. The sample sizes (SS) and associated implementation costs required for precise estimation of incidence (I) are driven by the prevalence (P), I, the mean duration of recent infection (MDRI), and the false recent rate (FRR), which vary with HIV subtype and ART coverage. We sought to evaluate the potential applicability of a RITA combining the limiting antigen immunoassay (LAg) and viral load (VL) to assess the impact of population-level prevention interventions (PLPI) nationally and in key populations (KP). We also describe features of new RITAs that would enable broader implementation for these and other applications.

**Methods:** SS were calculated using [publicly available spreadsheet tools](#) and R customizations, for assessment of the impact of an intervention that reduces I by 50%, with power 0.8 at alpha 0.05, with correction for design effect. We used an MDRI of 130 days (d) and FRR of 0.25% for LAg+VL (cutoff 1000 copies/ml), and compared this to MDRI 160 d (LAg+VL cutoff 100 copies/ml) and 240 or 280 d as aspirational targets. Surveys were considered feasible if SS was below 30,000 (national) or 5000 (KP). National P and I data for 2014 as reported by [UNAIDS](#) were used.

**Results:** Target SS for African countries with P>4% or I>0.3% for PLPI, and for one KP are shown in Table 1. With LAg+VL<sub>1000</sub>, PLPI surveys were feasible in 6 countries. If the MDRI could be increased to 280 d, SS would be reduced by approximately 60%, and surveys were feasible in an additional 5 countries. In countries with non-C subtypes, such as Cameroon, Kenya, Tanzania and Uganda, SS may be underestimated due to FRR>0.25%, but the impact of elevated FRR is dampened at longer MDRI. KP surveys in young women in South Africa (a high prevalence/incidence KP example) required SS >5000 but were feasible with MDRI of ≥240 d.

**Conclusions:** The currently available RITA of LAg+VL has limited applications for national PLPI surveys unless SS larger than 30,000 are considered; outside of high I settings it may generate imprecise estimates that are not able to detect large reductions in I. Similarly, LAg+VL surveys may not be feasible in KP surveys due to SS requirements. Development of new incidence assays with longer MDRI (≥240 d) and low FRR are needed to enable broader and more cost-effective use in national surveys, as well as for applications in PLPI and KP surveys.

**Table 1. Target Sample Sizes for African Countries with Prevalence > 4% (FRR = 0.25%)**

Country/Population	Predominant Subtype(s)	Estimated incidence (annual %)	Estimated prevalence (%)	Target sample size for indicated MDRI (days)			
				130	160	240	280
Lesotho	C	2.01	23.4	13,241	10,485	6,741	5,722
Swaziland	C	1.89	27.7	15,541	12,221	7,772	6,576
South Africa	C	1.27	18.9	20,272	15,976	10,189	8,626
Botswana	C	1.40	25.2	20,866	16,324	10,295	8,686
Namibia	C	0.91	16.0	27,860	21,875	13,869	11,719
Zimbabwe	C	0.92	16.7	27,979	21,944	13,889	11,730
Mozambique	C	0.74	10.6	30,926	24,440	15,650	13,261
Zambia	C	0.75	12.4	31,874	25,091	15,971	13,510
Uganda	A, D*	0.60	7.25	35,843	28,445	18,328	15,559
Malawi	C	0.45	10.0	54,091	42,256	26,571	22,391
Cameroon	A, CRF02**	0.38	4.77	55,160	43,759	28,174	23,910
Tanzania	A, C, D*	0.26	5.34	86,916	68,147	43,090	36,370
Kenya	A, D*	0.23	5.30	100,246	78,337	49,274	41,523
South Africa YWAG†	C	2.54	11.4	8,312	6,687	4,405	3,766

\*elevated FRR \*\*FRR unknown †YWAG: young women and girls aged 15-24; key population survey

**518 Acute Retroviral Syndrome: Useful for Guiding Testing for Acute HIV Infection?**

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**Background:** Diagnostic testing for HIV is often initiated with a 4<sup>th</sup> generation immunoassay or an HIV rapid antibody test. While recent healthcare guidelines recommend 4<sup>th</sup> generation HIV testing to detect acute HIV infection (AHI), the recommended p24 antigen based methods are limited by imperfect sensitivities for antibody negative AHI. Field-based testing programs generally rely on point-of-care rapid HIV antibody testing only, with specific AHI testing limited to persons presenting with signs and symptoms (S/Sx) consistent with an acute retroviral syndrome. However, the proportion of persons with AHI presenting symptomatic for their diagnostic test remains unknown. The objective of this study was to determine the proportion of persons with AHI presenting symptomatic for their diagnostic test.

**Methods:** This is a prospective observational cohort study in persons diagnosed with AHI (defined as negative HIV antibody test in the presence of detectable HIV-1 RNA) who were enrolled in the San Diego Primary Infection Resource Consortium (SD PIRC) between June 2007 and December 2014. We analyzed S/Sx on or immediately prior to the day of HIV-1 nucleic acid testing (NAT; without pooling) in 90 individuals diagnosed with AHI in a community-based program, offering universal (i.e. independent of S/Sx) AHI screening in San Diego, California.

**Results:** Forty-seven (52%) of the 90 persons identified with AHI reported ongoing S/Sx (median 6, IQR 4-8) at the time of HIV-1 RNA screening. Another 25 (28%) reported S/Sx that had occurred during the 14 days prior that had resolved by the time of the first positive NAT test and 12 (13%) reported S/Sx that started after the test, while only 6 (7%) reported no S/Sx.

Viral loads were significantly higher (p=0.001) and CD4/CD8 ratios lower (p=0.047) in those 72 individuals reporting S/Sx before or at the time of NAT screening compared to those 18 that did not have S/Sx prior to HIV-1 NAT screening (Table).

**Conclusions:** HIV diagnostic testing strategies that limit AHI testing to those presenting with S/Sx fail to identify about half of persons with AHI. In contrast, HIV-1 NAT provided to persons with S/Sx during the two weeks before the test may identify 80% of AHI cases. Such an approach may help maximizing the yield of AHI diagnoses in field-based testing programs, where universal 4<sup>th</sup>-generation testing frequently is not available.

Characteristics	Total Individuals	Symptoms prior to NAT test	Asymptomatic prior to NAT test	p-value (displayed if below 0.1)
Number of clients	90	72	18	-
Overall Number of Signs and Symptoms in those Symptomatic	5 (3-7; n=84)	5 (4-7)	5 (2-6; n=12)	n.s.
Duration Symptoms (days; median, IQR, range)	9 (5-13; n=79)	10 (6-13; n=67)	4 (3-7; n=12)	p=0.001
CD4+ cell count, cells/μL (median, IQR)	435 (298-597)	435 (302-586)	448 (257-615)	n.s.
CD4+/CD8+ cell ratio (median, IQR)	0.731 (0.474-1.160)	0.711 (0.433-1.016)	1.111 (0.575-1.466)	p=0.047
Viral load log10 RNA (median, IQR)	5.435 (4.455-6.270)	5.756 (4.809-6.405)	4.497 (3.218-5.011)	p=0.001

**Table 1:** Comparison of characteristics of signs and symptoms, CD4 count, and VL between those with symptoms prior to NAT testing versus those without.

**519 Comparative Sensitivity of 8 HIV Rapid Tests in HIV-Positive Patients**

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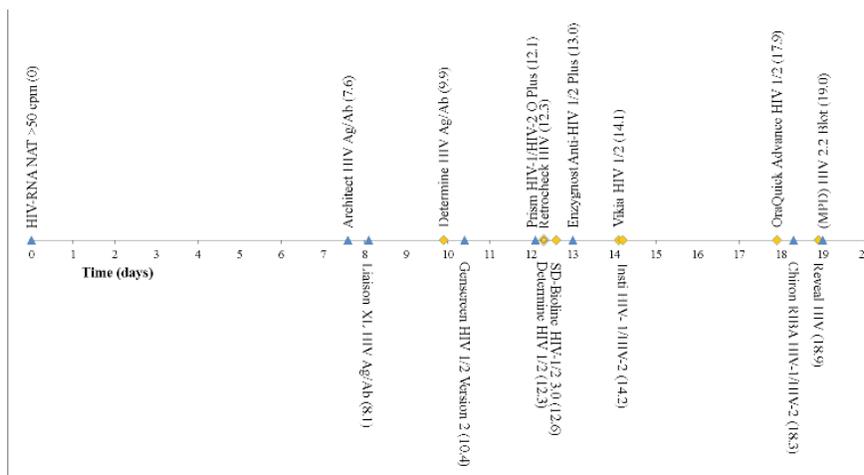
**Background:** HIV rapid test devices (HIV-RTD) often show a wide range of performances due to different HIV assay generations, test technologies and specimen types.

**Methods:** Test sensitivity of eight HIV-RTDs including assay generations 2nd (IgG sensitive), 3rd (IgG/IgM sensitive) and 4th (Antigen/Antibody) assays and different test formats was evaluated head-to-head in a cross-sectional study with 206 confirmed HIV positive patients paired for serum/plasma, whole blood and oral fluid, and in 10 seroconversion panels (105 serial samples). Comparison included laboratory-based HIV screening and confirmation tests established in the EU.

**Results:** The sensitivity of HIV-RTDs in patients was 98.1%-99.5% with serum/plasma, 97.1%-99.5% with whole blood and 92.7% with oral fluid. False-negative results with serum/plasma were from recent infection. With whole blood, two HIV-RTDs were less sensitive in recent infection than with serum and one HIV RTD was false negative also in chronic infection. With oral fluid there were 15 false-negative results from recent and chronic infection. 4th generation HIV-RTD was not superior to 3rd generation HIV RTDs in patients. The window periods of the HIV-RTDs relative to HIV-RNA in the seroconversion panels ranged from 9.9 to 18.9 days, and correlated with the order of sensitivity of the tests on the HIV patients.

The figure illustrates the findings on time to detection of HIV infection, compared to HIV-RNA (window period) for the HIV rapid tests and the laboratory-based HIV assays (mean values).

**Conclusions:** The sensitivity of HIV-RTDs showed significant differences. The range included state-of-the-art HIV antibody detection, low sensitivity for the identification of recent infection and false-negative results including in chronic infection. The sensitivity with oral fluid in this study was less suitable. The use of rapid tests with reasonable sensitivity therefore requires careful selection: In a situation following recent exposure to HIV and if individuals have received either early antiretroviral therapy, or have failed on post- or pre-exposure prophylaxis, a negative result with oral fluid should be viewed with caution, even after a 3 months period has passed.



**520 Comparison of Methods for HIV Incidence Estimation in a Cohort With Subtype C HIV**

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**Background:** Accurate methods for cross-sectional incidence estimation are needed for HIV surveillance and HIV prevention research. This is particularly true for southern Africa, an HIV-1 subtype C endemic region, where the burden of HIV is highest worldwide.

**Methods:** Samples were tested from a cohort of 176 seroconverters (1600 samples) from Zimbabwe (subtype C endemic area) followed for up to 9.9 years after seroconversion (median 6.9 years, interquartile range: 5.9 to 7.8) from the FHI 360 Hormonal Contraception and HIV trial. Three HIV avidity assays were analyzed alone at predetermined assay cutoffs, in combination with a viral load test (cutoff for recent infection >1000 copies/ml), or as part of a multi-assay algorithm (MAA). The assays evaluated were: the Limiting Antigen Avidity assay (LAG-Avidity; Sedia); a modified 3<sup>rd</sup> generation Genetic Systems (GS) HIV 1/2 + O EIA (JHU-3<sup>rd</sup> BioRad); and a modified 4<sup>th</sup> generation GS HIV Combo Ag/Ab EIA (JHU-4<sup>th</sup> BioRad). The MAAs evaluated were previously optimized for incidence estimation in subtype B epidemics; these included JHU-3<sup>rd</sup> BioRad and LAG-Avidity alone or JHU-3<sup>rd</sup> BioRad, LAG-Avidity, CD4 count, and a viral load test (cutoff >400copies/ml). Performance characteristics evaluated were: the mean duration of recent infection (MDRI, the average time individuals appeared recently infected using a time window of 2 years); and the false recent rate (FRR, the frequency of being identified as recently infected for samples from individuals infected >2 years).

**Results:** The MDRI and FRR for the different assays and algorithms are presented in the table below. A number of testing methods had MDRI >120 days and FRR <2%, which are the minimum performance characteristics needed for a cross-sectional incidence assay.

**Conclusions:** All algorithms that included a viral load test met minimum performance guidelines. Both JHU-3<sup>rd</sup> BioRad <40% AI and JHU-4<sup>th</sup> BioRad <80% AI had an MDRI >140 days and an FRR <1% without the need of an additional viral load test. All two-assay algorithms that included an avidity assay plus viral load met minimum performance characteristics with MDRI ranging from 133 to 214 days and FRRs ranging from 0 to 1%. The MAA that included LAG-Avidity + JHU-3<sup>rd</sup> BioRad + CD4 count + viral load had an MDRI of 7.5 months and an FRR of 1%, well within the minimum performance characteristics specified by the WHO/UNAIDS working group for cross-sectional incidence assays.

**Table. Comparison of different methods for HIV incidence estimation in a cohort with subtype C HIV.**

Method	MDRI (95% CI)	FRR (# positive/ # tested)
LAG-Avidity <1.5 OD-n	164 (151-178)	3.4% (3/671)
LAG-Avidity <1.5 OD-n + VL>1000 cps/ml	140 (127-156)	1.0% (7/671)
JHU-3 <sup>rd</sup> BioRad AI <40%	145 (133-157)	0.1% (1/671)
JHU-3 <sup>rd</sup> BioRad AI <40% + VL>1000 cps/ml	133 (121-147)	0.0% (0/671)
JHU-3 <sup>rd</sup> BioRad AI <80%	235 (220-250)	2.7% (18/671)
JHU-3 <sup>rd</sup> BioRad AI <80% + VL>1000 cps/ml	214 (200-230)	1.0% (6/672)
JHU-4 <sup>th</sup> BioRad AI <80%	163 (152-175)	0.3% (2/671)
JHU-4 <sup>th</sup> BioRad AI <80% + VL>1000 cps/ml	148 (137-163)	0.0% (0/671)
MAA1	140 (129-154)	0.1% (1/672)
MAA2	226 (212-242)	1.0% (7/672)

Table footnotes: OD-n = normalized optical density, AI = avidity index  
 MAA1: JHU-3<sup>rd</sup> BioRad <40% AI + LAG-Avidity <2.8 OD-n  
 MAA2: JHU-3<sup>rd</sup> BioRad <85% AI + LAG-Avidity <2.9 OD-n + CD4 >50 cells/mm<sup>3</sup> + VL >400 cps/ml

**521 4th Generation Rapid Tests Improve Detection of Acute Infection in MTN-003 (VOICE)**

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**Background:** The greatest risk of antiretroviral resistance from pre-exposure prophylaxis is conferred by use during undetected acute infection. Early, accurate identification of infection in HIV prevention trials is critical for protection of human subjects, data quality and study efficiency. We evaluated pre-seroconversion plasma from the VOICE Study (MTN-003) to determine if 4th generation antigen/antibody (Ag/Ab) rapid tests would have detected HIV infection earlier than the 3rd gen rapid tests used in the trial.

**Methods:** VOICE evaluated the safety and effectiveness of oral tenofovir disoproxil fumarate (TDF), oral TDF-emtricitabine (FTC) and vaginal tenofovir 1% gel for prevention of HIV infection in 5029 women from 15 clinical sites in South Africa, Uganda and Zimbabwe. Participants were monitored monthly for seroconversion with 1 or 2 3rd gen HIV rapid tests (Alere Determine™, OraQuick Advance® and/or Trinity Biotech™ Unigold®). Pre-seroconversion plasma collected closest to the date of the 1st positive rapid was tested for HIV-1 RNA (Roche TaqMan) and with Bio-Rad GS HIV Combo Ag/Ab EIA and re-tested with Unigold and OraQuick. The 4th gen FDA-approved Alere Determine™ HIV-1/2 Ag/Ab Combo (FDA-Combo) was compared with the Conformité Européenne (CE)-Marked Alere™ (CE-Combo) which is marketed to be optimized for non-B subtypes. We also evaluated Multispot® (MS), Western Blot (WB), and Geenius™ (all Bio-Rad) as confirmatory tests. McNemar's test was used to calculate statistical significance.

**Results:** Of 231 plasma samples collected 12-91 days (median 55) before detection of seroconversion, 68 had RNA >200 cp/mL, and of those 57 tested negative by both Unigold and OraQuick. Of these 57, 30(53%) were reactive by 4th gen EIA. 16(28%) were reactive on the CE-Combo (1 Ab; 9 Ag; 6 Ag/Ab reactive). MS confirmed only 1 of 16 acute infections while WB (9 indeterminate [ind]) and Geenius (2 HIV-2 ind) confirmed none. 4(7%) samples were reactive by FDA-Combo (2 Ab; 2 Ag; 0 Ag/Ab reactive) of which none were confirmed by MS, WB (3 ind) or Geenius (1 HIV-2 ind). CE-Combo detected significantly more infections than FDA-Combo (p<0.002).

**Conclusions:** In VOICE, 28% of infections missed by current 3rd gen rapid tests would have been identified with the use of CE-marked Alere HIV Combo. Geenius, MS and WB were all insensitive (<10%) in confirming infections detected by 4th gen assays. An improved diagnostic algorithm that includes 4th gen rapid tests with HIV RNA testing will be essential for efficiently identifying seroconverters.

**522 Usefulness of Rapid Tests for HIV Diagnosis in the ANRS IPERGAY PrEP Trial**

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**Background:** Pre-exposure prophylaxis (PrEP) implementation will lead to more frequent HIV testing. Rapid tests are likely to be used especially in resource limited countries, and our aim was to assess their usefulness in the setting of the ANRS IPERGAY PrEP trial.

**Methods:** In the ANRS IPERGAY trial, a 4<sup>th</sup> generation (4thG) antigen/antibody immunoassay (ARCHITECT HIV Ag/Ab Combo®, Abbott) and/or plasma Viral Load (pVL) (AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0) were used for HIV diagnosis at screening and during follow-up. We used stored sera to perform the following tests at the date of diagnosis: pVL, rapid test (VIKIA® HIV1/2, Biomérieux) and HIV-1 western blot (WB, GS HIV-1 Western Blot®, Biorad). We defined 3 stages of HIV infection according to the number of WB antibodies (Ab): chronic ( $\geq 7$  Ab), recent ( $1 \leq Ab < 7$ ) and acute (0 Ab). HIV-1 subtype was determined after phylogenetic analysis of the RT sequence.

**Results:** Overall, 31 HIV-1-infected patients were diagnosed during the ANRS IPERGAY trial. Stored sera were available for 27 cases of HIV infection. Overall, the 4thG was positive in 25 (93%) (median index 52), rapid tests in 15 (56%) and positive WB ( $\geq 1$  Ab) in 16 (59%) patients. Median pVL was 5.16 log<sub>10</sub> copies/ml. HIV-1 subtype B was identified in 16/25 (64%) cases.

Sensitivity of rapid tests was 100% (95%CI: 54-100) for chronic infection, 70% (95%CI: 35-93) for recent infection and only 18% (95%CI: 3-52) for acute infection (p < 0.002). Of note, among the 12 positive sera with the 4thG assay but with negative rapid tests, 8 were retested at the follow-up visit (median: 5 days) and 4/8 became positive with rapid tests.

**Conclusions:** Rapid tests were able to adequately detect chronic infection at screening but largely failed to diagnose acute HIV infection in people at high risk enrolled in a PrEP trial. 4<sup>th</sup> G assays should be used in settings where PrEP is implemented to avoid missing acute HIV infection, with the risk of selecting drug resistance and of ongoing HIV transmission.

HIV-1 infection	n	Positive rapid test (n)	HIV-1 RNA median [IQR]	4thG Index median [IQR]	no. antibodies median [IQR]
Chronic	6	6	5.15 [4.17-5.23]	424 [186-440]	8.5 [7-9]
Recent	10	7	4.45 [3.74-5.54]	13.6 [4.7-26.1]	4 [1-6]
Acute	11	2	6.94 [5.11-7.00]	55.9 [1.1-144.0]	0 [0-0]
Total	27	15	5.16 [4.17-6.94]	52.2 [4.7-177.6]	1 [0-7]

**523 Use of an Automated Assay to Identify HIV Infections in a Population Survey in Africa**

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**Background:** HPTN 071 (PopART) is a community-randomized HIV prevention trial in Zambia and South Africa (SA) that includes a cohort of >30,000 adults. Initial HIV testing is performed in-country using a 4<sup>th</sup> generation test (Abbott ARCHITECT HIV Ag/Ab Combo Test, one run per sample); special procedures for sample and data management are used in-country to optimize data quality. HIV status is confirmed in the United States (US). We evaluated performance of the Abbott test as an HIV screening test.

**Methods:** Samples that were initially reactive with the Abbott test (Abbott POS) were tested in the US with a second 4<sup>th</sup> generation test (BioRad Combo); 10% of samples that were initially non-reactive with the Abbott test (Abbott NEG) were retested in the US using the Abbott test. Samples with discordant results (in-country and US results did not agree) were tested with the BioRad Multispot test, the BioRad Geenius test, and an HIV RNA test (cutoff of 40 or 400 copies/mL).

**Results:** 10,390 samples were tested in the US (5,563 from Zambia, 4,827 from SA; 7,695 Abbott POS; 2,695 Abbott NEG). 56 samples had discordant results. For the Abbott POS samples: 52/7,695 (0.62%) were non-reactive with the BioRad Combo test (25 from Zambia, 27 from SA). Further testing revealed that 4 were HIV-positive (the original Abbott test result was correct), 48 were HIV-negative (the original Abbott test results were false positive results; 1 case involved a sample mixup). For the Abbott NEG samples: 4/2,695 (0.15%) were reactive when retested with the same assay. One had a signal/cutoff (S/C) ratio of 1.25 and was non-reactive on a second repeat test. The other two had S/C ratios of 4.77 and 10.38; both were reactive with the BioRad Combo test, but had undetectable HIV RNA and non-reactive Multispot and Geenius tests. All 4 were classified as HIV-negative. Overall, 48/7,695 Abbott POS samples had an initial false positive test result and 0/2,695 Abbott NEG samples had a false negative result (sensitivity=100%; specificity=98.3%).

**Conclusions:** The Abbott Combo test performed well when screening for HIV infection in a large, population-level survey in Africa. Special data and sample management procedures likely contributed to the high quality of initial HIV screening test results in this study. Quality assurance testing was helpful for confirming HIV infection status, especially for those with an initially reactive Abbott Combo test result.

#### 524 HIV Testing in the US PrEP Demonstration Project: rEIA vs Antigen/Antibody vs RNA

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**Background:** Ensuring individuals are HIV-uninfected prior to pre-exposure prophylaxis (PrEP) initiation and continuation is critical to minimize the risk of developing resistance, but the optimal HIV testing algorithm for PrEP is not yet known. We evaluated the performance of rapid blood EIA (rEIA), lab-based p24-antigen/antibody (4<sup>th</sup> generation), and RNA tests in detecting HIV infection at PrEP screening (n=635), and initiation and follow-up (n=557) in the MSM and transgender women (TGW) enrolled in the US PrEP Demonstration Project, an open label study of FTC/TDF PrEP in San Francisco, Miami, and Washington DC.

**Methods:** MSM and TGW interested in PrEP underwent screening, followed by enrollment within 45 days, when PrEP was initiated. All participants (ppts) were HIV tested at screening, enrollment, and follow-up (weeks 4, 12, 24, 36, 48) with rEIA and 4<sup>th</sup> generation tests. Pooled RNA was performed at all visits in San Francisco, with quantitative or qualitative RNA only at enrollment in DC and Miami, respectively. Any positive test was confirmed using local testing algorithms.

**Results:** At screening there were 16 (2.5%) ppts with a reactive rEIA and/or 4<sup>th</sup> generation test, 15 of whom were confirmed to be HIV infected. At enrollment 3 ppts had HIV infection: all were rEIA (-), 4<sup>th</sup> generation (-) and RNA (+), with viral loads of 120, 3343, and 51 copies/mL (all pre-treatment); 2 started ART as soon as the (+) RNA was known, and all viral loads were positive on repeat testing. Of 2 infections during follow-up, both were rEIA (+)/4<sup>th</sup> generation (+) at the visit with first evidence of infection; both had low or undetectable drug levels at seroconversion and no evidence of resistance on standard and ultrasensitive genotyping assays. During follow-up, of 2680 rapid EIAs there were 6 false positives in 2 ppts, for a specificity of 99.78% and a positive predictive value (PPV) of 25%; of 2673 4<sup>th</sup> gen tests there were 3 false positives in 2 ppts, for a specificity of 99.89% (PPV 50%). There were no false positive RNA tests at screening, enrollment or follow-up.

**Conclusions:** Rapid EIA and Lab-based 4<sup>th</sup> generation testing detected most HIV infection before PrEP initiation. Acute HIV infection should be ruled out with an RNA test before starting PrEP, if available, particularly in clients with recent exposure; low viral loads should not be assumed to be false positives. In this cohort, with high PrEP adherence, rapid EIA and lab based 4<sup>th</sup> generation tests were adequate to detect HIV infection during follow-up.

#### 525 Comparison of 4 HCV Viral Load Assays at High Viral Load

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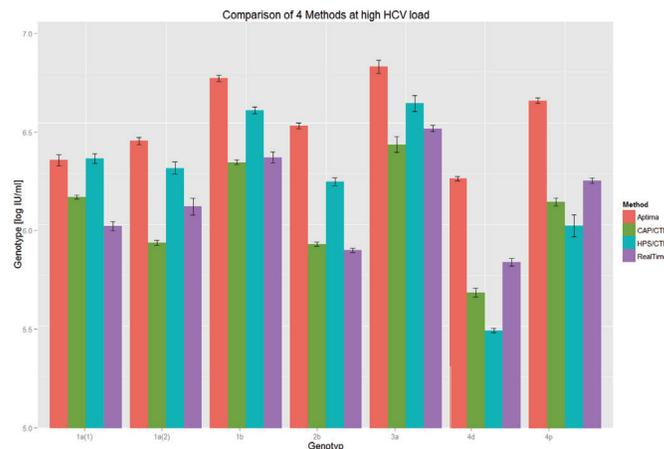
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**Background:** Hepatitis C virus (HCV) load is the most important surrogate marker for HCV replication. The height of viral load can be predictive for treatment success. The combination of sofosbuvir and ledipasvir has proven to be highly effective in treatment of HCV infection. While standard treatment duration of this combination is 12 weeks, in case of genotype 1 and a viral load below 6 Mio IU/ml, the treatment duration can be reduced to 8 weeks, reducing adverse effects and lower the price of this highly effective but costly treatment option. While in the clinical trials the Roche high pure/Cobas Taqman (HPS/CTM) was used, this assay is not common in clinical routine. We therefore compared four different quantitative HCV assays, the Abbott RealTime HCV (ART), the Cobas Ampliprep/TaqMan HCV v2 (CAP/CTM2), Cobas TaqMan HCV v2 for use with the High Pure System, v2.0 (HPS/CTM2) and the new Hologic Aptima HCV Quant Dx (HAC) at high viral load.

**Methods:** Seven clinical samples with different genotypes (2 x 1a, 1b, 2b, 3a, 4d and 4p) were tested with ART and diluted to a target concentration between 500.000 IU/ml and 3.000.000 IU/ml. All samples were tested in each assay in five replicates, resulting in overall 140 viral load measurements. Mean viral load and coefficients of variation were compared between all the assays.

**Results:** As expected for the high viral load used for testing, the coefficients of variation (CV) were low. Calculated on the logarithmic values CV were between 0,29 and 1,84%. HPS/CTM2 results were 1.8 times higher than ART results for genotype 1 samples. CAP/CTM2 results for the same samples were in mean not different to the ART results. HAC were 1.3 times higher than HPS/CTM2 in the genotype 1 samples. HAC samples for all genotypes, except genotype 4, were quantified in mean 1.4 times higher than in HPS/CTM2. Genotype 4 was under quantified by HPS/CTM2 and CAP/CTM2. ART results (genotype 4 excluded) were 1.2 times higher than CAP/CTM2.

**Conclusions:** The HAC assay showed in genotype 1 the closest correlation to the HPS/CTM2. While the CAP/CTM2 shows better performance on genotype 4 than HPS/CTM2, detection in genotype 4 is still better with HAC and ART. Despite their calibration to the WHO HCV RNA international standards, the assays showed significantly different quantitative results in this high viral load range. Therefore, results obtained with assays used in clinical trial, cannot be easily translated to clinical routine.



## 526 HCV Ag Core's Screening Performance in Mono-Infected, HIV- and HBV-Coinfected Patients

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**Background:** HCV chronic infection diagnosis currently relies on anti-HCV antibody (HCV-Ab) detection. As it cannot differentiate an active infection from a resolved one, its diagnosis must be confirmed by HCV-RNA measurement which is scarcely available in resource-limited settings. Quantifying HCV core antigen (AgC), a marker of viral replication, could shorten this algorithm if used as a one-step tool.

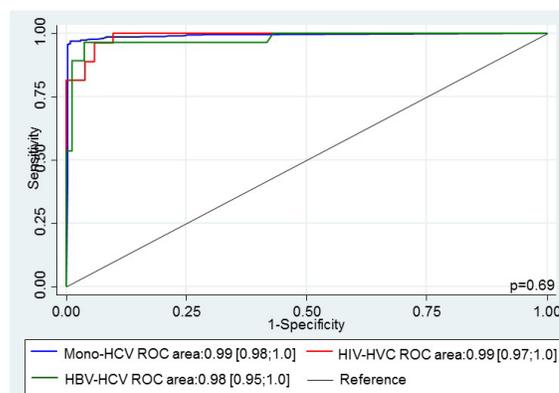
The aim of this study was to assess the performance of the AgC quantification for the diagnostic of chronic HCV in a serum bank of HCV mono- and HCV-HBV or HCV-HIV co-infected patients from Cameroon and the influence of co-infections on the test's results.

**Methods:** The quantification of the AgC was performed by an automated assay (Abbott Diagnostics) in 465 HCV-Ab negative samples and 544 HCV-Ab and HCV-ARN positive samples (n=1020) collected in patients from the Pasteur Center of Cameroon. Its performance was assessed by calculating its sensitivity and specificity, and building ROC curves in order to compare its results to the gold standard (ELISA and/or PCR) with the Area Under the Curve (AUC).

**Results:** Among these 1009 sera, 335 were un-infected, 489 were HCV mono-infected, 27 of 78 HIV-infected were co-infected with HCV and 28 of 107 HBV-infected were HCV-HBV. No statistical association was found between the AgC level and our covariates (age, gender, HBV or HIV co-infection). The correlation between AgC and HCV ARN was good in the mono-infected group ( $r=0.75$ ,  $p<0.00001$ ) and in the HIV co-infected group ( $r=0.83$ ,  $p<0.00001$ ) but lower in the HBV co-infected one ( $r=0.58$ ,  $p<0.001$ ). The assay had a sensitivity of 95.7% and a specificity of 99.7% in the mono and un-infected group, corresponding to an AUC of 0.99 (95% CI: 0.98-1.0). In the HBV and HIV-infected groups it has a sensitivity of 96.4% and 100%, a specificity of 96.2% and 88.2%, an AUC of 0.98 (95% CI: 0.95-1.0) and 0.99 (95% CI: 0.97-1.0), respectively. No significant difference between the three AUC was observed ( $p=0.69$ ). In the mono-infected group the PPV was 98.1%, the NPV 99.3%,  $LR+=319$  and the  $LR-=0.043$ . In the HBV and HIV-infected groups we respectively found a PPV of 80.2% and 57.6%, a NPV of 99.4% and 100%.

**Conclusions:** AgC quantification displayed high specificity and sensitivity; in addition neither HIV nor HBV coinfection influenced its discrimination capacity. Then it represents a reliable HCV diagnosis tool and, being less costly than viral load tests, could ease HCV screening, notably in resource-limited settings.

Figure 2: ROC curves of the performance of AgC quantification for the diagnostic of chronic hepatitis C in HCV mono-infected and HCV uninfected, HIV-infected and HBV-infected patients



## 527 Prognostic Value of Transient Elastography and FIB-4 in HIV/HCV Coinfection

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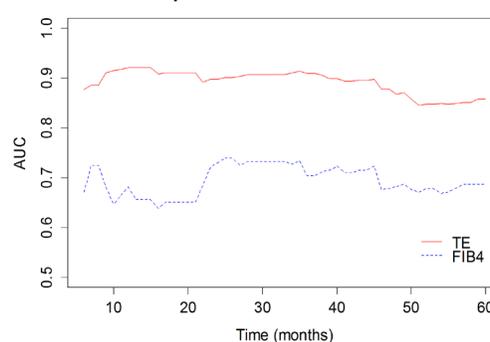
**Background:** FIB-4 outperforms liver biopsy as a predictor of outcomes in patients with HIV/HCV coinfection. Our aim was to compare the prognostic value of transient elastography (TE) and the FIB-4 index in patients with HIV/HCV coinfection.

**Methods:** The study was carried out in 3 institutions. We identified patients with at least 1 determination of liver stiffness who were both HIV+ and HCV-RNA+ and had compensated chronic hepatitis C. Baseline was the date of the first TE determination. The main outcome was liver-related events (LRE), namely, liver decompensation (DEC) or hepatocellular carcinoma (HCC), whichever occurred first. We used ROC curves and time-dependent ROC curves (ROC(t)) (Biometrics 2000; 56:337-344) to determine the ability of TE and FIB-4 to predict outcomes. We also assessed the association between advanced fibrosis—TE ( $\geq 9.5$ ) or FIB-4 ( $\geq 3.25$ )—and LRE using multivariate Cox regression analysis taking into account death as a competitive risk. The variables for adjustment were age, sex, HIV transmission category, CDC clinical category, CD4+ cell nadir, alcohol intake, and achievement of SVR.

**Results:** The study sample comprised 1,159 patients who met the inclusion criteria and had undergone determination of TE and FIB-4 between 24/09/2003 and 01/01/2015. After a median follow-up of 5.8 years, 255 patients achieved sustained viral response (SVR), 65 died, and 75 had LRE (67 DEC and 17 HCC). Baseline fibrosis by TE was  $\leq 7.1$  in 539 patients,  $>7.1$  and  $<9.5$  in 182 patients, and  $\geq 9.5$  in 438 patients. Fibrosis by FIB-4 was  $\leq 1$  in 453 patients,  $>1$  and  $<3.25$  in 520 patients, and  $\geq 3.25$  in 186 patients. The AUROCs (95%CI) for LRE for TE and FIB-4 were 0.854 (0.805–0.902) and 0.670 (0.595–0.743), respectively ( $P\leq.001$ ). The AUROCs for overall death (OD) and the composite end-point of OD/LRE (whichever occurred first) were also significantly higher for TE than for FIB-4. The **figure** shows time-dependent AUC plots for LRE for TE and FIB-4 up to 5 years; the estimated AUROC(t) at 3 and 5 years for TE and FIB-4 was 0.919 and 0.713 and 0.852 and 0.694, respectively. The adjusted hazard ratio (95%CI) of LRE was 18.7 (9.00–38.7) for advanced fibrosis assessed by TE ( $P<.001$ ) and 5.36 (3.22–8.93) for advanced fibrosis assessed by FIB-4 ( $P<.001$ ).

**Conclusions:** TE outperformed FIB-4 as a predictor of clinical outcomes. These findings support the prognostic role of TE in patients with HIV/HCV coinfection.

Time-depnt AUC Plot Liver-Related Event



**528 Variation in Liver Fibrosis Staging Using FibroTest, FIB-4, and APRI Scores**

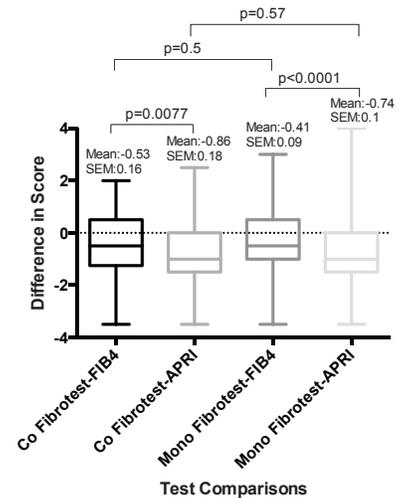
**Aurielle Thomas<sup>1</sup>**; Charisse Ahmed<sup>2</sup>; Chloe Gross<sup>3</sup>; Rachel Silk<sup>3</sup>; Elizabeth Akoth<sup>3</sup>; Eleanor M. Wilson<sup>4</sup>; Angie Price<sup>3</sup>; Shyam Kottlil<sup>5</sup>; Henry Masur<sup>6</sup>; Sarah Kattakuzhy<sup>3</sup>  
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**Background:** Patients with Hepatitis C (HCV) infection develop varying degrees of hepatic fibrosis, and fibrosis stage affects multiple aspects of HCV care. Increasingly, non-invasive methods are used to determine the stage of liver fibrosis. The objective of this analysis was to analyze three common staging methods from a large urban cohort and determine their degree of correlation among HCV mono-infected and HIV/HCV co-infected patients.

**Methods:** Analysis was completed by a chart review of 263 patients from the ASCEND study, a phase IV multi-site trial of community-based hepatitis C treatment. FibroTest, FIB-4, and APRI were obtained for each patient using standard equations, and all were adjusted to a four-point scale for comparison. Serum biomarkers used for FIB-4 and APRI scoring were obtained within 30 days from FibroTest. Statistical analysis was completed using nonparametric Wilcoxon rank sum and column statistics were performed in Graphpad Prism 6.0.

**Results:** The patient cohort was predominantly African American, with a mean age of 59, and included 61 HIV/HCV co-infected and 203 HCV mono-infected patients. Overall, mean FibroTest score was higher than both APRI (mean difference -0.77+/- .09) and FIB-4 (mean difference -0.44+/- .08). The calculated difference between FibroTest and APRI was significantly higher (p<0.0001) when compared to the mean difference between FibroTest and FIB-4. In contrast, the mean difference in between FIB-4 vs APRI was 0.33 (SEM:0.06). In co-infected patients, the calculated mean difference of both FibroTest-FIB-4 and FibroTest-APRI was not significantly different (p=0.5, p=0.57) compared to mono-infected patients.

**Conclusions:** Overall, this analysis demonstrates significant differences in liver fibrosis staging between FibroTest, FIB-4, and APRI scores in an urban cohort representative of the HCV epidemic, including HIV/HCV co-infected patients. There was minimal variation between APRI and FIB-4, and these scores were significantly lower than calculated FibroTest, with no effect of HIV co-infection on these relationships. In the era of non-invasive staging, more research on the correlation of each scoring system with long-term outcomes is required to determine optimal staging method.



**529 New Predictive Index Based on the Combination of Liver Stiffness and CTP**

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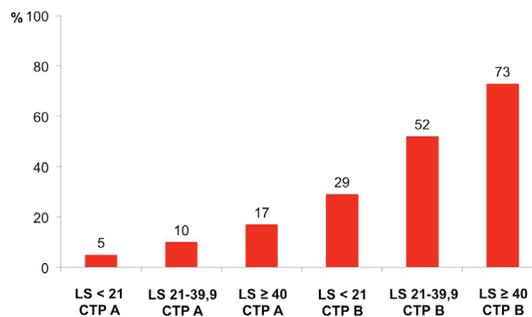
<sup>1</sup>Hosp Universitario de Valme, Sevilla, Spain; <sup>2</sup>IMIBIC, Córdoba, Spain; <sup>3</sup>Hosp La Línea de la Concepción (AGS Campo de Gibraltar), La Línea de la Concepción, Spain; <sup>4</sup>Complejo Hospario de Huelva, Huelva, Spain; <sup>5</sup>Hosp Universitario Virgen de la Macarena, Seville, Spain; <sup>6</sup>Hosp Universitario Virgen de la Victoria, Málaga, Spain; <sup>7</sup>Complejo Hospario de Jaén, Jaén, Spain; <sup>8</sup>Hosp de La Línea de la Concepción, Cádiz, Spain

**Background:** Liver stiffness (LS) predicts clinical outcome in patients with cirrhosis. However, LS is not being routinely used for some clinical decision-making, which still mainly relies on the Child-Turcotte-Pugh (CTP) or the MELD scores. Our objective was to develop a new predictive index, which includes LS, and to evaluate its ability to predict events in HIV/HCV-coinfected patients with compensated cirrhosis.

**Methods:** Prospective study of 446 HIV/HCV-coinfected patients with a new diagnosis of cirrhosis and no previous liver decompensation (LD). The time from diagnosis to LD and to liver-related death (LRD), as well as its predictors, were evaluated. A new score based on the combination of LS and CTP was built. The ability of the new score to predict outcomes was compared to that of other classical scores, by means of the comparisons of the AUROC and the integrated discrimination improvement (IDI) between models.

**Results:** Median (IQR) follow-up of 49 (25-68) months. 80 (17.9%) patients had a LD. Variables independently associated with LD: age, HBV, SVR, AIDS, CTP stage B (vs A) (AHR 4.18, p<0.0001) and baseline LS (comparison group: LS < 21 kPa) (LS 21-39.9 kPa: AHR 2.48, p=0.005; LS ≥ 40 kPa: AHR 3.68, p<0.0001). LS yielded a better performance than MELD (IDI 3.3%; p=0.01) and a similar performance than CTP (IDI 0.13%; p=0.9) to predict LD. Consequently, LS and CTP were combined in a new predictive score. 3-year probability of LD increased across stages of the new index: stage 1 (CTP A and LS < 21 kPa) 5%, stage 2 (CTP A and LS 21-39.9 kPa) 10%, stage 3 (CTP A and LS ≥ 40 kPa) 17%, stage 4 (CTP B and LS < 21 kPa) 29%, stage 5 (CTP B and LS 21-39.9 kPa) 52% and stage 6 (CTP B and LS ≥ 40 kPa) 73% (p<0.0001). A new multivariate analysis including the new index demonstrated its independent association with LD (stage 1: comparison group): stage 2 [AHR 2.9; p=0.002], stage 3 [AHR 3.5; p=0.001], stage 4 [AHR 3.7; p=0.2], stage 5 [AHR 10.4; p<0.0001] and stage 6 [AHR 20.7; p<0.0001]. The AUROC of this model was higher than that of the model based on the CTP stage (AUROC 0.612 vs AUROC 0.575; p=0.06). LRD occurred in 37 (8.3%) patients. 3-year probability of LRD increased from stage 1 to 3 (stage 1: 1%, stage 2: 4%, stage 3: 10%) and was significantly higher in stages 4 to 6 (stage 4: 29%, stage 5: 27%, stage 6: 28%).

**Conclusions:** The combination of LS and CTP stage in a new predictive index improves the ability of CTP to predict clinical outcome in HIV/HCV-coinfected patients with compensated cirrhosis.



**530 Liver Stiffness Predicts Variceal Bleeding in HIV/HCV-Coinfected Patients**

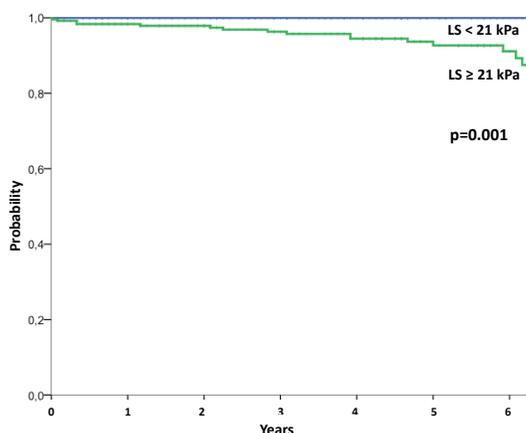
**Nicolás Merchante**<sup>1</sup>; Antonio Rivero-Juárez<sup>2</sup>; Francisco Téllez<sup>3</sup>; Dolores Merino<sup>4</sup>; María José Ríos<sup>5</sup>; Guillermo Ojeda-Burgos<sup>6</sup>; Mohamed Omar Mohamed-Balghata<sup>7</sup>; Juan Macías<sup>1</sup>; Antonio Rivero<sup>8</sup>; Juan A. Pineda<sup>1</sup>; for the HEPAVIR-Cirrhosis Study Group  
<sup>1</sup>Hosp Universitario de Valme, Sevilla, Spain; <sup>2</sup>IMBIC, Córdoba, Spain; <sup>3</sup>Hosp La Línea de la Concepción (AGS Campo de Gibraltar), La Línea de la Concepción, Spain; <sup>4</sup>Complejo Hospario de Huelva, Huelva, Spain; <sup>5</sup>Hosp Universitario Virgen de la Macarena, Seville, Spain; <sup>6</sup>Hosp Universitario Virgen de la Victoria, Málaga, Spain; <sup>7</sup>Complejo Hospario de Jaén, Jaén, Spain; <sup>8</sup>Hosp Universitario Reina Sofía, Córdoba, Spain

**Background:** A previous study has shown that a liver stiffness (LS) < 21 kiloPascals (kPa) has a 100% negative predictive value (NPV) to exclude the presence of esophageal varices (EV) at risk of bleeding in HIV/HCV-coinfected patients. Consequently, upper gastrointestinal endoscopy (UGE) for the screening of EV could be avoided in these patients. However, this strategy has not been widely accepted due to concerns about its safety. Our objective was to assess the predictive value of LS to predict the risk of variceal bleeding in HIV/HCV-coinfected patients with compensated cirrhosis.

**Methods:** Prospective cohort study of 461 HIV/HCV-coinfected patients with a new diagnosis of cirrhosis, based on the presence of LS ≥ 14 kPa, and no previous decompensation of liver disease. All patients underwent a UGE for the screening of EV at entry in the cohort before November 2009. From this date, UGE was not recommended by the cohort protocol in patients with LS < 21 kPa. The time from diagnosis of cirrhosis to the emergence of a variceal bleeding episode, as well as the predictors of this outcome were evaluated.

**Results:** At baseline, 206 (45%) had a LS < 21 kPa whereas 255 (55%) had a LS ≥ 21 kPa. In 311 (67%) patients, at least 1 UGE was done. EV at risk of bleeding were present in 26 (8%) of them. During follow-up, 417 UGE were performed in 311 patients. The median (IQR) elapsed time between LS assessment and UGE examination was 21 (-12, 78) days. EV at risk of bleeding were present in 2 (2%) UGE examinations of patients with a LS < 21 kPa whereas it was found in 43 (13%) UGE examinations of patients with a LS ≥ 21 kPa (p=0.008). These two patients with a LS < 21 kPa and high-risk EV harboured a LS of 20.2 and 20.9 kPa, respectively. After a median (IQR) follow-up of 49 (24-68) months, 16 (3.5%, 95% confidence interval: 1.8-5.1) patients developed a first variceal bleeding episode. In all cases, baseline LS was ≥ 21 kPa. Thus, the NPV of a LS < 21 kPa to predict a bleeding episode during follow-up was 100%. At the moment of the bleeding episode, LS was also above this threshold.

**Conclusions:** Baseline LS identifies HIV/HCV-coinfected patients with compensated cirrhosis with a very low risk of presenting a variceal bleeding episode. In fact, no individual with baseline LS < 21 kPa developed this outcome. Our results confirm that UGE can be safely spared in patients with LS < 21 kPa, provided that LS maintains below this threshold.



**531 Hepatitis C Screening and Linkage to Care at a Comprehensive Health System**

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**Background:** The Centers for Disease Control and Prevention (CDC) and the United States Preventive Services Task Force (USPTF) recommend screening for Hepatitis C (HCV) among patients born between 1945 and 1965. With the advent of novel highly effective therapies, we evaluated the current HCV screening rates along with linkage to care for patients with active disease.

**Methods:** We used the Henry Ford Health System records to create a retrospective cohort of patients born between 1945 - 1965 seen at 21 internal medicine clinics between July 2014 and June 2015. Patients previously screened for HCV and those with established disease were excluded. We studied patient socio-demographic and medical conditions along with provider-specific factors associated with likelihood of screening. Patients who tested positive were reviewed to assess appropriate linkage to care and treatment.

**Results:** 47,304 patients were included in our study cohort and 40,561 patients met inclusion criteria. A total of 8,657 (21.3%) were screened. Screening rates were found to be higher among men (p < 0.001) and African Americans (p < 0.001). The rates were lower in patients with multiple comorbidities (p < 0.001) and fewer clinic visits (p < 0.001). Practice setting influenced screening rates as patients seen in residency teaching clinics were more likely to be screened (p < 0.001). Patient electronic health engagement was associated with higher screening rates (p < 0.001). Among patients who were screened, 117 (1.4%) patients tested positive. After excluding patients without active viremia, 78% of patients were referred to a Hepatitis C specialist and 50% were successfully evaluated. On follow-up, 27% of HCV positive patients received treatment with Direct Acting Anti-virals.

Medicaid patients were less likely to be treated (p < 0.05) along with a trend towards a decrease in likelihood of treatment among patients with lower income. Electronic health engagement was again a significant factor that increased the odds of treatment (p < 0.05).

**Conclusions:** HCV screening rates are suboptimal with a significant influence of sociodemographic and provider-specific factors. Furthermore, patients who tested positive had inadequate linkage to care with a major disadvantage for Medicaid and low income patients. This accentuates the need for a more robust and equitable care delivery system. The study also highlights a promising role for patient's engagement in electronic health portals through active linkage at multiple phases of the care cascade.

Factors Influencing Screening		
Variable	Odds Ratio	P- value
African American Race	1.34	< 0.001
Male Gender	1.18	< 0.001
Electronic Health Engagement	1.24	< 0.001
Residency Teaching Clinic	1.20	< 0.001
Medical Comorbidities	0.87	< 0.001
Number of Office Visits	1.42	< 0.001
Factors Influencing Treatment		
Variable	Odds Ratio	P- value
Medicaid Coverage	0.21	< 0.05
Electronic Health Engagement	2.73	< 0.05

### 532 Influence of Hepatitis C Virus Screening on Emergency Department Length of Stay

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**Background:** In April 2014, we integrated triage nurse hepatitis C virus (HCV) screening into emergency department (ED) clinical operations. We utilized a laboratory-based testing protocol and native staffing to offer, perform, and disclose results. Because of concerns that HCV screening would increase ED length of stay (LOS), our protocol did not require patients to wait for their HCV test results prior to discharge. The objective of this study was to assess the impact of this integrated HCV screening protocol on ED LOS.

**Methods:** In this retrospective cohort study we analyzed prospectively collected timestamp data for all discharged patients. The primary outcome compared the median LOS in minutes between patients who completed HCV screening and those who did not. We additionally stratified patients based on whether or not other laboratory testing was done, using complete blood count (CBC) tests as a surrogate. Length of stay was defined as the time between triage (timestamped when intake begins) and discharge (timestamped when discharge instructions are printed).

**Results:** Over the 1-year study period, LOS was calculated for 69,639 (96%) of the 72,338 visits for which patients were discharged. The LOS for visits that included HCV screening ( $n = 2,864$ ) was 151 minutes (IQR 66 to 251) compared with 119 minutes (IQR 48 to 221) for visits that did not include HCV screening ( $n = 66,775$ ) ( $p < 0.001$ ). Among the 49,726 visits in which no CBC testing was performed, there was a significant difference in LOS between the 1,701 visits that included HCV screening (86 minutes, IQR 38 to 158) and the 48,025 visits that did not (77 minutes, IQR 34 to 150) ( $p < 0.001$ ). However, among the 19,913 visits in which CBC testing was performed, there was no significant difference in LOS between the 1,163 visits that also included HCV screening (240 minutes, IQR 173 to 339) and the 18,750 visits that did not (242 minutes, IQR 170 to 347) ( $p = 0.68$ ).

**Conclusions:** We show that an integrated HCV screening program modestly prolongs overall ED LOS. However, among patients undergoing other blood tests, HCV screening had no significant effect on LOS. Emergency departments must consider whether the public health benefit of screening justifies the impact on quality metrics, such as LOS, which has been shown to influence the ability to provide timely acute care. Future programs may consider routinely offering HCV screening to patients who are undergoing laboratory testing.

### 533 Devising a Strategy to Control the HCV Epidemic in British Columbia, Canada

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**Background:** In 2012, less than 1% of the estimated 12,000 hepatitis C (HCV) infected people who inject drugs (PWID) in British Columbia (BC) received HCV treatment, despite accounting for 80% of all new HCV infections. The recent approval and availability of high-efficacy and tolerable HCV medications will make it possible to treat a large number of individuals who were previously ineligible for treatment. Reinfection risk remains an issue, particularly among PWID, and it is still not clear if individuals who achieve sustained virologic response gain some degree of subsequent immunity. Reinfection risk could also be mitigated by engaging individuals in harm reduction programs.

**Methods:** We designed a deterministic compartmental mathematical model of HCV disease transmission fit to the PWID population in BC, based on treating a fixed number of individuals per year. We calculated the difference in incident cases with respect to the status-quo, as a function of both the number of PWID treated per year, and varying rates of reinfection risk. We defined the threshold for HCV control as the minimum number of PWID treated per year required to offset the number of new incident cases, with removals taken into account.

**Results:** The control threshold at year five (Figure 1A) ranged from 128 PWID treated per year, assuming 0% reinfection risk, to 178 when there is 100% reinfection risk, i.e., equal to naïve uninfected PWID. At ten years (Figure 1B), the threshold varied between 121 and 240 individuals treated. We simulated the change in incident cases (Figures 1C-1D) when treating 100 or 300 PWID per year, for varying rates of reinfection risk reduction. In the first scenario (100 treated per year, Figure 1C), after 20 years of constant treatment uptake, the number of incident cases was reduced between 1% and 16%. In the second scenario (300 treated per year, Figure 1D), the number of incident cases decreased between 16% and 54%.

**Conclusions:** The availability of highly efficacious treatments holds great promise to disrupt the course of the HCV epidemic. Treating the PWID population is crucial to controlling the epidemic, as the majority of new infections occur within this population, but reinfection risk remains a concern. Our simulations show that treating a minimum of 200 to 300 PWID per year will lead to HCV control, and this will be substantially accelerated if the potential for HCV reinfection is minimized through the deployment of harm reduction programs.

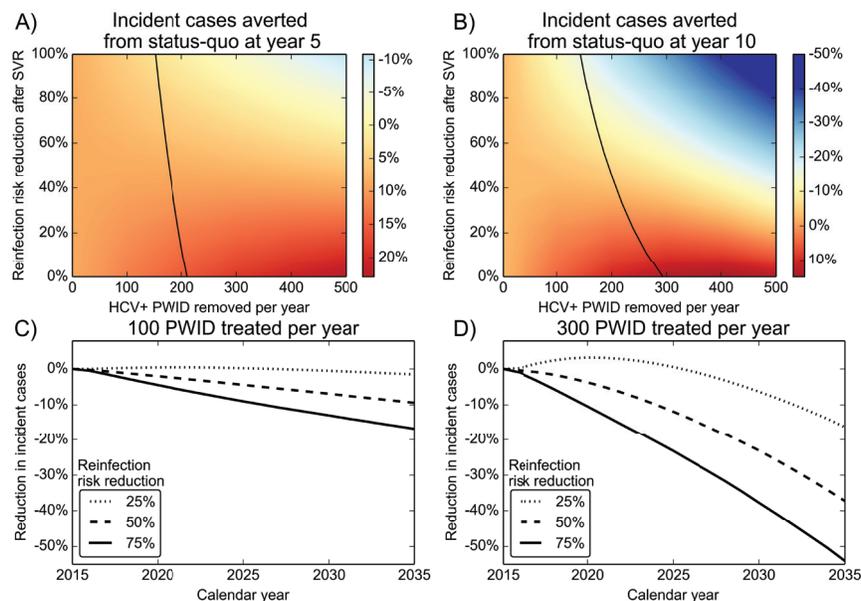


Fig. 1. Figures 1A and 1B show the difference in incident cases averted from baseline as a function of the number of PWID treated per year and the reinfection risk compared to naïve individuals. The black curve is the disease control threshold. Figures 1C and 1D show the change in incident cases on either side of the threshold, for varying reinfection risks.

**534 Project INSPIRE: A Comprehensive Care Coordination Program for HCV Infection**

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**Background:** New York City (NYC) has an estimated 146,500 Hepatitis C (HCV)-infected residents and <10% have been treated. HCV infection is a complex disease coexisting in patients with co-morbid conditions such as substance abuse, HIV, and mental illness, making treatment in a supported setting critical. Project INSPIRE, a Round II Innovation Award from the Centers for Medicaid and Medicare Services, was designed by the NYC Health Department to offer comprehensive care coordination and to develop a payment model for currently unpaid care coordination services.

**Methods:** The goals of Project INSPIRE are to improve HCV cure rates and patient self-sufficiency through evidence-supported care coordination services. Program components include a comprehensive assessment and care plan, clinic-based care coordination services, health promotion, and medication adherence support. INSPIRE medical providers participate in case conferences with care coordinators and are trained via weekly tele-mentoring sessions on HCV care. INSPIRE aims to enroll 3,200 patients, initiate treatment for 75%, and achieve a cure for 50% of cirrhotic and 90% of non-cirrhotic patients. Program data will be used to support rigorous evaluation activities and develop a payment model, in collaboration with two managed care organizations.

**Results:** Between January – August, 2015, 919 HCV-positive patients were enrolled. The majority of participants are male, (n=570, 62%) Hispanic (n=408, 44%) or Black (n=319, 35%). The median age was 56, and 693 (75%) were born between 1945-1965. In the first year, 664 enrollees (72%) completed HCV medical evaluation, and 655 (71%) received a comprehensive assessment. Of those assessed, 426(65%) have past or present IV drug use, 222 (34%) are co-infected with HIV, and 35 (5%) have serious mental illness. Of 580 eligible treatment candidates, 311 (54%) initiated HCV treatment. To-date, 31 INSPIRE treatment candidates have achieved SVR-12 and the rest are still undergoing treatment. Project INSPIRE experts conducted 54 tele-mentoring sessions with 123 attendees.

**Conclusions:** Project INSPIRE is successfully enrolling high need patients and starting many on treatment with the expectation that the vast majority will be cured. Over the next two years, DOHMH will work with project partners to develop cost estimates and a payment model that, if adopted by CMS, will be instrumental in making these services available and sustainable nationwide.

**535 HCV Treatment As Prevention Will Require Massive Scale-up to See Prevention Benefits**

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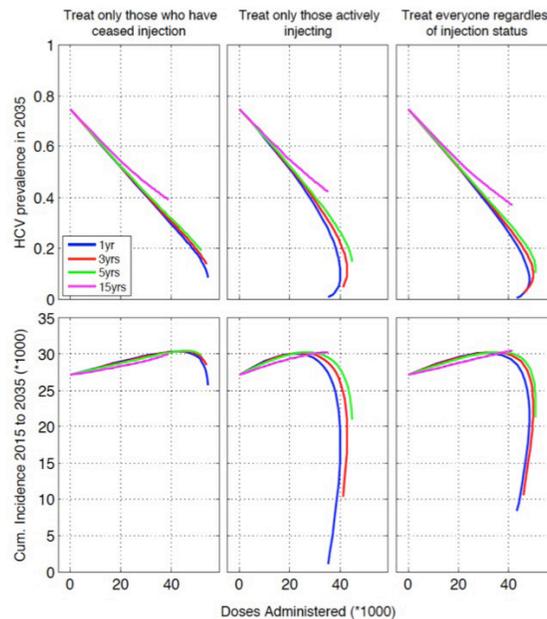
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**Background:** Modeling has suggested HCV prevalence reductions of >50% with widespread treatment in people who inject drugs [PWID]). However, there has been inadequate consideration of 1) how much transmission can be targeted by treatment given realistic delays and empirical data on age specific hazards; and 2) population-level mixing characterizing counterfactual transmission if those treated remain untreated and continue to infect. We explore the impact of treatment strategies on HCV incidence using data from multi-decade studies of HCV transmission among PWID in Baltimore, Maryland.

**Methods:** We developed an age-specific compartmental model of HCV transmission in a community of current and former PWID parameterized using empirical data from studies in Baltimore to obtain age-specific 1) HCV prevalence from 1988-2008; 2) rates of injection initiation, cessation and relapse; and 3) mortality. We varied contact matrices from random to fully age-specific. We compared strategies from conservative: 1) treating only abstinent PWID ~ 15 years after infection to aggressive: 2) treating all PWID regardless of injection 1-5 years after infection with and without harm reduction scale-up. We estimated reductions in incidence/prevalence over 20 years.

**Results:** Our model supports that widespread HCV treatment can have significant positive impact – reduction in prevalence of 40% with 20,000 treatment courses over 20 years. At this level, HCV prevalence decrease varied little by who was treated (active, abstinent vs. all) or when they received it (1-15 years after infection, Figure). Further, at coverage <88% of the PWID population, almost all prevalence reduction was due to direct effects of curing people. Indirect effects were negligible because the hazard of HCV infection is so high that significant treatment is needed to reduce it. In order to impact transmission (indirect effects), treatment needs to be scaled to >90% of the population (>40,000 doses) targeted 1-3 years after infection with simultaneous harm reduction scale up. Even at these levels, only 0.8 incident cases are averted per treatment.

**Conclusions:** To truly impact HCV transmission in PWID, treatment programs need to be aggressive in treating large numbers of PWID almost immediately after HCV acquisition and comprehensive by integrating harm reduction. Given the vast amount of treatment need to impact transmission, programs should prioritize clinical considerations and the relative impact of harm reduction.



**Figure.** HCV prevalence in the year 2035 and cumulative incidence from 2015 to 2035 in response to different intervention strategies that vary by 1) number of persons treated per 1000 population (x-axis); the delay between infection and treatment (colored lines); and different states of injection from A) PWID who have ceased injection only; B) only actively injecting PWID; and C) all PWID regardless of current injection (columns).

**536 Is HCV Elimination Possible? A Modeling Study of HIV-Positive MSM**

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**Background:** Hepatitis C virus (HCV) infections are common among HIV positive men who have sex with men (MSM). Acute and chronic HCV have traditionally been treated with peginterferon containing regimens. During the last year, direct-acting antivirals (DAAs) for chronic HCV treatment have become available. Contrary to peginterferon, DAAs have limited side-effects and high cure rates. Widespread use of DAAs could cure patients and prevent onward transmission, in turn reducing the HCV epidemic. Unfortunately, DAAs are expensive and treatment can be deferred until later stages of chronic infection. The aim of this study was to assess the epidemiological and economic impact of providing DAAs to all co-infected MSM compared to deferring DAAs until fibrosis stage F2 or F3.

**Methods:** A deterministic mathematical model was calibrated to the Dutch HCV epidemic among HIV-infected MSM. We determined the epidemiological impact from 2015 to 2030 of providing DAAs (89%-100% cure rate) to all diagnosed patients. In the counterfactual scenarios, patients were treated with peginterferon and ribavirin in the acute stage. In patients that failed or refused, treatment with DAAs was initiated in stage F2 or F3. The costs, cost per infection averted and incremental cost-effectiveness ratios (ICER) were calculated from a societal perspective using a DAA price of €50,000 for a 12-week course.

**Results:** Compared to deferring DAA treatment to stage F2 or F3, treating all patients will 1) avert 1060 infections (F2) or 1081 infections (F3), 2) reduce the incidence rate from 12/1000 to 5/1000 person-years (compared to a stable incidence of 12/1000 for F2 or F3) and 3) reduce the prevalence from 5% to 1.4% (compared to an increase to 6.5% for F2 or 7.6% for F3). Treating all patients will cost society €31 million (€27000 per infection averted) or €39 million (€32000) compared to deferring DAA-treatment to stage F2 or F3, respectively. The ICER is €53,000 (F2) or €58,000 (F3) per quality-adjusted life year (QALY). The economic impact depends on the price of DAAs: the costs ranges between €14 million (defer to F2, DAA-price of €30,000) and €50 million (F3, €60,000) and the ICER range between €24,000 (F2, €30,000) and €73,000 per QALY (F3, €60,000).

**Conclusions:** Treating all diagnosed patients with DAAs will strongly reduce, but not eliminate, the HCV-epidemic among HIV-infected MSM. The major determinant for cost-effectiveness is the price of DAAs.

**537 Primary Care Provider Interest in Treating HCV: A Mixed-Methods Study**

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**Background:** There are over 3 million Americans infected with hepatitis C (HCV). Despite recent advances in HCV treatment, a major barrier to care remains a limited number of providers. Provision of HCV therapy by primary care providers (PCPs) could expand access by increasing the pool of treating clinicians. We characterize willingness and self-efficacy of PCPs to become HCV treaters in a city with high HCV prevalence.

**Methods:** 271 PCPs were identified from 4 large federally qualified health centers and community clinics affiliated with a large academic center in Baltimore, MD. We administered an internet-based survey from September 2014 to February 2015; followed by qualitative interviews with 20 randomly selected PCPs. The survey assessed provider demographics, clinical practice site and agreement with the statement "HCV treatment should be provided by PCPs in the all oral HCV DAA era" based on a 5-point Likert scale. A composite score was created for PCP self-efficacy based on five questions on aspects of HCV care for a maximum score of 20. Factors associated with agreement were examined using odds ratios (OR). Qualitative transcripts were read by 3 investigators and themes identified.

**Results:** 129 (48%) PCPs responded. The majority (71%) had an MD/DO degree, were white (60%), currently screen patients for HCV (88%) and refer patients for specialist care (86%). 14 (11%) had treated ≥1 patient for HCV in the prior year. Only 5 (4%) had a score of ≥15 consistent with high self-efficacy of being skilled in HCV care, and 36 (26%) a score of ≥10 consistent with average self-efficacy. Most reported no/limited knowledge of HCV care. Although only 20% agreed treatment should be provided by PCPs, 61% were interested in more HCV training. Willingness to provide treatment was strongly linked to having a high proportion of HCV-infected patients (>20% vs ≤20%; OR 4.5; 95% CI 1.7-11.6) and availability of other services at the primary care site including HIV treatment (OR 7.0; 95% CI 2.7-17.8), substance abuse treatment (OR 2.7; 95% CI 1.1-6.7), and mental health services (OR 4.8; 95% CI 2.0-12.1). HCV care barriers identified in qualitative interview include limited PCP knowledge of HCV care, access to treatment specialists, competing patient health care needs, and medication cost.

**Conclusions:** These data suggest that the largest impact of PCP involvement in HCV care will be achieved by initially focusing HCV training on PCPs with a high burden of HCV infected patients and existing systems to support HCV care.

**538LB High Efficacy of HCV Treatment by Primary Care Providers: The ASCEND Study**

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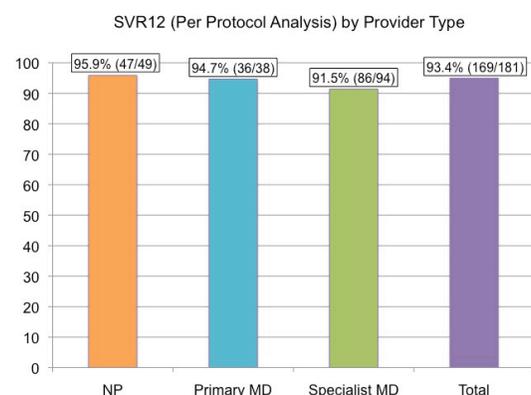
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**Background:** The hepatitis C (HCV) care cascade in the US is limited by the number of specialists able to treat HCV. Given the advent of directly acting antiviral therapy, we conducted a large-scale, longitudinal trial to evaluate the efficacy and safety of primary care driven HCV treatment

**Methods:** In this multi-center, open label, phase IV clinical trial, chronic HCV-infected patients of community health centers in Washington DC were identified by their providers, consented, and distributed in a non-randomized manner to receive treatment from either a specialist (ID/Hepatology), primary care physician (PCP), or nurse practitioner (NP). Providers underwent uniform training on IDSA-AALSD therapeutic guidelines. Patients were treated with ledipasvir and sofosbuvir (LDV/SOF) as per label. The primary outcome was defined as unquantifiable HCV RNA viral load 12 weeks after completion of therapy (SVR12). Adherence to visits at 4, 8, and 12 weeks (all -7 to +14 days), were categorized by a composite score of attendance. Statistical analysis included chi-squared or Fisher's exact test and logistic regression using SAS, version 9.3

**Results:** 600 patients began treatment with LDV/SOF from May to November 2015, with follow up ongoing. 14 patients discontinued treatment early, including 4 due to adverse events and 1 death unrelated to study participation. Patients were predominantly black (96%) and genotype 1a (72%); 24% were HIV/HCV-coinfected, 18% were treatment experienced, and 20% were CPA cirrhotic, with comparable baseline characteristics between provider groups. Of 181 patients with available results, 169 achieved SVR12 (93.4% per protocol; 86.7% intention-to-treat including early discontinued). Of 12 patients with virologic failure, 1 had breakthrough and 11 had relapse. There was no significant difference ( $p=0.67$ ) between per protocol SVR12 and provider type: NPs (47/49;95.9%), PCPs (36/38;94.7%), and specialists (86/94;91.5%). HIV status had no impact on SVR12 or SVR12 by provider type. Of 419 patients who completed 12 weeks of therapy, composite adherence was significantly associated with provider type: 50% in NPs, 41% in PCPs, and 19% in specialists ( $P<0.001$ )

**Conclusions:** For the first time, we demonstrate that HCV treatment administered independently by PCPs and NPs is safe and effective, inclusive of challenging subpopulations of the epidemic, and within the largest African-American cohort described to date. Community-based non-specialist providers could significantly expand the scale of HCV therapy



**539LB Effect of Baseline Resistance-Associated Variants on SVR With the 3D Regimen Plus RBV**

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**Background:** The 3D regimen (ombitasvir/paritaprevir/ritonavir [paritaprevir identified by AbbVie and Enanta] and dasabuvir) + RBV, which target HCV NS3, NSSA and NSSB, respectively, is highly effective and is approved in the US and EU for the treatment of HCV GT1 infection. To assess the prevalence of baseline resistance-associated variants (RAVs) and their impact on SVR rate for this regimen, we used next generation sequencing (NGS) to evaluate baseline samples from patients enrolled in the Phase 3 studies of GT1a treatment-experienced (SAPPHIRE-II) and cirrhotic (TURQUOISE-II) patients.

**Methods:** NGS was employed using Illumina MiSeq on baseline samples from 332 GT1a patients (n=118 in TURQUOISE-II; n=214 in SAPPHIRE-II) all of whom received 3D + RBV. Patients who discontinued treatment early for non-virologic reasons were excluded. The prevalence and impact of baseline RAVs in NS3, NSSA, and NSSB were determined using a threshold of NGS detection ranging from 1% to 20%. The impact of baseline RAVs on treatment outcome was determined by comparing SVR rates in patients with or without detectable RAVs.

**Results:** 320/332 (96%) patients in this sample set achieved durable SVR (SVR<sub>12</sub>). Baseline variants at positions 80, 155, 156 and 168 in NS3; 28, 30, 58, and 93 in NSSA; and 316, 414, 448, 556, and 561 in NSSB were evaluated. Using a NGS threshold of 1%, NSSA RAVs were present in 22% of patients; at a sensitivity of 15% (comparable to population sequencing), ≥1 NSSA RAVs were present in 38 (11%) patients. Using the prevalence data at the 15% NGS threshold, similar SVR rates were seen in patients with or without ombitasvir-specific RAVs (95% vs. 97%). RAVs in NS3 and NSSB were rare (1 and 2%, respectively). All 3 patients with a baseline NS3 RAV achieved SVR, while an NSSB RAV was seen in 1 of the 12 virologic failure patients. The highly prevalent Q80K polymorphism in NS3 was assessed and had no impact on SVR rate (96% with Q80K vs. 97% without Q80K).

**Conclusions:** As most current HCV therapies include an NSSA inhibitor, the high prevalence of baseline NSSA RAVs is of particular importance. Patients treated with the 3D regimen + RBV achieved similarly high SVR rates, regardless of presence or absence of baseline NSSA RAVs.

**540 HCV IgG Antibody Avidity as a Biomarker to Estimate Population-Level Incidence**

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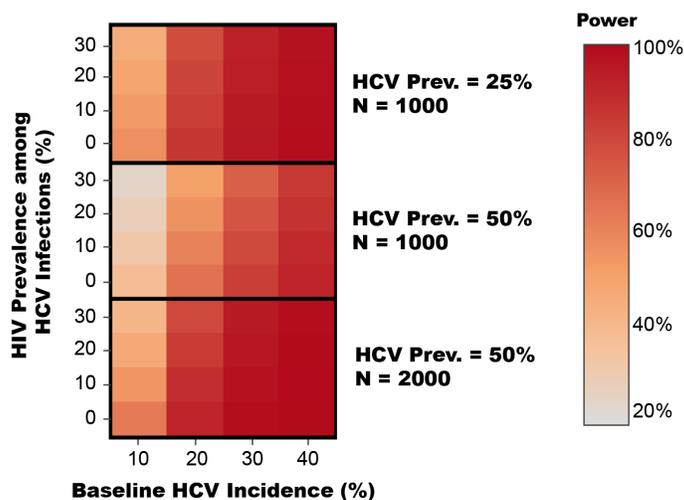
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**Background:** Sensitive methods to estimate population-level incidence are needed to monitor the trajectory of the HCV epidemic. We demonstrate how HCV IgG antibody avidity, a biomarker associated with recent infection, can be used to estimate population level HCV incidence.

**Methods:** From 568 people who inject drugs and are enrolled in prospective cohort studies in Baltimore, Maryland, we tested 997 serum or plasma samples [n=233 collected <2 years post-HCV seroconversion and n=764 collected >2 years post-HCV seroconversion]. HCV IgG antibody avidity was determined using a modified Ortho 3.0 HCV ELISA test system. Avidity-based testing algorithms were evaluated by their: (1) mean duration of recent infection (MDRI), the average time an individual is identified as recently infected by a given testing algorithm, (2) false recent rate, the proportion of samples collected >2 years post-HCV seroconversion misclassified as 'recent', (3) sample size needed to precisely estimate incidence in various simulated epidemics (relative standard error <30%), and (4) power to detect a 50% reduction in incidence between serial cross-sectional surveys.

**Results:** A multi-assay algorithm consisting of an avidity index <30% followed by detection of HCV viremia (RNA>500 IU/mL) had an MDRI of 147 days (95%CI: 125-195), and a false recent rate of 0.7% (95%CI: 0.2-1.8) and 7.6% (95%CI: 4.2-12.3) among HIV negative and positive persons, respectively. In several simulated high-risk populations (HCV incidence >10%), this multi-assay algorithm required less than 1000 individuals to estimate incidence. This multi-assay algorithm also had >80% power to detect a 50% reduction in HCV incidence in various epidemic scenarios where baseline incidence was >20% (see Figure 1; N=1000-2000). Avidity-based algorithms consistently required smaller sample sizes than current approaches (viremic detection among seronegative individuals).

**Conclusions:** Integrating HCV avidity-based testing algorithms into current surveillance systems may improve the capacity to accurately estimate incidence among high-risk populations. This methodology may serve as an effective tool to rapidly assess the impact of interventions. Algorithms to improve performance in HIV coinfecting individuals should be explored.



**Figure 1. Power to detect a 50% reduction in HCV incidence between serial cross-sectional surveys.**

**541 HCV Phylogenetic Analysis Complements HIV Contact Tracing in an HIV Outbreak Setting**

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**Background:** In January 2015, a cluster of HIV infections was detected in rural Indiana among persons who reported injecting the prescription opioid oxycodone. We conducted molecular analyses of HCV sequences from a subset of patients co-infected with HIV/HCV in this outbreak investigation.

**Methods:** As a component of the HIV outbreak response, persons at-risk for HIV or HCV (e.g., persons who inject drugs [PWID]) were also tested for HCV. HCV NS5b gene sequencing was performed on HCV antibody-reactive specimens with detectable HCV RNA. HCV clusters were defined when NS5b sequences were the same genotype and highly genetically related (>96% nucleotide identity). Persons infected only with HCV were cross-referenced with HIV contact investigation data to determine if they had been identified as a contact of an HIV-infected case-patient in this outbreak.

**Results:** From October 2014 – August 2015, HCV antibody testing was conducted for 647 at-risk persons, of whom 570 (88.1%) had a reactive HCV result and 126 (19.5%) were also HIV co-infected. The NS5b genomic region was amplified in a subset of 270 specimens (25.0% HIV-coinfected) that had detectable HCV RNA. NS5b subtype 1a (n=192) was most common, followed by 3a (n=56), 2b (n=16), and 1b (n=6). Overall, 132 (48.9%) specimens clustered in one of two large HCV NS5b clusters (Cluster 1, n=89; Cluster 2, n=43) that included persons infected with HCV alone (n=90) and those co-infected with HIV (n=42) [Figure]. All 42 HIV co-infected patients in these clusters were linked to the HIV outbreak. Of the persons infected only with HCV within these clusters, 52 (57.8%) were known contacts and 38 (42.2%) were not known contacts of HIV-infected patients in this outbreak. Of the 38 not identified as contacts, 19 (50%) resided outside of the outbreak county. Compared with HCV-infected persons not in an HCV cluster, a greater proportion of those in HCV clusters 1 and 2 resided within the HIV outbreak county (79.6% vs 42.9%,  $p<0.01$ ) and were known contacts of HIV-infected persons in the outbreak (71.2% vs 42.9%,  $p<0.01$ ).

**Conclusions:** In this analysis, many persons infected only with HCV had HCV strains that phylogenetically clustered with those from persons dually infected with HIV/HCV, yet one-half of those infected only with HCV were not identified as needle-sharing contacts in the HIV outbreak investigation. HCV phylogenetic analysis among PWID may provide information complementary to contact-tracing in identifying networks of persons at risk for HIV acquisition.

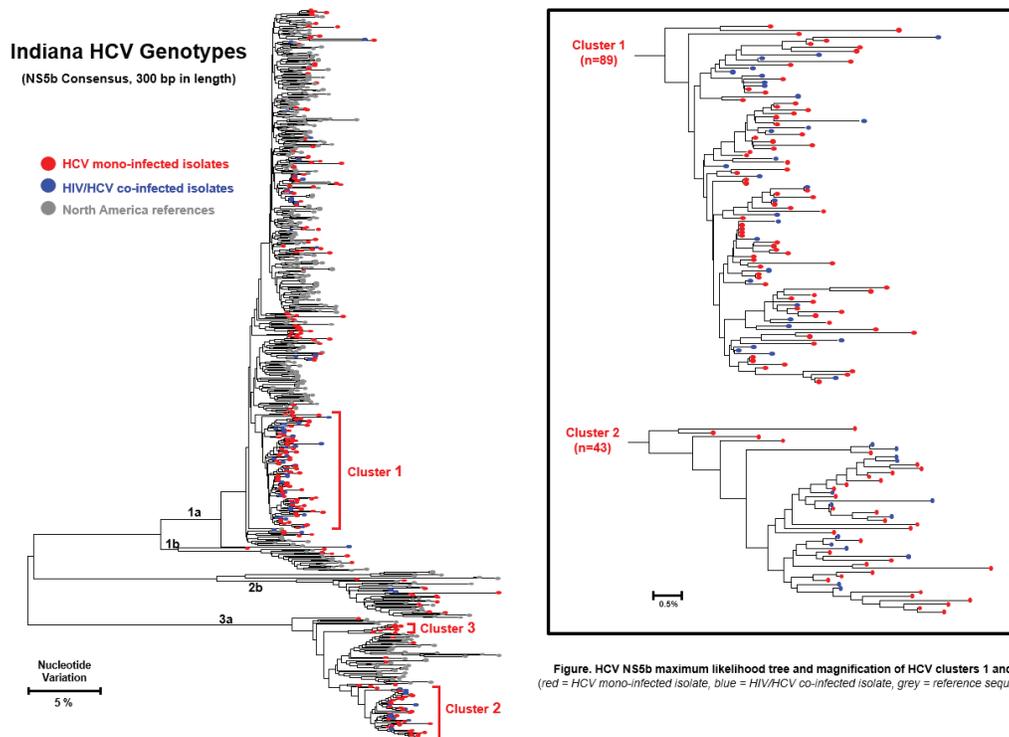


Figure. HCV NS5b maximum likelihood tree and magnification of HCV clusters 1 and 2 (red = HCV mono-infected isolate, blue = HIV/HCV co-infected isolate, grey = reference sequence)

#### 542 Limited Overlap in Transmission Clusters of HIV and HCV Among MSM in the Netherlands

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**Background:** HIV-1 and hepatitis C virus (HCV) circulate among men who have sex with men (MSM). We conducted a study to investigate the overlap of these epidemics among MSM in the Netherlands using a phylogenetic approach.

**Methods:** Data were derived from the observational ATHENA and MOSAIC cohort studies. We included 5,038 MSM who were diagnosed with a HIV subtype B infection between 1981 and 2014. HIV subtype was based on the availability of a *pol* sequence. Of them, 562 (11.2%) were (ever) coinfected with HCV (until October 2014). Time from HIV diagnosis to HCV infection was calculated using the Kaplan-Meier method. HCV NS5B sequences were available for 126/562 (26.7%) coinfected MSM, allowing phylogenetic analysis of both HIV and HCV. HIV phylogenetic clusters were defined as having  $\geq 10$  sequences with a bootstrap value  $>90$  and a median pairwise distance within the clade smaller than the 5<sup>th</sup> percentile threshold of the pairwise distances in the whole tree. We investigated the presence of HIV clusters that had an increased risk for HCV infection.

**Results:** In total, 118 HIV phylogenetic clusters were identified. These clusters included 3,084/5,038 (61.2%) MSM infected with HIV subtype B, and 97/118 (82.2%) clusters contained  $\geq 1$  HCV infection. Median HIV-1 cluster size of those with past or present HCV infection was comparable to those with no history of HCV. Median time between HIV diagnosis and HCV diagnosis was 3.3 years (IQR: 1.0-7.3), but decreased over time. HCV NS5B sequences were obtained from 150 HCV infections among 126 MSM; 21 MSM had  $\geq 1$  reinfection. Among 51 HIV clusters with  $\geq 2$  HCV sequences, 14 clusters contained HCV strains of concordant genotypes, but only 8/14 HIV clusters with  $\geq 2$  HCV sequences contained two HCV strains of the exact same HCV lineage. Ultimately, 19/150 (12.7%) coinfected MSM clustered on both HCV and HIV phylogeny.

**Conclusions:** In this study, HCV infection was not confined to specific HIV clusters, indicating there are no specific HIV clusters with elevated risk of HCV infection. When multiple HCV infections were present within an HIV cluster, concordance of HCV phylogeny was relatively uncommon, even among those with concordant HCV genotype. One explanation may be that HCV spreads in MSM networks that differ from the HIV transmission networks. The median duration from HIV diagnosis to HCV infection of 3.3 years suggests that these HCV networks are established some time after HIV infection.

543 Tracing the Origin of HCV NS3 Q80K Among HIV-Infected MSM in the Netherlands

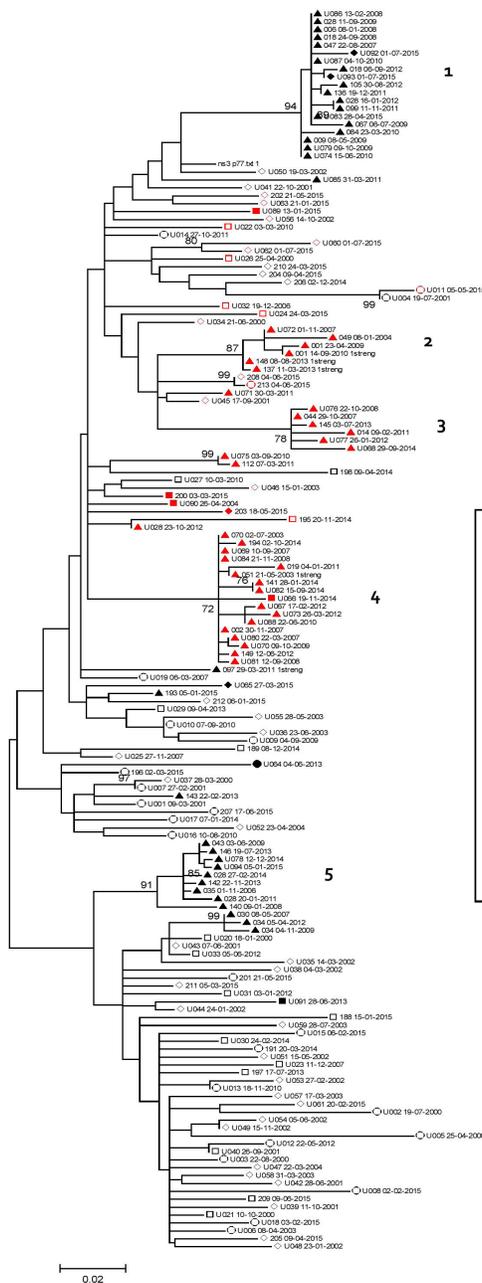
Astrid M. Newsum<sup>1</sup>; Cynthia K. Ho<sup>2</sup>; Faydra I. Lieveid<sup>3</sup>; Thijs J. van de Laar<sup>4</sup>; Jan T. van der Meer<sup>5</sup>; Anne M. Wensing<sup>3</sup>; Greet J. Boland<sup>3</sup>; Joop E. Arends<sup>5</sup>; Maria Prins<sup>1</sup>; Janke Schinkel<sup>1</sup>  
<sup>1</sup>PH Service of Amsterdam, Amsterdam, Netherlands; <sup>2</sup>Academic Med Cntr, Amsterdam, Netherlands; <sup>3</sup>Univ Med Cntr Utrecht, Utrecht, Netherlands; <sup>4</sup>Sanquin Blood Supply Fndn, Amsterdam, Netherlands; <sup>5</sup>Cntr of Infectious Diseases and Immunology Amsterdam, Academic Med Cntr, Amsterdam, Netherlands

**Background:** The naturally occurring Q80K polymorphism in the nonstructural protein 3 (NS3) of hepatitis C virus (HCV) has been associated with a reduced response to simeprevir (a 'second wave' first generation HCV protease inhibitor) / pegylated-interferon/ribavirin triple therapy. This polymorphism is transmissible between hosts, and its prevalence varies geographically and per risk group. We describe the prevalence of Q80K among the major HCV risk groups in the Netherlands, e.g. HIV-1 coinfected men who have sex with men (MSM) and people who inject drugs (PWID). Using phylogenetic analysis, we examined whether the presence of Q80K was linked to specific HCV transmission networks.

**Methods:** Stored blood samples from 150 HCV genotype 1a infected patients attending two large Dutch medical centers, AMC Amsterdam and UMC Utrecht, were used for this study. Data on transmission route and HIV-status were extracted from patient records. At the time of sampling, all patients were treatment-naïve for NS3/4A protease inhibitors. A 611 bp fragment of the NS3 genomic region including the Q80 amino acid position was amplified and sequenced. The NS3 maximum likelihood (ML) phylogeny was reconstructed using MEGA v6 with the HKY+G substitution model and 1000 bootstrap replicates.

**Results:** Of the 150 patients, 45% was coinfected with HIV-1, 39% was MSM (all HIV-1 coinfected), 17% PWID, 14% had other risk factors including blood transfusion and for 30% the route of transmission was unknown. The Q80K polymorphism was present in 35% of patients and throughout the time span of sample collection (2000-2015). Q80K was most prevalent among MSM (52%), followed by persons with other or unknown risk factors (30%) and PWID (8%). Robust clustering in the ML phylogenetic tree (figure 1) was only observed for MSM; 5 clusters supported by high bootstrap values were identified. Q80K was present in 100% of sequences in 3 out of these 5 clusters. The largest cluster included 17 patients. Interestingly, sequences did not cluster according to treatment center.

**Conclusions:** The Q80K polymorphism naturally occurs in 35% of our study population and has persisted over at least 15 years. Among HIV-1 coinfected MSM, the prevalence of Q80K was highest and distinct transmission networks with and without Q80K were identified. This suggests a founder effect, with the introduction and expansion of Q80K variants in this key population which potentially jeopardizes future treatment with simeprevir of HIV-1 coinfected MSM.



**Figure 1: ML phylogenetic tree of the Q80K polymorphism.**

**Colors:**  
 Filled red = Q80K and HIV-1 coinfection  
 Border red = Q80K without HIV-1 coinfection  
 Filled black = HIV-1 coinfection without Q80K  
 Border black = no HIV-1, no Q80K

**Symbols:**  
 Triangle = MSM  
 Circle = PWID  
 Square = Other risk factors  
 Diamond = Risk factor unknown

Only bootstrap support values  $\geq 70$  are shown. The branch lengths represents nucleotide substitutions per site.

Numbers next to the tree represent MSM clusters supported by high bootstrap values.

Poster Abstracts

**544 Incidence of Hepatitis C Virus Infection in the HIV Outpatient Study Cohort 2000-2013**

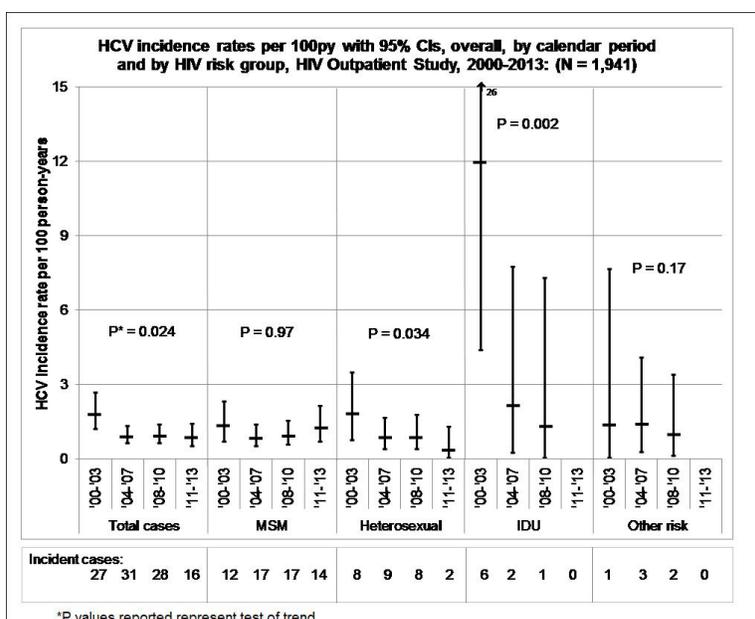
**Taraz Samandari**<sup>1</sup>; Kate Buchacz<sup>1</sup>; Carl Armon<sup>2</sup>; Dana Franklin<sup>2</sup>; Rachel Hart<sup>2</sup>; Joan S. Chmiel<sup>3</sup>; John T. Brooks<sup>1</sup>; Ellen Tedaldi<sup>4</sup>; for the HIV Outpatient Study Investigators  
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**Background:** Recent data on incidence of hepatitis C virus infection (HCV) in the United States (U.S.) are limited. We examined the rates and correlates of incident HCV in a multi-site U.S. cohort of HIV-infected patients in care.

**Methods:** We studied HIV Outpatient Study (HOPS) participants seen in 9 HIV-specialty clinics. We selected patients who had ≥1 clinical encounter between 2000 and 2013 and ≥2 HCV-related tests; their first negative HCV antibody (Ab) test in that interval defined the baseline date for each patient's analysis and the follow-up accrued until the later of either the last negative HCV Ab test or the midpoint between the last negative HCV Ab test and the first positive HCV Ab, viral load, or genotype test. We assessed rates of incident HCV per 100 person-years (py) overall and by demographic and HIV risk strata using chi-square test of trend over time: 2000-2003, 2004-2007, 2008-2010, and 2011-2013. Risk factors for incident HCV were analyzed using multivariable Cox proportional hazards models.

**Results:** The 1,941 eligible patients (median age 40 years, 23% female, 61% with men who have sex with men as an HIV risk factor (MSM) and 3% with injection drug use [IDU] risk) had a median of two post-baseline HCV Ab tests during observation (interquartile range: 1-4). There were 102 (5.2%) incident HCV infections for an overall incidence of 1.07 (0.87-1.30) per 100py. HCV incidence tended to be higher in earlier calendar years and among persons with IDU risk than among other risk groups; over time, HCV incidence decreased among heterosexuals and persons with IDU risk, but not among MSM (p for trend=0.97) (Figure). In multivariable analyses of 2000-2013 data, factors associated with incident HCV infection were having IDU risk (adjusted hazard ratio [aHR] 3.76, 95% confidence interval [CI] 1.76-8.03 compared with heterosexual risk), and having no insurance (aHR 1.84, 95% CI 1.09-3.11 compared with private insurance).

**Conclusions:** Among HIV-infected patients in care with repeated HCV screening tests during 2000-2013, we observed a high (1% per year) rate of incident HCV infection that over time declined among heterosexuals and persons with IDU risk but remained unchanged among MSM who accounted for the majority of observed events. Rates were also elevated among those with no insurance. Our data support recommendations for annual HCV screenings for HIV-infected persons with MSM risk to enable HCV diagnosis and treatment, if co-infected.



**545 Sexual Behaviour is Associated With Recently Acquired HCV in HIV/HCV Coinfected MSM**

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<sup>5</sup>Holdsworth House Med Practice, Sydney, Australia

**Background:** Epidemics of HCV infection have emerged in HIV-positive men-who-have-sex-with-men (MSM) in many countries over the past decade. Knowledge of sexual and drug use behaviour in these populations may help target health strategies and interventions.

**Methods:** The Control and Elimination of HCV from HIV-infected individuals within Australia (CEASE-D) cohort study aims to characterise socio-demographic, clinical and behavioural features of individuals with HIV/HCV co-infection. This analysis included individuals enrolled between July 2014 and August 2015 who had completed the screening behavioural questionnaire regarding sexual behaviour, drug use and HCV knowledge. Logistic regression analysis assessed factors associated with recently acquired (<2 years) HCV.

**Results:** 175 HIV/HCV infected individuals (mean age 47 years [SD 9], MSM 89%, cART 97%) were included. Median time since HCV infection was 7 years (IQR 2-15) in MSM and 16 years (IQR 10-24) in the remainder (p<0.001), with 25% and 0% having recently acquired HCV (p=0.014). Injecting drug use (IDU, 53%) and sexual exposure with a partner of the same sex (33%) were the predominant modes of HCV acquisition. IDU ever and within 6 months were reported by 81% and 37%, respectively, with amphetamines the most commonly injected drug (ever 72%). Age at first injecting was older in MSM (27 years [IQR 20-35] vs 17 years [IQR 16-21], p=0.001). Knowledge regarding behaviours associated with HCV transmission was variable; 83% and 73% correctly identified needle sharing and sharing other drug paraphernalia as high risk, whereas fisting, sex toys and group sex were identified as risk factors by 51%, 46% and 52%. Only 69% were aware of the potential for HCV reinfection. In the MSM population (n=155), 34% had a regular male partner (RMP) (HIV-positive 65%) and 66% casual male partners (CMP). Current high-risk sexual behaviour was reported by the majority (condom-less anal intercourse 74%, group sex 35%). While 74% 'always' or 'sometimes' disclosed their HIV status, only 41% disclosed their HCV status. Recently acquired HCV in MSM was associated with younger age (AOR 0.9, 95% CI 0.9, 1.0; p=0.002), full or part time employment (AOR 3.0, 95% CI 1.1, 8.0; p=0.028), sexual acquisition (AOR 13.6, 95% CI 4.5, 41.2; p<0.001) and CMPs (AOR 4.0, 95% CI 1.2, 13.4; p=0.023).

**Conclusions:** Sexual behaviour was associated with recently acquired HCV in HIV/HCV co-infected MSM. Limited knowledge around sexual transmission risk and HCV status disclosure is concerning.

## 546 Prevalence and Factors of HCV Infection Among HIV-Negative and HIV-Positive MSM

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**Background:** Given recent treatment advances for and HIV-related implications of hepatitis C virus (HCV) infection, we sought to identify factors associated with past/current and incident HCV infection within a prospective cohort of gay, bisexual, and other men who have sex with men (MSM) in Metro Vancouver, Canada.

**Methods:** Eligible participants were recruited from 2012-2015 using respondent-driven sampling, were aged  $\geq 16$  years of age and reported recent sex with another man.

Participants completed study visits every 6 months that included a computer-assisted self-interview on demographics, sexual and substance use behaviors, and a nurse-administered clinical questionnaire. A rapid HIV test was administered and a venous blood sample was taken for HCV-antibody serology. We used logistic regression and generalized estimating equations to identify factors associated with prevalent HCV antibodies at enrolment and incident HCV infection at follow-up, respectively. Relative risks (RR) and 95% confidence intervals (CI) are shown. RDS-adjusted population parameters are provided for baseline data.

**Results:** Of 774 participants, 2.0% (15/551) of HIV-negative and 28.3% (50/223) of HIV-positive MSM were HCV seropositive at enrollment. Of these, 56/65 (86.2%) were aware of their diagnosis, but only 5 HIV-negative and 17 HIV-positive MSM reported prior HCV treatment, with only 2 and 7 of those reporting treatment success. Factors associated with HCV seropositive at enrolment, stratified by HIV status are shown in Table 1. Of 534 participants with follow-up data, we observed 5 HCV-seroconversions for a calculated incidence rate of 0.50 per 100 person-years (95% CI: 0.21-1.21). All incident HCV infections were among single, HIV-positive, gay-identified MSM who did not work as escorts and had never been treated for HCV. Incident HCV infections were associated with older age (RR=1.06, 95% CI: 1.01-1.12), recent crystal methamphetamine use (RR=10.62, 95% CI: 1.77-63.64), and a greater number of recent anal sex partners (RR=1.01, 95% CI: 1.01-1.02). Notably, only 1 of 5 with HCV seroconversion reported recent injection drug use, and as such was not associated with HCV seroconversion (RR=3.01, 95% CI: 0.35-26.15, p=0.32).

**Conclusions:** New cases of HCV infection indicate a potential shift to sexual transmission among HIV-positive gay men based on the lack of association with recent injecting behavior. Crystal methamphetamine use remains a strong factor associated with HCV seropositivity and predictor of new infection.

**Table 1. Multivariable logistic regression of prevalent HCV-antibodies at enrollment separately for HIV-negative and HIV-positive MSM**

	HIV-Negative AOR (95%CI)	HIV-Positive AOR (95%CI)
<b>Race/ethnicity</b>		
White	1.00	1.00
Aboriginal (indigenous)	<b>7.76 (1.88-31.99)</b>	2.92 (0.96-8.88)
Other	N/A	0.30 (0.04-2.29)
<b>Sexual identity</b>		
Gay	1.00	1.00
Other	<b>6.51 (1.73-24.52)</b>	<b>3.74 (1.23-11.40)</b>
<b>Injecting drug use</b>		
No	1.00	1.00
Yes	<b>6.56 (1.59-27.12)</b>	<b>9.94 (3.75-23.78)</b>
<b>Currently lives in downtown core</b>		
No	Not selected	1.00
Yes		<b>5.00 (1.16-20.00)</b>
<b>Any condomless anal intercourse with an unknown HIV status partner, P6M</b>		
No	Not selected	1.00
Yes		<b>3.04 (1.20-7.67)</b>

## 547 The Impact of Host Genes on the Risk of Acquiring Hepatitis C Virus Infection

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**Background:** Injecting drug use (IDU), receiving untested blood (-products) as well as high risk sexual behavior amongst HIV-1 infected men having sex with men (MSM) are major risk factors for acquiring HCV. Approximately 10-20% of HCV multiple exposed individuals remain uninfected (MEU), whilst the remainder become infected (MEI). We hypothesize that host factors play a role in HCV susceptibility. Aim of our study was to identify polymorphisms in host genes and their promoters that encode for proteins that modulate virus entry into cells; CD81, Scavenger receptor 1, low-density lipoprotein receptor, Claudin-1, Occludin and Niemann-Pick C1-like 1 (NPC1L1). In addition, we studied two genes that are thought to be involved in HCV transmission, the dendritic cell specific ICAM-grabbing non-integrin (DC-SIGN) and DC-SIGN related (DC-SIGNR), since they have been reported to capture and transfer HCV to hepatocytes.

**Methods:** HCV exposed individuals from two observational cohorts were selected. From the MSM observational study of acute infection with HCV (MOSAIC) 30 HIV-1 infected MEU cases and 32 HIV-1 infected MEI controls were selected based on reported high risk sexual risk behavior. From the Amsterdam Cohorts Studies (ACS) IDU cohort, 40 MEU cases and 22 MEI controls were selected who injected drugs for  $\geq 2$  years. 65 SNPs in the selected genes were determined by sequencing or SNP assays.

**Results:** In the MSM cohort, we observed an association of three DC-SIGN promoter SNPs with HCV infection; rs2287886 GG, rs735240 AA and rs735239 GG are the protective genotypes (OR: 0.35 95% CI= 0.12 to 0.97, OR: 0.23 95% CI= 0.07 to 0.69 and OR: 0.23 95% CI= 0.06 to 0.85, respectively). No associations were found within the IDU cohort. Additionally, we found three SNPs in NPC1L1 to be associated with HCV susceptibility. When combining the MSM and IDU cohorts, rs217434 TT, rs2072183 CC and rs41279633 CC appeared as protective genotypes (OR: 0.38 95% CI= 0.17-0.83, OR: 0.31 95% CI= 0.14-0.68, OR: 0.43 95% CI= 0.19-0.99, respectively). This association was maintained when the cohorts were analyzed separately.

**Conclusions:** Our results support the hypothesis that DC-SIGN plays a role in HCV acquisition and indicates for the first time that this may relate to infection via sexual but not IDU exposure. Additionally, our results identify that variations within the NPC1L1 gene associate with HCV susceptibility, independent of the transmission route.

548 **Liver Disease Progression in a Community-Based Sample of HCV-Infected PWID**

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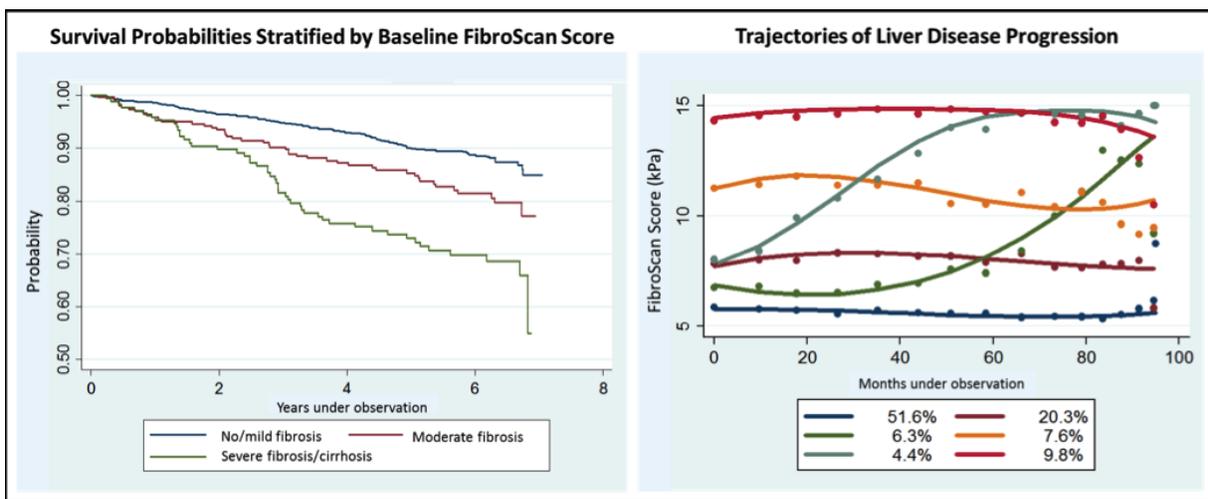
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**Background:** Highly efficacious all oral direct-acting antiviral (DAA) therapy is curative against HCV infection. However, because of cost, DAA is denied to many individuals without cirrhosis based on the assumption that liver fibrosis progression can be safely monitored until cirrhosis is detected. We tested that assumption by examining the natural history of HCV infection in a large, largely untreated cohort in whom fibrosis was uniformly monitored.

**Methods:** 1278 HCV-infected people who inject drugs (PWID) were followed from 2006-2014 as part of the ALIVE study in Baltimore, MD. Liver fibrosis was ascertained semi-annually by transient elastography (FibroScan) using previously validated cutoffs ( $\leq 8.0$ : no/mild fibrosis, 8.0-12.3: moderate fibrosis;  $> 12.3$ : severe fibrosis/cirrhosis). Cox regression was used to determine the association between fibrosis and mortality. Semi-parametric growth mixture modeling was used to characterize long-term patterns of fibrosis among those with  $> 5$  FibroScan scores (N=743). Persons with patterns consistent with progression to cirrhosis were subsequently compared to those with low/stable scores.

**Results:** Median age was 49; 68% were male. Persons with cirrhosis had the highest mortality risk (6.25 per 100 PY); however mortality in persons with moderate fibrosis (3.51 per 100 PY) at baseline was also significantly higher compared to those with no fibrosis (2.04 per 100 PY;  $p < 0.001$  and  $p = 0.004$ , respectively). After adjustment for age, HIV, alcohol use, and other comorbidities, those with baseline severe fibrosis/cirrhosis (mortality rate ratio [MRR]: 2.04; 95% confidence interval [CI]: 1.43, 2.92) and moderate fibrosis (MRR: 1.39; 95% CI 0.97, 1.99) had higher mortality than those with no fibrosis. Over 8 years, out of the total number of people without cirrhosis, 14.9% had a pattern consistent with progression to cirrhosis. Only high HCV viral load ( $> 6 \log_{10}$  IU/mL) differentiated those who progressed versus those with low/stable FibroScan scores across follow-up (OR 5.14, 95% CI 1.79 – 14.77) compared to undetectable HCV viral load. However, high HCV viral load had low prognostic accuracy (ROC=0.63).

**Conclusions:** Over the course of nearly 8 years, liver staging was generally stable, with 10% progressing to cirrhosis. Our data do not support restricting access to HCV treatment based on disease stage because mortality is increased even at moderate stages and progression to cirrhosis cannot be reliably predicted.

549 **Protective Effect of Coffee Intake on Mortality of French HIV/HCV-Infected Patients**

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**Background:** HIV-HCV co-infected patients are particularly concerned by liver disease due to immune activation/inflammation, exposure to antiretroviral agents and evolution of HCV co-infection. Polyphenols contained in coffee have several hepato-protective properties. Elevated coffee consumption has already been associated with a reduced risk of insulin resistance and lower levels of liver enzymes in co-infected patients (Carrieri et al. CID 2014, Carrieri et al. JHEPAT 2013). However its association with overall mortality is unknown. This study aimed to investigate the effect of coffee consumption and other behaviors on mortality risk in HIV-HCV co-infected patients.

**Methods:** The ANRS C013 HEPAVIH cohort is a French nationwide prospective cohort of HIV-HCV co-infected patients with medical and psycho-social/behavioral data collection using self-administered questionnaires at enrolment (M0) and every 12 months thereafter until M60. The present study's outcome was all-cause mortality reported between M0 and M60. We used a Cox proportional hazards model to study the effect of coffee consumption on mortality.

**Results:** Over a median [IQR] follow-up of 5.0 [3.9-5.8] years, 77 deaths occurred among 1,035 eligible patients, corresponding to a mortality rate [95% CI] of 1.64 [1.31-2.05]/100 person-years. Leading causes of death were: related to HCV (including hepatocellular carcinoma, n=33, 43%), to cancers (unrelated to AIDS or HCV) (n=9, 12%), and to AIDS (n=8, 10%). Elevated coffee consumption ( $\geq 3$  cups/day) was reported by 26.3% of the patients at M0. It was, as a time-varying covariate, significantly associated with a 50% reduced risk of mortality (HR [95% CI] = 0.5 [0.3-1.0],  $p = 0.045$ ), after adjustment for gender and other time-varying factors as follows: precarious housing, having a steady partner, alcohol and tobacco use, HIV stage, CD4+ cell count  $\leq 200/\text{mm}^3$ , HCV treatment status (ongoing treatment: HR [95% CI] = 0.9 [0.4-2.1]; treated but not cured: 0.6 [0.3-1.3]; treated and cured: 0.2 [0.1-0.5]; treatment naive: reference).

**Conclusions:** This study indicates a possible protective effect of elevated coffee intake on mortality in HIV-HCV co-infected patients. This association is independent of HIV immunological status and HCV clearance. As this effect may be mediated by coffee compounds having anti-inflammatory and anti-fibrotic properties, these results underline the need of evaluating the benefits of coffee extracts and supplementing dietary intake of other anti-inflammatory compounds in this population.

550 **Statin Use and Cirrhosis Progression in an HIV/HCV Coinfected Population**

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**Background:** Metabolic risk factors such as diabetes and obesity have previously been described in patients with liver disease who progress to cirrhosis and hepatocellular carcinoma (HCC). Much less is known about the role of hypertension and lipid abnormalities on liver fibrosis. In addition, the role of statins in preventing cirrhosis in HCV has been

demonstrated in HCV mono-infected individuals and may be due to anti-proliferative effects. We sought to evaluate the association of statin use and progression to cirrhosis in patients with HIV/HCV coinfection.

**Methods:** We used the health information from the HIV Clinical Care Registry, which is a comprehensive clinical VA database on all HIV-infected Veterans nationwide. Patients included were ≥18 years and had ≥1 confirmed positive HIV test or ICD-9 code and confirmed HIV-infected by the HIV registry. HCV infection was confirmed by positive HCV RNA or genotype test. Cirrhosis (primary outcome) was defined by ICD-9 code or AST to platelet ratio > 2. Variables including diabetes (DM), hypertension (HTN), low-HDL, obesity, and statin use were based upon ICD-9 codes, laboratory tests, and presence of drug prescription. Predictors of cirrhosis included demographics, HIV-specific lab values, statin drug use, and components of metabolic syndrome were analyzed using Cox proportional hazard regression analysis with SAS version 9.1 (SAS Institute Inc, Cary, NC). These variables were modeled as time dependent.

**Results:** There were 6033 HIV/HCV co-infected patients in our cohort (excluding cirrhosis diagnosis prior to HIV diagnosis); 2313 developed cirrhosis. Demographic data can be found in the table. Factors associated with cirrhosis were older age at HIV diagnosis, greater comorbidities, and CD4 count below 200 cells/uL. Greater time with undetectable HIV viral load was protective. Metabolic risk factors significantly associated with cirrhosis development were diabetes and low-HDL. Statin use was found to be significantly protective against cirrhosis (HR 0.73, CI 0.58-0.92).

**Conclusions:** Cirrhosis development was greater in the HIV/HCV co-infected patients who were diagnosed with HIV later in life and had greater immunosuppression. Metabolic risk factors were prevalent in the cohort, and diabetes and low-HDL were each associated with progression to cirrhosis. HIV VL suppression was protective of liver fibrosis. Statin use was independently protective against cirrhosis development, which suggests statins may be beneficial as adjunctive therapy in the group.

Demographic Information, N = 6033 (HIV/HCV Co-infected) *deaths removed		
	Cirrhosis, N= 2331	No Cirrhosis, N=3710
Age, years (median)	44	46
Age, years		
<40	740 (12.27)	944 (15.65)
40-50	1129 (18.71)	1759 (29.16)
>50	444 (7.36)	1017 (16.86)
Race		
White/other/unknown	633 (10.49)	913 (15.13)
Black	1451 (24.05)	2565 (42.52)
Hispanic	229 (3.8)	242 (4.01)
Duration of follow-up, yrs (mean)	4.16	7.83
Deyo Co-morbidity Score		
0	Reference	
1	256 (4.24)	535 (8.87)
≥2	160 (2.65)	425 (7.04)
Era of HIV Diagnosis		
Pre-cART	905 (15)	1035 (17.16)
Early cART	940 (15.58)	1353 (22.43)
Late cART	468 (7.76)	1332 (22.08)
Ever cART	1815 (30.08)	3189 (52.86)
CD4 Value		
>350	1109 (18.47)	2201 (36.99)
200-349	506 (8.5)	744 (12.5)
<200	626 (10.52)	775 (13.02)
Percent Undetectable		
>80%	451 (7.48)	1079 (17.88)
40-80%	445 (7.38)	1091 (18.08)
0-40%	577 (9.56)	976 (16.18)
HCV treatment		
SVR	73 (1.21)	109 (1.81)
No SVR	254 (4.21)	290 (4.81)
BMI, maximum		
<25	894 (15.02)	1359 (22.83)
>25, ≤ 30	936 (15.73)	1448 (24.33)
>30	462 (7.76)	853 (14.33)
Co-morbid HCC	91 (1.51)	22 (0.36)
Diabetes	347 (5.75)	622 (10.31)
Alcohol	1342 (22.24)	2218 (36.76)
Hypertension	1046 (17.34)	2256 (37.39)
Low-HDL	1266 (20.98)	2407 (39.9)
Statin (ever)	182 (3.02)	767 (12.71)

551 **Statin Type and Dose Reduce the Risk of Cirrhosis and HCC in HCV Infected Patients**

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**Background:** HMG-CoA reductase inhibitors (statins) have been associated with delayed fibrosis progression and a reduced risk of hepatocellular carcinoma (HCC), in patients with chronic hepatitis C virus (HCV) infection. The potency and optimal dose for this statin-mediated effect have not yet been defined. We investigated the impact of statin type and dose upon fibrosis progression and the development of incident HCC.

**Methods:** Within the Electronically Retrieved Cohort of HCV Infected Veterans (ERCHIVES), a national Veterans Affairs (VA) database, we identified all subjects initiated on anti-HCV therapy. Patients were followed annually from 2001 to 2014, and incident cases of cirrhosis and HCC were identified. Statin administration was defined according to the World Health Organization definition of cumulative defined daily dose (cDDD) [1], with "use" defined as >28 cDDD. Multivariable Cox proportional hazard regression models were used to examine the relationship between statin use and the development of cirrhosis as well as HCC.

**Results:** A total of 9,135 subjects with confirmed HCV were included. We identified 1649 cases of cirrhosis and 239 cases of incident HCC. Statin use was associated with a 44% reduction in risk of cirrhosis (adjusted HR 0.6, 95% CI 0.53, 0.68, p<0.0001). A significant dose-response relationship between statins and cirrhosis was observed: the adjusted HR of fibrosis progression with statin use of 28-89 cDDDs, 89-180 cDDDs, and >180 cDDDs were 0.74 (95% CI 0.59, 0.93), 0.71 (95% CI 0.59, 0.88), and 0.6 (95% CI 0.53, 0.68), respectively, compared to non-users. Mean change in FIB-4 score with atorvastatin (n=944) and fluvastatin (n=34) was -0.17 and -0.13 respectively, p=0.04 after adjustment for baseline FIB-4 score and established predictors of cirrhosis. Statin use was also associated with a 49% reduction in risk of developing incident HCC, compared to non-users (adjusted HR 0.51, 95% CI 0.36, 0.72). A similar dose-response relationship was observed.

**Conclusions:** In patients with chronic HCV infection, statin use appears to reduce the risk of fibrosis progression and decrease the risk of incident HCC, in a dose-dependent manner. Atorvastatin and fluvastatin are each associated with significantly reduced fibrosis progression to cirrhosis, compared to other types of statins. Further prospective clinical studies with clinical and histological endpoints are needed.

Table 1. Hazard ratio (HR) of fibrosis progression to cirrhosis, according to cumulative defined daily dose (cDDD) of statin medications

Variable (N)	Fibrosis progression <sup>1</sup> N (%)	Unadjusted HR		Adjusted HR*	
		β coefficient	HR (95% CI)	β coefficient	HR (95% CI)
Statin use <sup>2</sup> (4165)					
• cDDD 28-89 (543)	81 (14.92%)	-0.44	0.65 (0.52, 0.81)	-0.31	0.74 (0.59, 0.93)
• cDDD 90-180 (815)	122 (14.97%)	-0.42	0.66 (0.54, 0.79)	-0.34	0.71 (0.59, 0.86)
• cDDD >180 (2807)	381 (13.57%)	-0.58	0.56 (0.50, 0.63)	-0.51	0.6 (0.53, 0.68)
No Statin use (4970)	1065 (21.43%)		1		1

\*HR, hazard ratio; HR adjusted for age, sex, race, smoking history, ETOH abuse history, body mass index (BMI), diabetes, baseline FIB-4 score, Metformin use, angiotensin converting enzyme (ACE) inhibitor use, other lipid-lowering agent use, prior completed HCV treatment, and attainment of sustained virological response (SVR).

<sup>1</sup>Fibrosis progression: defined as FIB-4 score > 3.5 at any time during the study observation period, in patients with baseline FIB-4 scores < 3.5.

<sup>2</sup>Statin use: defined as ≥28 cDDD of statin medications, over the study observation period

### 552 Assessment of Hepatic Antifibrotic Effect of Cenicriviroc in Patients With HIV

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**Background:** Chronic liver disease is frequently observed in HIV-infected patients and is multifactorial. Attenuation of fibrotic progression may improve liver-related morbidity and mortality. Cenicriviroc (CVC) is an oral, dual antagonist of CCR2/CCR5, which are involved in key pro-inflammatory and fibrogenic pathways. We evaluated effects of 2 doses of CVC on serum hepatic fibrosis biomarkers in HIV+ subjects treated in a Phase 2b study (NCT01338883).

**Methods:** Patients with CCR5-tropic HIV-1 were randomized to receive CVC 100 mg (n=59), CVC 200 mg (n=56) or Efavirenz (EFV) (n=28), each combined with emtricitabine/tenofovir for 48 weeks. The Enhanced Liver Fibrosis (ELF) biomarker index was evaluated in a subset of patients who completed 48 weeks of treatment and had paired baseline and 48-week samples. The ELF index has been validated previously in patients with NASH, HCV and HBV infection, and by our lab in HIV patients with liver disease. The ELF index was calculated from the results of 3 serum biomarkers of collagen and extracellular matrix deposition: hyaluronic acid, propeptide of type III procollagen, and tissue inhibitor of metalloproteinase-1.

**Results:** Paired baseline and 48-week samples were randomly selected for 72/100 subjects completing the study: CVC 100 mg arm (n= 20/42), CVC 200 mg arm (n= 36/41) and EFV controls (n= 16/17). No subjects were coinfecting with HCV or HBV. Among subjects receiving CVC 100 mg and 200 mg, the ELF scores at baseline were  $9.80 \pm 0.96$  and  $10.53 \pm 2.12$  and after 48 weeks were  $9.93 \pm 1.00$  and  $8.28 \pm 0.09$ , respectively. Subjects who received EFV had a mean ELF score of  $9.13 \pm 0.98$  at baseline and  $9.28 \pm 1.06$  after 48 weeks. ELF scores decreased significantly in patients who received CVC 200 mg after 48 weeks of treatment ( $p < 0.0001$ ) but remained unchanged in patients who received EFV or CVC 100 mg. HIV suppression was similar in all groups.

**Conclusions:** Daily administration of CVC 200 mg for 48 weeks was associated with a significant decrease in specific biomarkers of hepatic fibrosis encompassed by the ELF index. This was not observed in control subjects treated with EFV nor with the lower dose of CVC (100 mg) (with caveat that  $< 50\%$  of paired samples tested to date). Clinical trials of CVC are underway in adults with NASH and liver fibrosis, using a new single tablet formulation of CVC 150 mg providing drug levels comparable to CVC 200 mg in the HIV Phase 2 trial. Evaluation of CVC in HIV patients who are at risk of liver fibrosis progression is warranted.

### 553 Statin Therapy Reduces Liver Fat Measured by Computed Tomography in Patients With HIV

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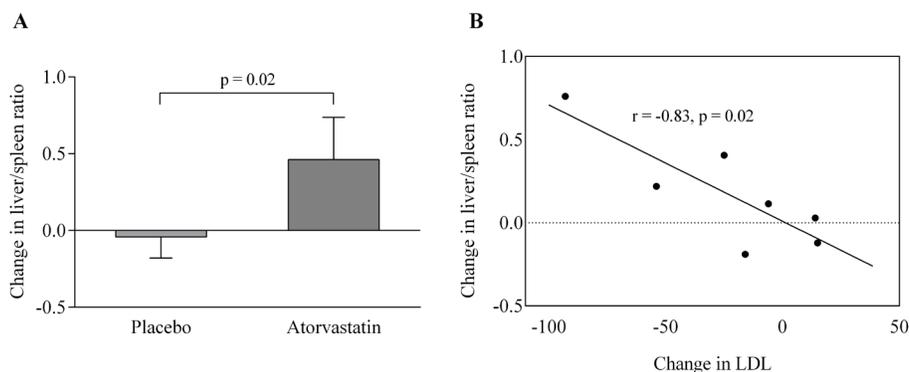
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**Background:** Nonalcoholic fatty liver disease (NAFLD) is highly prevalent among people living with HIV. Among non-HIV patients, limited studies suggest potential benefit of statins in patients with NAFLD. Non-contrast computed tomography (CT) allows for the measurement of liver density (attenuation). Liver density is inversely correlated to fat content and has been validated as an accurate, reproducible means to characterize hepatic steatosis. Thus, we leveraged data obtained using CT in a trial of statin therapy, focusing on a subgroup with NAFLD to determine the effect of a statin on liver fat. We hypothesized that statins would reduce hepatosteatosis.

**Methods:** We had previously performed a randomized, double-blind, placebo-controlled trial in HIV patients on stable anti-retroviral therapy with subclinical coronary atherosclerosis and LDL-cholesterol  $< 130$ mg/dL. Forty HIV-infected subjects were enrolled and assigned to placebo (n=21) or atorvastatin (n=19) for 12 months. Patients with AST or ALT  $3 \times$  upper limit of normal and active liver disease were excluded. Subjects underwent CT imaging with measurements of liver and spleen attenuation, and metabolic assessments including fasting lipids, anthropometric measurements and cross-sectional abdominal CT to assess VAT at baseline and end of the study.

**Results:** NAFLD was identified in 9 participants at baseline using a liver-to-spleen attenuation ratio cutoff  $< 1$ , in whom these analyses were performed. Among these subjects, liver-to-spleen attenuation ratio increased in the atorvastatin group,  $0.46 \pm 0.27$  compared to a mean decrease of  $-0.04 \pm 0.14$  in the placebo group ( $p = 0.02$ ) (Figure Panel A), indicating a reduction in hepatosteatosis with atorvastatin. Atorvastatin reduced liver fat without a change in BMI or VAT. The change in liver-to-spleen attenuation ratio was significantly associated with change in LDL ( $r = -0.83$ ,  $p = 0.02$ ) (Figure Panel B), but not other lipid, metabolic or inflammatory parameters.

**Conclusions:** To our knowledge, this is the first report suggesting an effect of statin therapy to reduce liver fat in the HIV population. The change in liver fat significantly correlated with reduction in LDL by statin therapy. These data provide rationale for future larger trials in HIV patients with NAFLD to assess the potential beneficial effects of statins in reducing liver fat.



**Figure.** A) Comparison of change in liver/spleen attenuation ratio between atorvastatin and placebo groups in patients with NAFLD at baseline. Bar denotes mean and error bar denotes standard deviation. B) Linear regression between change in liver/spleen ratio and direct LDL. Pearson correlation coefficient =  $-0.83$  ( $p = 0.02$ ).

### 554 PEth Improves Detection of Alcohol and Associated Mortality Among HIV+/HCV+

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**Background:** Abstinence from alcohol is recommended for individuals co-infected with HIV and hepatitis C (HIV+/HCV+) making self-report subject to social desirability bias. Phosphatidylethanol (PEth), an abnormal phospholipid formed only in the presence of ethanol, can detect exposure for up to 14 days. Any detectable amount suggests exposure, but the most commonly used cut off is 8-19 ng/mL. Higher cutoffs, 20-80 ng/mL, have been proposed for harmful use. Using data from the Veterans Aging Cohort Study, we evaluate agreement between PEth and self-report (AUDIT-C) among HIV+/HCV+ and whether PEth demonstrates a stronger association with mortality.

**Methods:** HIV+/HCV+ participating in the Biomarker Study who reported having had a drink in the past 12 months or had a detectable PEth were included (n=663). We restricted analysis to current drinkers to avoid confounding from those who stopped due to illness. Because any alcohol use may have health effects among HIV+/HCV+, any detectable value of PEth (>0 ng/mL) was considered indicative of exposure and a threshold of 8+ ng/mL as harmful. PEth thresholds were compared with self report of "any use over 12 months" and an AUDIT-C score of 4+ for men (3+ for women) indicated harmful use. We used separate Cox regression to estimate mortality hazard ratios by exposure using PEth (0, 1-7, 8-49, 50-99, 100+ ng/mL) and AUDIT-C (0, <4, 4-5, 6-7, 8+). Models were adjusted for age, race, and disagreement between measures.

**Results:** Agreement for any exposure was 59%; despite a shorter time frame (14 days), detectable PEth classified 91% of the sample as exposed compared with 68% by self report of use in the past year. When PEth 8+ ng/mL (45%) was compared with AUDIT-C for harmful use (29%), agreement improved to 72%. Both PEth and AUDIT-C were associated with mortality (Figure below), but PEth demonstrated stronger associations throughout its range. Finally, self-report did not enhance the association between alcohol and mortality beyond that identified by PEth ( $p>0.2$ ).

**Conclusions:** Among HIV+/HCV+, PEth is more sensitive than AUDIT-C for detecting alcohol exposure associated with mortality and thresholds for clinically important exposure may need to be reduced. Based on a dried blood spot, PEth offers a practical, direct measure of exposure.

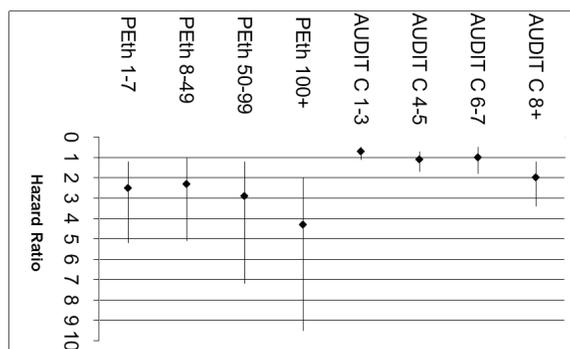


Figure. Association of Self Report (AUDIT-C Score) and a Direct Biomarker (PEth in ng/mL) with Mortality Among HIV+/HCV+

#### 555 HLA-B18 As a Risk Factor of Progression to Severe Liver Fibrosis in HIV/HCV Patients

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**Background:** Chronic infection by hepatitis C virus (HCV) is characterized by progressive liver fibrosis. Although several factors have been found to be associated with faster liver fibrosis progression, a biomarker with sufficient predictive value to impact clinical decision making has not been identified. The aim of our study was to analyze the influence of HLA-B molecules on liver fibrosis progression in HIV/HCV patients without cirrhosis.

**Methods:** A retrospective longitudinal study included HIV/HCV patients in follow-up between 2007 and 2014. Inclusion criteria were: a) at least two determinations of Liver Stiffness Measurement (LSM); b)  $\geq 12$  months of total follow-up; c) active HCV infection. Exclusion criteria were: a) HCV treatment previous to follow-up or during the first 12 months of the study; b) cirrhosis at baseline, defined as an  $LSM \geq 14.6$  kPa. Analytical, demographic and clinical variables were collected. LSM cutoffs applied were: <6.5 kPa (F0-F1); 6.5-9.4 kPa (F2); 9.5-14.5 kPa (F3) and  $\geq 14.6$  kPa (F4). Outcome variables were: 1) fibrosis stage progressed at least one stage; 2) fibrosis progressed up to severe liver fibrosis (F3-F4). Patients were censored at: a) an event of interest; b) initiation of HCV treatment; c) loss of follow-up; d) end date of study.

**Results:** One-hundred and four patients constituted the study population. The patient distribution according to liver fibrosis was as follows: F0-F1, 62 (59.6%) patients; F2, 22 (21.2%) patients; and F3, 20 (19.2%) patients. The median (IQR) follow-up period for these patients was 54.5 (IQR: 26.2-77) months. Forty-five patients (43.3%) showed an increase in the degree of fibrosis (time to event: 29 [IQR: 14-49.5] months). Patients bearing HLA-B18 more frequently had liver fibrosis and had a faster liver fibrosis progression rate (73.3%; time to event 24 [IQR: 8-29] months) than HLA-B18neg patients (38.2%; time to event 34.5 [IQR: 14.7-51.2] months). This association was also observed in the development of F3-F4 among F0-F2 patients (HLA-B18pos: 69.2%; time to event = 18 [6.5-37] months vs. HLA-B18neg: 28.2%; time to event = 37 [IQR: 19-52] months).

**Conclusions:** HIV/HCV patients carrying allele HLA-B18 were more likely to progress more rapidly to developing advanced and severe fibrosis (F3-F4) than HLA-B18neg patients. These results could help make decisions about the timing of HCV therapy in F0-F2 patients at risk of accelerated progression.

#### 556 Impact of PNPLA3 Variants on the Liver Histology of 168 HIV/HCV Patients

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**Background:** A genome-wide association study recently identified a non-synonymous sequence variation (rs738409 C to G) encoding an isoleucine-to-methionine substitution at position 148 in the adiponutrin/patatin-like phospholipase-3 (PNPLA3) gene, which appears to be the strongest determinant of human steatosis. The I148M variant of PNPLA3 has been found to be independently associated with liver steatosis and disease progression in NAFLD, alcoholic liver disease, CHC and HCC.

**Methods:** This study analyzes the impact of PNPLA3 variants on liver histology of 168 HIV/HCV coinfecting patients naïve to HCV-treatment. A pathologist unaware of the patients' condition graded liver fibrosis and necroinflammation (Ishak) and steatosis (Kleiner). Patients were tested for PNPLA3 variants and genotyped for the PNPLA3 rs738409 C to G variant underlying the I148M substitution. All were HBsAg-negative and stated no alcohol abuse.

**Results:** The mean age was 40.6(37.6-44.1), 72.6% were males, 42% showed HCV-genotype 3, 38.9% HCV-genotype 1 and 79.2% were receiving HAART. The 79 patients with the PNPLA3 p.148I/M or M/M variants more frequently showed severe steatosis (score 3-4) than the 89 with PNPLA3 p.148I/I, 43% vs. 24.7% ( $p=0.001$ ), whereas no difference was observed in the degree of necroinflammation or fibrosis. Compared with 112 patients with lower scores, 56 with severe steatosis showed higher BMI ( $p=0.03$ ), higher rate of HCV-genotype 3 (55.6% vs. 35.2%,  $p=0.01$ ) and PNPLA3p.148I/M or M/M (60.7% vs. 39.3%,  $p=0.01$ ) and lower CD4+ cells/mm<sup>3</sup> [514.00(390.5-673.0) vs. 500.00(399.0-627.0)];  $p=0.002$ ). At multivariate analysis, BMI ( $p=0.01$ ), HCV-genotype 3 ( $p=0.006$ ), CD4+ cell count ( $p=0.005$ ) and the PNPLA3 p.148I/M or M/M variants ( $p=0.01$ ) were found to be independent predictors of severe liver steatosis.

Besides, the PNPLA3p.148I/M or M/M variants and CD4+ cell count were the only independent predictors of severe steatosis in patients with HCV-genotype non-3.

**Conclusions:** This is the first study to show that among patients HIV/HCV coinfecting patients the PNPLA3 p.148I/M or M/M variants has substantially less impact on steatosis in those with HCV-genotype 3 than in non-genotype 3 infected patients.

**557 The Impact of Cannabinoid Receptor2-63 Variants in Liver Biopsy of HIV/HCV Patients**

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**Background:** This is the first study analyzing the impact of the rs35761398 variant of the CNR2 gene leading to the substitution of Gln (Q) of codon 63 of the cannabinoid receptor 2 (CB2) with Arg (R) on the clinical history of chronic hepatitis in HIV/HCV coinfecting patients.

**Methods:** One hundred and sixty-six consecutive HIV/HCV coinfecting patients, naïve for HCV-treatment were enrolled. A pathologist unaware of the patients' condition graded liver fibrosis and necroinflammation (Ishak). All patients were screened for CBR2 rs35761398 polymorphism by a TaqMan assay.

**Results:** Of the 166 HIV/HCV coinfecting patients, 72.9% were males, 42.5% were infected with HCV-genotype 3, 74.1% had a history of previous intravenous drug use. The median age was 40.58 years and the immunological condition was quite good (median CD4+ cells/mm<sup>3</sup> =507, IQR: 398.0-669.5). Thirty-five (20.8%) patients were naïve for HAART and 131(78.9%) were on HAART. The CBR2-RR variant was detected in the 45.8% of patients, QR in 38.55% and QQ in 15.66%. The mean degree of necroinflammation (HAI) was 5.4±3.0 (SD), of fibrosis 2.3±1.6 and steatosis 1.7±1.3. The 64 subjects with CBR2-QR variant and 76 CBR2-RR variant more frequently than the 26 patients with CBR2-QQ variant had a history of previous IVDU (76.79% and 73.3%, respectively vs. 22.41%, p < 0.01). Patients with CBR2-RR showed a high degree of HAI (>9) more frequently than those with CBR2-QQ or CBR2-QR than in those (38.9% vs. 11.5% and 14.1%, respectively, p<0.001). No other significant difference was observed in demographics and in laboratory and histological data. The 37 patients with moderate or severe HAI (>9), compared with the 129 patients with a lower HAI score showed higher serum level of AST (p=0.000016), ALT (p=0.00039), and ALP (p=0.008) and higher degree of fibrosis and steatosis (3.59±1.48 vs 1.94±1.42 p<0.0001 and 2.03±1.26 vs 1.59±1.31, p=0.03, respectively). The association between the CBR2-RR variants and a HAI > 9 was also analyzed in a multivariate analysis considering also the CD4+ > 500 cell/mL, fibrosis, HAART regimen and HAART naïve, age as covariants. Both the severe fibrosis (p=0.0001) and the CBR2-RR variant (p=0.03) were found to be independently associated with severe necroinflammation.

**Conclusions:** The CBR2-RR variant was identified as an independent predictor of severe necroinflammation in HIV/HCV coinfecting patients with chronic hepatitis.

**558 Poorly Controlled HIV Infection Is a Risk Factor for Liver Fibrosis in CNICS Cohort**

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**Background:** Liver disease is a major cause of morbidity among HIV-infected persons. There is limited information about the extent to which HIV disease severity influences liver disease progression, particularly early in the disease course when interventions may have the greatest impact.

**Methods:** We determined the incidence and predictors of advanced hepatic fibrosis measured by the FIB-4 index in a large and diverse population of HIV-infected patients without significant liver disease at baseline (FIB-4 <1.45). We used Cox proportional hazards analysis to examine factors associated with progression to FIB-4 ≥3.25, stratified by hepatitis C (HCV) status.

**Results:** A total of 14,198 HIV-infected patients in care between January 2000 and March 2014 were included in the analysis, the majority of whom were male (82%) and had sex with men (58%) as a transmission risk factor. The prevalence of HCV coinfection was 15% and alcohol use disorder 9%. Progression to advanced fibrosis occurred in 1,386 patients (10%) in a median of 3 years during a total of 61,904 person-years of follow-up (PYFU) for an incidence of 2.2 per 100 PYFU overall and 4.7 per 100 PYFU among HIV-HCV coinfecting patients. In multivariable analysis, HCV coinfection (adjusted hazard ratio [aHR] 1.85, 95% CI 1.63-2.11), HBV coinfection (aHR 1.45, 95% CI 1.17-1.81), alcohol use disorder (aHR 1.36, 95% CI 1.17-1.58) and diabetes (aHR 1.87, 95% CI 1.56-2.25) were associated with progression to advanced fibrosis. In addition, patients with lower time-varying CD4 count were more likely to progress, with the greatest risk in those with CD4 <100 cells/mm<sup>3</sup> (aHR 6.93, 95% CI 5.80-8.27) compared with CD4 ≥500 cells/mm<sup>3</sup>. An increasing gradient of risk was also observed among patients with higher time-varying HIV viral load (VL), with the greatest risk in those with VL ≥100,000 copies/ml (aHR 2.60, 95% CI 2.19-3.08) compared with those who were suppressed. We observed similar findings for both HIV monoinfected and HIV-HCV coinfecting patients in stratified analyses.

**Conclusions:** We found that both lower CD4 count and higher HIV VL were significantly associated with progression to advanced hepatic fibrosis, independent of the risk associated with traditional factors including HCV and HBV coinfection, alcohol, and diabetes. Our findings suggest that early treatment of HIV infection could mitigate liver disease.

**Table. Factors Associated with Progression to Advanced Liver Fibrosis Among Patients with Baseline FIB<1.45**

Factor	Overall (n=14,198)			HIV-monoinfected (n=12,532)			HCV-coinfected (n=1,666)		
	aHR	95% CI	P Value	aHR	95% CI	P Value	aHR	95% CI	P Value
Male sex	1.02	0.88-1.17	0.82	1.00	0.85-1.18	0.97	1.09	0.84-1.42	0.53
Race (reference: White)									
Black	0.93	0.83-1.05	0.26	0.91	0.79-1.04	0.18	1.00	0.79-1.27	1.00
Hispanic	0.95	0.80-1.14	0.59	0.96	0.79-1.16	0.68	0.82	0.53-1.28	0.39
Other	0.76	0.56-1.04	0.09	0.74	0.51-1.05	0.09	0.88	0.47-1.63	0.69
Chronic hepatitis C	1.85	1.63-2.11	<0.001	--	--	--	--	--	--
Chronic hepatitis B	1.45	1.17-1.81	0.001	1.54	1.21-1.96	<0.001	1.08	0.64-1.82	0.77
Alcohol use disorder	1.36	1.17-1.58	<0.001	1.43	1.18-1.72	<0.001	1.30	1.01-1.68	0.04
Diabetes mellitus*	1.87	1.56-2.25	<0.001	2.01	1.64-2.47	<0.001	1.30	0.82-2.03	0.26
CD4 count*, cells/mm <sup>3</sup>									
≥500 (referent)	--	--	--	--	--	--	--	--	--
350-500	1.29	1.10-1.53	0.002	1.31	1.07-1.59	0.007	1.23	0.89-1.72	0.22
200-349	1.90	1.62-2.24	<0.001	1.89	1.57-2.29	<0.001	1.80	1.33-2.45	<0.001
100-199	2.88	2.39-3.48	<0.001	3.29	2.66-4.08	<0.001	1.75	1.29-2.79	0.001
<100	6.93	5.80-8.27	<0.001	7.93	6.48-9.71	<0.001	3.97	2.71-5.82	<0.001
HIV viral level*, copies/ml									
<500 (referent)	--	--	--	--	--	--	--	--	--
500-9999	1.27	1.07-1.52	0.007	1.43	1.17-1.74	<0.001	0.83	0.56-1.24	0.36
10,000-99,999	1.43	1.22-1.66	<0.001	1.40	1.17-1.68	<0.001	1.45	1.09-1.93	0.01
≥100,000	2.60	2.19-3.08	<0.001	2.76	2.27-3.35	<0.001	2.04	1.41-2.94	<0.001
Baseline FIB-4 per unit	3.89	3.23-4.69	<0.001	4.30	3.47-5.32	<0.001	2.66	1.81-3.91	<0.001

\* Time-varying

**559 MiR-122 and -200a in Exosomes of ART+ HIV-1 Infected Individuals With Liver Disease**

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**Background:** Liver disease is one of the main contributors to the increased levels of morbidity and mortality seen in the HIV-1 infected, ART-treated population. Circulating miRNAs, particularly those located inside exosomes, are seen as promising biomarkers for a number of human disease conditions including liver related diseases. Furthermore miRNAs carried in exosomes, as distinct from those bound to Argonaute proteins, are potentially functional.

In a previously analysed set of 126 cases, (individuals who died whilst on therapy during the SMART and ESPRIT trials) and 247 matched controls the levels of 21 miRNAs, measured in serum, showed no associations with mortality (all-cause, cardiovascular or malignancy related). In this study we further analysed these cases and controls to determine if these miRNAs associated with liver related morbidity and mortality.

**Methods:** Levels of the 21 circulating miRNAs were analysed, from the 373 cases and controls, to determine if there were any differences between HIV/HCV co-infected individuals (HIV/HCV) (n=82) vs HIV-mono-infected individuals (HIV-M) (n=291). Additionally levels of miR-122, -200a and let-7e were analysed in the 13 cases who died from liver related diseases (Hepatitis C/Hepatitis B or non-viral liver failure) and compared to their 25 matched controls. Exosomes were then purified and quantified (using nanoparticle tracking analysis) from the serum of 10 of the 13 liver related cases and their 19 matched controls. MiR-122, -200a, let-7e and cel-miR-39 were then quantified, by RTqPCR, in these samples.

**Results:** In the SMART and ESPRIT samples there was a clear increase in serum levels of miR-122 (p<0.001) and miR-200a (p<0.001) in the HIV/HCV group compared to the HIV-M group. These same two miRNAs were also significantly elevated in the cases that died from liver related mortality compared to their matched controls (p<0.001 and p<0.01). MiR-122 was also significantly increased in the purified exosomes of the cases compared to their matched controls (p<0.01). Exosome levels of miR-200a, while not statistically significant (p=0.17), showed a six-fold increase in the median of cases compared to controls.

**Conclusions:** These data indicate that in ART-treated individuals circulating levels of miR-122 and miR-200a are elevated and this increase is due to an increased quantity of these miRNAs inside exosomes. These miRNAs may provide novel biomarkers for liver disease, particularly HCV associated disease, in both HIV-1 and non-HIV-1 infected populations

**560 Liver Fibrosis in HIV Patients: Which Factors Play a Role?**

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**Background:** Liver-related death in HIV-infected individuals is about ten times higher compared to the general population, while the prevalence of significant liver fibrosis in HIV mono-infected patients amounts up to 15%. A better understanding of liver disease beyond the classic risk factors is needed, particularly in light of normal life expectancy under modern cART. The present study aimed to assess risk factors for development of hepatic fibrosis in HIV-patients.

**Methods:** Health trajectory, including clinical characteristics and liver fibrosis stage assessed by transient elastography were collected at inclusion and after one year follow-up. Liver stiffness values greater than 7.1kPa were considered as significant fibrosis, while values greater than 12.5kPa were defined as severe fibrosis. Logistic regression and cox-regression uni- and multivariate analyses were performed to identify independent factors associated with liver fibrosis.

**Results:** 432 HIV-patients were included, of which 80% were HIV mono-infected, 16% were anti-HCV-positive (54% SVR and 46% HCV-RNA positive), and 5% were HBsAg-positive. Significant liver fibrosis was detected in 10% of HIV mono-infected, in 37% of HCV and 18% of HBV co-infected patients. Patients with history of SVR after successful HCV therapy did not show a significantly higher rate of abnormal liver stiffness. The presence of diabetes mellitus (OR=5.4), adiposity (OR=4.6) and the FIB4-score (OR=3.3) were independently associated with significant fibrosis in HIV mono-infected patients. Importantly, cumulative cART duration protected, whereas persistent HIV viral replication promoted the development of significant liver fibrosis along the duration of HIV infection. After one year follow up 13% of HIV mono-infected patients presented abnormal liver stiffness values. Per treatment naive year the risk of developing significant fibrosis rose by 12%.

**Conclusions:** Our findings strongly indicate that besides known risk factors like metabolic disorders, HIV may also have a direct effect on fibrogenesis. Successful cART leading to complete suppression of HIV replication might protect from development of liver fibrosis.

**561 NASH Is Associated With a Unique Biomarker Signature in HIV-Infected Adults**

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**Background:** Nonalcoholic fatty liver disease (NAFLD) and its progressive form, nonalcoholic steatohepatitis (NASH), are seen at high rates in HIV-infected patients. In HIV-negative populations, NAFLD is associated with visceral adiposity, increased plasma inflammatory biomarkers, and elevated risk of cardiovascular disease. This study assessed the association of NASH with plasma inflammatory biomarker levels in HIV-infected adults with NASH.

**Methods:** Plasma levels of C-reactive protein (CRP), tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-6, CXCL10 (IP-10), CCL2 (MCP1), IL-8, soluble CD14 (sCD14), and soluble CD163 (sCD163), were measured in cryopreserved samples from HIV-infected adults with suppressed HIV viremia on antiretroviral therapy (ART) and liver biopsy-proven NASH (n=38), HIV-infected adults with  $\geq 2$  years of normal aminotransferases and suppressed HIV viremia on ART and no known liver disease (n=40), and HIV-negative healthy controls (n=39). Participants with current or prior diagnosis of chronic viral hepatitis were excluded. Non-parametric methods were used to compare the groups and for pairwise comparisons.

**Results:** HIV-infected adults with NASH had elevated levels of sCD163, TNF $\alpha$ , CXCL10 and IL-8 compared to HIV-positive and HIV-negative control groups (p<0.001 for all comparisons). IL-6, CCL2 and sCD14 levels were elevated in HIV-infected patients (n=78) compared to HIV-negative controls but were not significantly different in HIV-infected patients with and without NASH. CRP levels did not differ between groups. Findings remained significant after adjustment for body mass index.

**Conclusions:** In HIV-infected patients, NASH is associated with increased levels of inflammation. In other HIV-infected cohorts, elevations in these biomarkers predict non-AIDS related morbidity and mortality. The contribution of NASH to HIV-related inflammation and associated comorbidities warrants further investigation.

**562 Early Mortality Risk of HIV/Hepatitis B Virus Coinfected Patients Initiating ART**

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**Background:** Hepatitis B virus (HBV) co-infection, common in HIV-infected patients in sub-Saharan Africa, is associated with impaired immunological recovery while on antiretroviral treatment (ART), and worse clinical outcomes, even in the context of effective ART. A clear association between HBV co-infection and early mortality has not been shown in African settings and the impact of tenofovir-containing ART on outcomes is unknown.

**Methods:** The prevalence of hepatitis B surface antigen (HBsAg) was determined in a cohort of patients enrolling in an ART programme in Kenya between 2003 and 2012. Clinical outcomes, immunological and virological responses to ART were compared between HIV mono-infected and HIV/HBV co-infected patients using Cox regression. The impact of tenofovir-containing ART, phased in over the observation period, on outcomes was determined in an analysis adjusting for confounders relating to time and indication.

**Results:** 7,155 patients were enrolled in the cohort and followed for 12,408 person years. HBsAg was detected in 451/7155 (6.3%, 95%CI 5.8-6.9%) of patients. HBsAg prevalence was higher in men than women (9.2% versus 5.0%, p<0.001) and increased with age. HBsAg positivity was associated with increased risk of death in crude analysis (HR 1.84, 95%CI 1.4-2.5, p<0.001). Among those initiating ART (n=6,214), HBsAg positive patients (n=419) had significantly impaired immunological recovery within the first year of ART

compared to HBsAg negative patients (median CD4 cell count increase 110 cells/mL vs 135 cells/mL,  $p=0.03$ ), despite similar rates of virological suppression (90% vs 88%,  $p=0.32$ ). HBsAg positivity remained an independent predictor of mortality in adjusted analysis (aHR 1.84, 95%CI 1.3-2.6,  $p=0.001$ ). Among patients initiating tenofovir-containing regimens ( $n=3125$ ), HBsAg positivity ( $n=350$ ) was no longer significantly associated with increased risk of mortality (aHR 1.45, 95%CI 0.9-2.2,  $p=0.1$ ) in contrast to the markedly increased risk in patients receiving non-tenofovir based regimens (aHR 3.32, 95%CI 1.8-6.2,  $p<0.001$ ),  $p$ -value for interaction = 0.03.

**Conclusions:** Hepatitis B co-infection was associated with impaired immunological responses to ART and increased risk of mortality in this large cohort of Kenyan patients initiating ART despite adequate HIV virological suppression and no evidence for severe liver disease. Use of tenofovir-containing regimens significantly reduced mortality risk in HIV/HBV co-infected patients.

**Table 1. Associations between Hepatitis B surface antigen status and outcome in a cohort of 7155 HIV-infected Kenyans**

	HBsAg +ve	HBsAg -ve	p-value	TDF	No TDF	p-value
Age (years)	38 (3245)	36 (3044)	<0.001	37 (3144)	36 (3043)	<0.001
Sex (% male)	46% (209)	31% (2057)	<0.001	34% (1034)	30% (951)	0.009
CD4 count (cells/L)	145 (61299)	167 (67347)	0.01	167 (69327)	137 (59276)	<0.001
HIV viral load (Log <sub>10</sub> Copies/mL)	4.76 (3.75-2)	4.59 (3.35-3)	0.07	4.64 (3.35-2)	4.73 (3.75-3)	<0.001
CD4 increase in year 1 (cells/L)	110 (41208)	135 (48251)	0.03	128 (43224)	140 (50267)	<0.001
HIV viral suppression in year 1 (% n)	90.4% (263)	88.5% (3784)	0.32	89.5% (1927)	87.8% (2118)	0.07
Mortality in year 1 (% n)	9.3% (42)	5.3% (356)	<0.001	4.8% (148)	4.1% (127)	0.16
HBsAg and Mortality	aHR of death	95% CI	p-value			
All patients	1.84	1.3-2.6	0.001	All adjusted analyses were restricted to patients initiating ART and adjusted for a sex, CD4 count at ART initiation, baseline creatinine and calendar year. When tenofovir use was included as an interaction term there was significant evidence of interaction ( $p=0.03$ ).		
Not on TDF	3.32	1.8-6.2	<0.001			
On TDF	1.45	0.9-2.2	0.1			

All results are median (interquartile range) or percentage (number) unless otherwise indicated. HBsAg = Hepatitis B surface antigen. TDF = tenofovir disoproxil fumarate. aHR = adjusted hazard ratio, derived from a Cox regression model.

### 563 Incidence and Risk Factors for Hepatitis B in HIV-Infected Adults in Rakai, Uganda

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**Background: Objective:** Coinfection with Hepatitis B (HBV) and HIV is common in sub-Saharan Africa (SSA) and accelerates progression of liver disease to cirrhosis and other complications. Vaccination of HIV infected adults for HBV is standard of care in developed countries but not commonly performed in SSA where HBV is primarily believed to be acquired in childhood and where there is a lack of HBV incidence data. We investigated the incidence and risk factors associated with HBV among HIV infected adults in Rakai, Uganda.

**Methods:** We screened stored sera from 944 HIV infected adults enrolled in the Rakai Community Cohort Study between September 2003 to March 2015 for evidence of HBV exposure using the anti-HBc marker. Serum from participants who tested anti-HBc negative (506) at the baseline round was tested over 3-7 consecutive survey rounds for either anti-HBc or anti-HBs sero-conversion. The time of HBV incidence was defined as the median date between the last anti-HBc or HBsAg negative sample and the first positive anti-HBs or HBsAg serum sample. All ART treatment regimens included lamivudine (3TC) or emtricitabine (FTC). Exact poisson incidence methods were used to estimate the incidence of HBV with 95% confidence intervals while the Cox proportional regression methods were used to estimate adjusted hazard ratios of ART use and other confounders.

**Results:** Forty six infections occurred (12 positive for both HBsAg and anti-HBc, 5 for HBsAg only and 29 for anti-HBc only) over 3,346.4 person years, incidence 1.37/100 person years. HBV incidence was significantly lower with ART use: 0.67 /100 person years with ART use and 2.58/100 person years in absence of ART ( $p<0.001$ ), and significantly decreased with age: 3.54 /100 pys if aged 15-29 years, 1.5/100 pys if aged 30-39 years and 0.48/100 pys if aged 40-50 years ( $p<0.001$ ). The adjusted hazard ratios of HBV incidence significantly differed by ART use: non ART use versus ART use, aHR=0.33(95% CI=0.2-0.6), and by age: 40-50 years versus 15-29 years, aHR=5.65(95% CI=2.1-15.1); 40-50 years versus 30-39 years, aHR=2.71(95% CI=1.1-). There was no statistical significant differences by gender, occupation, marital status or number of sex partners or baseline CD4 count.

**Conclusions: Conclusion:** Ongoing HBV transmission demonstrated by this study represents a potential opportunity for vaccine preventive strategies. The protective effect of 3TC/FTC adds to the existing benefit of scaling up ART globally.

### 564 Long-Term Changes in Liver Fibrosis in HIV and HIV/HBV Infected Nigerians on ART

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**Background:** There are limited longitudinal data on changes in liver fibrosis by transient elastography (TE) in HIV/HBV co-infected patients on HBV active antiretroviral therapy (ART) in sub-Saharan Africa. Correlations with HBV DNA levels have also not been performed.

**Methods:** This is a prospective analysis of ART-naïve, HIV and HIV/HBV co-infected adults ( $\geq 18$ ) at Jos University Teaching Hospital (JUTH), Nigeria, HIV Care and Treatment Center who underwent paired liver stiffness measurement (LSM) by TE at baseline and 36 months after ART initiation. All patients received the combination of either AZT or TDF + 3TC or FTC + NNRTI; 94% of HIV/HBV patients received both TDF + FTC. Cutoffs for Metavir scores were 5.9kPa (F2, moderate fibrosis), 7.6 kPa (F3, advanced fibrosis), and 9.4kPa (F4, cirrhosis). Multivariate (MV) regression models were constructed to identify factors associated with LSM decline, defined as  $\geq 1$  unit decrease in fibrosis stage from baseline, in all patients and HIV/HBV patients alone.

**Results:** 99 HIV and 74 HIV/HBV co-infected ART-naïve patients (Table 1) underwent TE between July 2011 and February 2012 and again following ART initiation between July 2014 and February 2015. After a mean interval of 26.1 ( $\pm 10.6$ ) months on ART, a significant decrease in mean LSM occurred in HIV (-0.2kPa,  $p<0.01$ ) and HIV/HBV patients (-1.9kPa,  $p<0.01$ ). A similar proportion of HIV and HIV/HBV patients had a decline in fibrosis  $\geq 1$  stage [15/22 (68%) vs. 28/42 (65%);  $p=0.81$ ]. 55 (59%) and 48 (65%) of HIV and HIV/HBV

patients respectively, achieved HIV RNA < 20 copies/mL and 52 (70%) HIV/HBV co-infected patients achieved HBV DNA < 116 copies/mL (lower limits of detection). In MV analyses, after adjusting for baseline LSM and other factors, female sex and higher baseline CD4 T-cell counts/mm<sup>3</sup>, were associated with LSM decline in all patients [F vs M: OR 3.9, (95% CI 1.2-13.3); p= 0.03; CD4 T-cell count, p=0.01] and HIV/HBV patients alone [F vs M: OR 9.0, (95% CI 1.6-48.8); p=.01; CD4 T-cell count, p <.01]. In HIV/HBV patients, HBV DNA level and HBeAg seropositive status were not associated with LSM decline.

**Conclusions:** Fibrosis regression, as measured by TE, appears mainly associated with gender and immune status in HIV and HIV/HBV infected Nigerians. The association between higher CD4 counts and LSM declines supports earlier ART initiation in both patient groups. The impact of antiviral treatment and HBV suppression on fibrosis changes requires further investigation.

Table 1: Patient Characteristics at Baseline (N=173)

	HIV Mono-infection (N=99)	HIV/HBV Co-infection (N=74)	p-value
Baseline			
Female	81 (81.8%)	45 (60.8%)	.002
Age, years	39.7 (9.8)	37.3 (9.0)	.100
BMI, kg/m <sup>2</sup>	29.8 (28.5)	26.2 (13.2)	.308
WHO stage ≥ 2*	24 (31.6%)	27 (48.3%)	.087
Any alcohol use	32 (32.3%)	33 (44.6%)	.099
Platelet count (x100k/ul)	243 (76)	235 (76)	.487
ALT, IU/L	26 (19)	54 (103)	.008
Hgb, g/dL	13.1 (5.3)	12.6 (4.0)	.426
HIV RNA, log <sub>10</sub> copies/mL	4.0 (1.0)	4.4 (1.1)	.005
CD4+ T-cells, cells/mm <sup>3</sup>	434 (172)	353 (195)	.004
HBV DNA, log <sub>10</sub> copies/mL	-	3.1 (3.2)	-
HBV DNA ≥ 3.3 log <sub>10</sub> copies/mL	-	24 (34.3%)	-
HBeAg Reactive*	-	14 (20%)	-
LSM, kPa	5.2 (1.5)	8.0 (6.1)	<.001
≤ F1 Fibrosis (<5.9 kPa)	77 (77.8%)	31 (41.9%)	<.001
F2 Fibrosis (≥5.9 – <7.5)	15 (15.2%)	21 (28.4%)	<.001
≥ F3 Fibrosis (≥ 7.6 kPa)	7 (7.1%)	22 (29.8%)	<.001

Continuous and categorical variables analyzed by Student's T-test and X<sup>2</sup> tests, respectively. Values expressed as mean (SD) or n (%). BMI: body-mass index; WHO stage: World Health Organization Clinical Staging for HIV/AIDS; ALT, alanine transaminase; Hgb, hemoglobin; HIV, human immunodeficiency virus; HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; LSM, liver stiffness measurement; kPa, kilopascal  
\*More than 5% data missing

565 **Changes in Viral Hepatitis Screening Practices Over Time in African HIV Clinics**

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**Background:** There is a major burden of HIV-hepatitis B virus (HBV) co-infection in sub-Saharan Africa and knowledge of HBV status is important to guide optimal selection of ART drug regimens. We aimed to describe changes in testing practices related to viral hepatitis over a 3-year period in HIV clinics in sub-Saharan Africa.

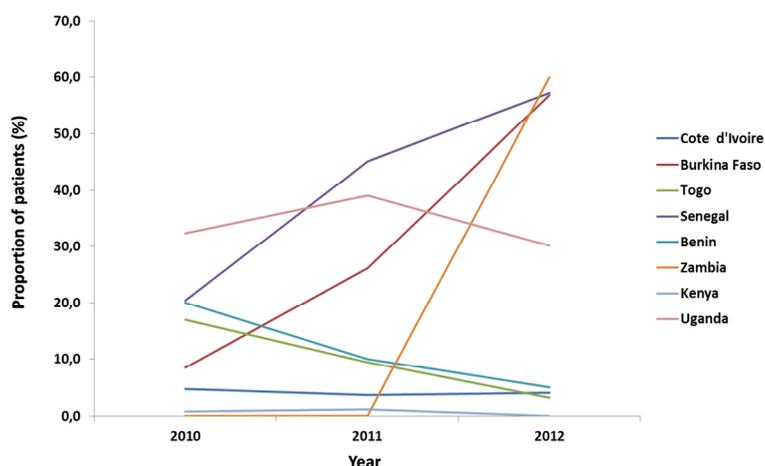
**Methods:** A medical chart review was conducted in 10 large urban HIV treatment centers in Côte d'Ivoire (3 sites), Burkina Faso, Bénin, Togo, Sénégal, Zambia, Kenya and Uganda (1 site each). Of the patients who started ART between 2010 and 2012, 100 per year were randomly selected from each clinic. Demographic, clinical and laboratory information were collected using a standardized questionnaire with a special focus on screening and management of HBV and hepatitis C virus (HCV) infections. We examined changes in the proportion of patients screened over time and identified predictors of testing in multivariable logistic regression models including sex, age, region (West, East and Southern Africa) and level of education (secondary/high school vs. lower).

**Results:** A total of 2,981 patients were included (median age 37 years, 64.2% female). Overall, only 471 (15.8%) patients had been tested for HBV, increasing over the years (10.4% in 2010, 14.6% in 2011 and 22.9% in 2012, p<0.001) although there were large differences in uptake across countries (Figure). Overall, 73 (15.5%, 95% confidence interval [CI]: 12.3-19.1) were HBsAg-positive. In 86.6% of patients screened, the HBsAg assay was the only test implemented, and 70.9% of the tests were performed before ART initiation. The

increase in HBV testing over time was largely due to programmatic changes and only 3.8% of tests were clinically-driven (elevated transaminases). In multivariable analysis, high education level (adjusted odds ratio 1.84, CI 1.47-2.30) and region (South vs. West: 1.43, CI 1.04-1.96; East vs. West: CI 1.17, 0.91-1.51) were associated with HBsAg screening. Among HBV-infected patients, 59 (80.9%) received tenofovir-containing ART. Only 30 (1.0%) patients were screened for HCV (19 in Senegal) and one of them (3.3%, 95% CI 0.84-17.2) was positive.

**Conclusions:** Conclusion: Between 2010 and 2012, the systematic screening for HBV infection in HIV-positive patients before ART initiation was limited in many African countries and differed widely across clinics. The increasing availability of HBsAg rapid tests and tenofovir as part of first-line ART should encourage HIV programs to improve HBV.

Figure. Proportion of patients screened for hepatitis B infection, by year and country



### 566 Tenofovir and the Incidence of Hepatocellular Carcinoma in HIV/HBV-Coinfected Persons

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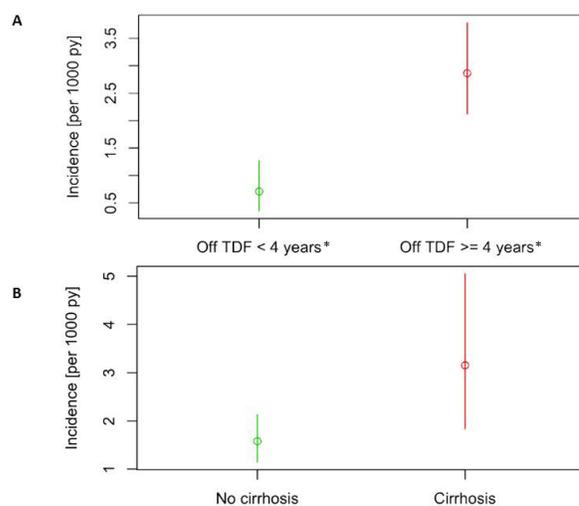
**Background:** Hepatocellular carcinoma (HCC) is a leading cause of death in HIV/hepatitis B virus (HBV)-coinfected patients. Current screening recommendations are based on incidence estimates in untreated HBV-infected patients and might be inadequate for HIV/HBV-coinfected individuals on antiretroviral therapy (ART). We explored the impact of tenofovir (TDF) on HCC incidence in a large collaboration of HIV cohorts including the Swiss HIV Cohort Study, Athena, EuroSIDA and ANRS CO3 Aquitaine.

**Methods:** We included all HBsAg-positive adults with complete ART history available. HCC incidence was described for the full population and compared between sub-groups according to the main demographic and clinical characteristics. We defined the cumulative time off TDF (either without any HBV-active ART or including only lamivudine [3TC]) as the main HBV therapy exposure variable. A binary variable was created according to the median follow-up (FUP) time on TDF (4 years). Liver cirrhosis was defined according to histology or as an AST-to-platelet ratio index (APRI) >1.5. We evaluated the association between cumulative time off TDF and the incidence of HCC using multivariable Poisson regression, adjusted for sex, ethnicity, hepatitis C virus (HCV) infection and liver cirrhosis.

**Results:** Of 3,593 HIV/HBV-coinfected patients included, 587 (16.3%) were female, 1,803 (50.2%) men who have sex with men, 2,876 (80.0%) Caucasians and 835 (23.2%) HCV-coinfected. Overall, 40.3% of the total FUP time was spent on TDF, 30.6% on 3TC only and 29.1% on ART without HBV-activity. Over 32,644 patient-years (py), 60 individuals (1.7%) developed an HCC, resulting in an overall incidence of 1.84 per 1,000 py (95% confidence interval [CI] 1.40-2.37). The incidence of HCC was highest in patients with >4 years of FUP off TDF (incidence rate ratio [IRR] 4.04, 95% CI 2.10-7.70) and in those with liver cirrhosis (IRR 3.04, 95% CI 1.83-5.04) (Figure). In adjusted analyses, there was a significant increase in the incidence of HCC per year off TDF (adjusted IRR [aIRR] 1.12, 95% CI 1.07-1.17), and patients with cirrhosis remained at higher risk of HCC (aIRR 2.85, 95% CI 1.70-4.79). During TDF therapy, the risk of HCC remained stable per additional year of FUP (aIRR 0.96, 95% CI 0.86-1.05).

**Conclusions:** Approximately 2% of patients developed an HCC over a median follow-up time of 8.4 years. HCC incidence increased with the length of FUP off TDF and was three times higher in cirrhotic compared to non-cirrhotic patients.

Figure: Incidence of hepatocellular carcinoma by (A) tenofovir (TDF) exposure and (B) cirrhosis status



\* The 4 year threshold corresponds to the median follow-up time on tenofovir

### 567 Dually Active HIV/HBV Antiretrovirals Protect Against Incident Hepatitis B Infections

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**Background:** The prevention of hepatitis B virus (HBV) transmission in HIV infected individuals is important as both viruses share common transmission modes and both have detrimental effects on each other's natural course. Vaccination against HBV remains the mainstay of preventing HBV acquisition both in HIV infected and uninfected patients. However owing to HIV's effect on the immune system, mounting and maintaining a protective immune response against HBV is sometimes unattainable with a success rate between 18%-71%. The strength of this study is twofold: (i) the ability to address confounding by condomless sex and (ii) sample size. We hypothesize that dually active antiretroviral therapy (DAART) against HBV and HIV (Tenofovir, Lamivudine, and Emtricitabine) has a protective effect against HBV but that the magnitude of the association could be confounded by behavioral and immune factors.

**Methods:** Patients with at least one negative serological marker (HBsAg, AntiHBC, or HBV-DNA) for HBV infection at baseline were included in the analysis. We excluded patients with a positive AntiHBs response, and their follow-up time after the first positive AntiHBs test. An incident case was then defined to be the presence of any of HBV serological markers following a negative baseline test. Both univariate and multivariate Cox proportional hazard models were utilized, with the outcome variable being an incident case of HBV infection and the explanatory variable being the proportion of observation time on DAART.

**Results:** We included 1826 patients from the Swiss HIV Cohort Study (988 heterosexuals (54%), 226 intravenous drug user (IDU) (12%) and 612 men who have sex with men (MSM) (34%). The total number of incident HBV cases was 180 of which 49% were in MSM. Most patients had only two tests (Median 2, IQR 2-3), and the median time between tests was 29 months (IQR 12-59).

Both univariable and multivariable analysis show a risk reduction of acquiring HBV for patients on DAART. DAART was associated with a lower HBV hazard ratio 0.4 (95% CI 0.2-0.6). DAART association was robust to adjustment (0.3, 0.2-0.5) for condomless sex,  $\sqrt{\text{CD4}}$  cell count, and patients' demographics. Condomless sex (1.4, 1-1.9), belonging to MSM (3.0, 2.0-4.3) or IDU (4, 2.6-6.5) transmission group were all associated with higher HBV incidence.

**Conclusions:** Overall, our study suggests that antiretroviral therapy, regardless of CD4 counts, has a strong beneficial public health impact that includes pre-exposure prophylaxis of an HBV co-infection.

## 568 Alcohol Use, Hepatitis B, and Liver Fibrosis Among HIV-Infected Persons in West Africa

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**Background:** Liver diseases represent a leading cause of mortality in people living with HIV (PLWHIV) in industrialized countries. Little is known about liver fibrosis and the associated factors among PLWHIV in sub-Saharan Africa.

**Methods:** A liver fibrosis screening program was undertaken in three HIV referral clinics in Côte d'Ivoire, Senegal and Togo, West Africa. PLWHIV attending their usual clinic appointment and who agreed to the study were consecutively enrolled and underwent a non-invasive assessment of liver fibrosis using both liver stiffness measure (LSM) with transient elastography and the aspartate aminotransferase to platelet ratio index (APRI). Significant liver fibrosis ( $\geq$ F2 METAVIR score) was defined as an LSM  $\geq$ 7.1 kPa among patients with an APRI score  $\geq$ 0.5. The short alcohol use disorder identification test (AUDIT-c) was administered to identify hazardous alcohol use (AUDIT-c  $\geq$ 4) and heavy alcohol use (AUDIT-c  $\geq$ 6) based on a 12-point score. All patients were tested for Hepatitis B virus (HBV) surface antigen (HBsAg) using a rapid test (Determine<sup>®</sup>) and if positive, followed by viral load quantification. A multivariate logistic regression model was used to identify factors associated with liver fibrosis using Odds Ratio (OR) with 95% Confidence Interval (CI). **Results:** A total of 807 PLWHIV (69.8% women) were included at a median age of 43 years [interquartile range (IQR): 36–50]. Their median CD4 count was 393 cells/mm<sup>3</sup> [IQR 234–563] and 682 (84.5%) were on antiretroviral therapy (ART). A significant liver fibrosis was reported in 5.3% (95% CI 3.8–6.7) of participants. At the time of the survey, 76 (9.5%) patients reported hazardous alcohol use and 32 (4.0%) heavy alcohol use. Of 74 (9.2%) HBsAg-positive patients, 63 (85.1%) were on ART and among them 37 (58.7%) were on a tenofovir (TDF)-based regimen. Among the 26 HBsAg-positive patients with a detectable HBV viral load, those exposed to TDF and/or lamivudine had lower rates of detectable HBV viral load (29.0%) compared to unexposed patients (66.6%) ( $p=0.01$ ). In multivariate analyses, heavy alcohol use [OR=4.5 (95% CI 1.8–11.1)], [Ref. AUDIT-c<4] and HBV infection [OR= 2.6 (95% CI 1.1–6.5)] were associated with significant liver fibrosis.

**Conclusions:** Heavy alcohol use was identified as an important determinant of liver fibrosis among PLWHIV in West Africa. Screening of alcohol use and specific interventions to prevent alcohol abuse should be proposed to PLWHIV, along with the recent WHO recommendations to screen for HBV infection.

## 569 Baseline IL-18 Level Is Associated With HBeAg Seroconversion in HIV/HBV Coinfection

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**Background:** In HIV/HBV co-infected patients, hepatitis B e antigen (HBeAg) seroconversion is associated with lower rates of developing cirrhosis and hepatocellular carcinoma. A previous animal study suggests that interleukin-18 (IL-18), a cytokine associated with interferon- $\gamma$  production and anti-viral defense, inhibits HBV replication. Recent studies also find that IL-18 polymorphisms are associated with immune control of HBV in HBV mono-infected patients. However, whether IL-18 is associated with HBeAg seroconversion in HIV/HBV co-infected patients is unknown.

**Methods:** We enrolled 35 treatment-naïve, HBeAg positive, HIV/HBV co-infected patients. HBV DNA, HIV RNA, CD4 cell count, HBV surface antigen (HBsAg) quantification (qHBsAg), HBeAg quantification (qHBeAg) and IL-18 levels were measured prior to, at 24 and 48 weeks of HBV-active antiretroviral therapy (ART). Primary endpoint was HBeAg seroconversion. Multivariate Poisson regression models with robust standard errors were used to determine factors associated with HBeAg seroconversion.

**Results:** Twenty-one patients received tenofovir (TDF)+ lamivudine (3TC) based ART while 14 patients received 3TC-based ART. Median baseline HBV DNA levels were 8.04 log IU/ml (upper limit of detection). After 48 weeks of treatment, 10 patients experienced HBeAg seroconversion. In comparison with non-seroconverters, seroconverters had higher median HIV RNA (5.22 vs. 4.58 log copies/ml,  $P=0.030$ ), lower median qHBsAg (3.97 vs. 4.76 log IU/ml,  $P=0.011$ ), lower median qHBeAg (1.61 vs. 3.01 log PEIU/ml,  $P=0.004$ ), and marginally higher median IL-18 (511.1 vs. 335.9 pg/ml,  $P=0.068$ ) prior to ART. In the multivariate regression model, IL-18 levels (RR 5.13 per 1 log pg/ml increase,  $P=0.004$ ), CD4 cell count (RR 1.92 per 100 cells/ $\mu$ l increase,  $P=0.011$ ), HIV RNA (RR 2.01 per 1 log copy/ml increase,  $P=0.001$ ) and qHBsAg (RR 0.17 per 1 log IU/ml increase,  $P=0.012$ ) were significantly associated with HBeAg seroconversion.

**Conclusions:** In HIV/HBV co-infected patients with HBeAg positivity, high IL-18 levels, CD4 cell count, HIV RNA load, as well as low qHBsAg prior to ART were associated with HBeAg seroconversion.

Table. Factors associated with HBeAg seroconversion.

Variables	Adjusted RR	95% CI	P values
CD4 cell count (per 100 cells/ $\mu$ l)	1.92	1.16, 3.18	0.011
HIV RNA (per 1 log copy/ml)	2.01	1.35, 3.00	0.001
qHBeAg (per 1 log PEIU/ml)	1.55	0.87, 2.78	0.136
qHBsAg (per 1 log IU/ml)	0.17	0.04, 0.68	0.012
IL-18 (per 1 log pg/ml)	5.13	1.68, 15.67	0.004
ALT>40 IU/l	2.04	0.81, 5.14	0.131

Poisson regression with robust standard errors was used to build this regression model. Multivariate regression was conducted in a backward stepwise fashion, with  $P>0.15$  removed from the models. Routes of transmission were also adjusted for in this multivariate regression model, while TDF use, sex, age, and HBV DNA were removed from this model during the stepwise regression.

## 570 HBsAg Mutations Correlate With HCC, Affect HBsAg Release, and Favor Cell Proliferation

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**Background:** To evaluate HBsAg genetic elements associated with HBV-induced liver cancer (HCC) and their impact on cell proliferation.

**Methods:** This study includes 133 HBV chronically infected patients (pts): 23 with HCC (73.9% HBV genotype D; 26.1% A), and 110 asymptomatic pts as control (72.7% D, 27.3% A). HBsAg mutations (detected in plasma samples) are defined according to the reference sequence of each specific genotype. Association of mutations with HCC is assessed by Fisher test. HBsAg ultra-deep sequencing (UDPS) is performed for 13 HCC- and in 24 non-HCC pts. HBsAg mutations are introduced into a 1.3x genome-length HBV genotype D. WT and mutated clones are transfected into Huh7 cells. Lysates and supernatants are harvested in triplicate daily until day 5 post-transfection, and HBsAg quantified by Alexiss assay. Mutations are also introduced into a pIRES II plasmid encoding HBsAg and GFP. Cell cycle is analysed by flow cytometry (DNA propidium iodide-staining) on transfected GFP+ cells at day 7 post transfection (4 experiments in triplicate).

**Results:** Two HBsAg mutations (alone or in association) significantly correlate with HCC: P203Q (17.4% [4/23] in HCC vs 0.8% [1/110] in non-HCC,  $P=0.003$ ), S210R (34.8% [8/23] in HCC vs 3.6% [4/110] in non-HCC,  $P<0.001$ ), P203Q+S210R (17.4%[4/23] in HCC vs 0% in non-HCC). They reside in transmembrane C-terminal domain critical for HBsAg secretion. By UDPS, the intra-patient prevalence of P203Q and S210R is  $\geq 50\%$  in 100% and 71.4% of HCC-pts, indicating their selection in the viral population. Conversely, in non-HCC pts carrying P203Q and S210R, their intra-patient prevalence exceeds 50% in only 1 patient. *In vitro*, the presence of P203Q, S210R and P203Q+S210R reduces the ratio of secreted/intracellular HBsAg compared to wt at each time point ( $P<0.05$ ), with the strongest decrease observed at 4 days (Ratio:4.4 $\pm$ 0.3 for wt; 2.2 $\pm$ 0.2 for P203Q; 3.5 $\pm$ 0.2 for S210R; 2.3 $\pm$ 0.2 for P203Q+S210R,  $P=0.05-0.005$ ). By flow cytometry, P203Q and P203Q+S210R significantly correlate with an increased % of cells in the S phase, indicating cell cycle progression: P203Q (26 $\pm$ 13%) and P203Q+S210R (29 $\pm$ 14%) compared to wt (18 $\pm$ 9%) ( $P\leq 0.01$ ).

**Conclusions:** Key mutations, in C-terminal HBsAg domain, correlate with HBV-related HCC *in vivo*. They affect HBsAg release and promote cell proliferation *in vitro*, suggesting their potential involvement in HCC development. Their detection may help identifying patients at higher HCC-risk that may deserve intensive liver monitoring, or early anti-HBV therapy.

#### 571 Liver Transplantation in HIV/HBV-Coinfected Patients: A Cohort Study

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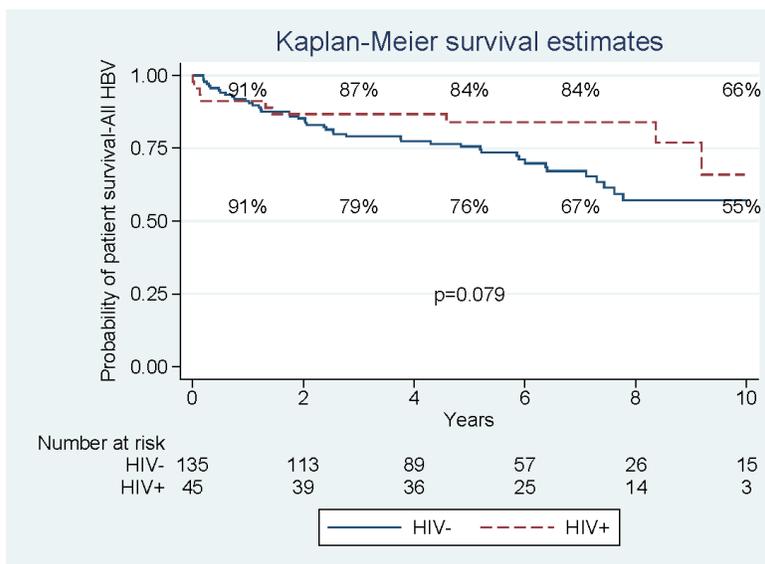
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**Background:** Prognosis of HIV/HBV coinfecting patients undergoing liver transplantation (LT) has been reported as satisfactory. However, this contention is based on reports including small series of patients. This study aims to determine patient and graft survival rates in a larger series of patients with HIV and HBV infection who underwent LT and compare them with those observed in HBV-infected LT recipients without HIV infection.

**Methods:** 271 consecutive HIV-infected patients who underwent LT between 2002 and 2012 and who were followed until December 2014 were matched with 813 LT recipients without HIV infection in 22 Spanish institutions. Matched criteria were: same site, age ( $\pm 12$  years), gender, calendar year ( $\pm 1$  year), and LT indication. Those patients with HBV infection constitute the present study cohort.

**Results:** 45 (17%) HIV/HBV-coinfecting LT recipients and 135 (17%) HBV-infected patients without HIV infection were included. 37 (82%) HIV-infected and 114 (84%) HIV-uninfected patients also had HCV infection. 17 (46%) HIV/HBV/HCV-coinfecting patients and 107 (95%) of HBV/HCV-coinfecting patients had detectable RNA HCV at LT ( $p<0.001$ ). After a median follow-up of 5.8 (IQR: 3.3-7.5) years, 9 (20%) HIV-infected patients and 46 (34%) HIV-uninfected patients died ( $p=0.081$ ). The main cause of death was post-LT HCV recurrence in both groups (22% each). Patient survival rates (95% CI) at 1, 3, and 5 years for HIV-infected vs. HIV-uninfected patients were 91% (78-97) vs. 91% (85-95), 87% (73-94) vs. 84% (69-92), and 84% (69-92) vs. 76% (67-82), respectively ( $p=0.079$ ) (Figure). 4 (9%) HIV-infected patients and 12 (9%) HIV uninfected patients underwent liver retransplantation. Graft survival rates (95% CI) at 1, 3, and 5 years for HIV-infected vs. HIV-uninfected patients were 87% (73-94) vs. 86% (79-91), 82% (68-91) vs. 73% (65-80), and 80% (64-89) vs. 70% (61-77), respectively ( $p=0.065$ ). Detectable serum HCV RNA at LT was the only factor associated with mortality in the whole series (HR 95% CI: 2.42, 1.18-4.95;  $p=0.015$ ).

**Conclusions:** HIV/HBV-infected patients undergoing LT have a patient and graft survival not different than those in HBV-infected patients without HIV infection. HCV coinfection with detectable HCV RNA at LT is associated with a worse outcome.



#### 572 Incidence and Risk Factors of Acute Rejection in HIV+ Liver Transplant Recipients

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**Background:** HIV-infected liver transplant (LT) recipients seem to have higher rates of acute rejection than recipients without HIV infection. We determined the incidence and risk factors of acute rejection in HIV-infected LT recipients in comparison with HIV-uninfected LT recipients

**Methods:** 271 consecutive HIV infected patients who underwent LT between 2002-2012 were matched with 813 patients without HIV infection (1:3 ratio) who underwent LT during the same period in 22 Spanish institutions. Other matched criteria were age ( $\pm 12$  years), gender, calendar year ( $\pm 1$  year), and LT indication. All acute rejections were biopsy-proven.

**Results:** After a median follow-up of 4.4 (IQR: 2.1-7.0) years, 85 (36%) HIV-infected and 151 (19%) HIV-uninfected patients developed acute rejection ( $p<0.001$ ). Cumulative incidence of acute rejection (95% CI) at 1, 3, and 5 years in LT recipients with and without HIV infection was 32% (26-38) vs. 16% (14-19), 33% (27-39) vs. 19% (16-22), and 33%

(27-39) vs. 19% (16-22), respectively ( $p=0.001$ ). The table shows the comparison of characteristics of patients with and without rejection in the whole series of HIV-infected and HIV-uninfected patients. HIV infection (HR 95% CI:1.85, 1.40-2.45;  $p<0.001$ ) and early calendar period of LT (2002-2007) (HR 95% CI:1.33, 1.02-1.72;  $p=0.035$ ) were the only factors independently associated with acute rejection. Among HIV-infected LT recipients, patients with rejection had a lower MELD score and older donor age compared to patients without rejection. HIV-related factors, such as history of opportunistic infections, CD4 cell count, serum HIV detectable viremia at LT or raltegravir-based initial post-LT antiretroviral therapy, were not associated with acute rejection. The development of acute rejection did not impact on survival. No significant differences were observed in the mortality rate in patients with vs. without acute rejection: 43% vs. 41% in the cohort of HIV-infected patients, and 36% vs. 32% in the cohort of HIV-uninfected patients, respectively.

**Conclusions:** HIV-infected LT recipients have a higher incidence of acute rejection, although the development of acute rejection did not influence the survival. HIV infection was an independently associated factor of acute rejection. In the cohort of HIV-infected LT recipients, HIV infection-related factors were not associated with acute rejection

**Table 1. Predictors of first biopsy-proven acute rejection in liver transplant recipients**

	Non-acute rejection n=848	Acute rejection n=236	Crude HR (95% CI)	p-value	aHR (95% CI)	p-value
<b>Pre-LT characteristics</b>						
Recipient age at LT, years (1-unit increase)*	48 (44;53)	47 (42;52)	<b>0.98 (0.96;0.99)</b>	<b>0.030</b>	0.99 (0.97-1.01)	0.471
Male gender	676 (80)	177 (75)	0.79 (0.59;1.06)	0.116	1.26 (0.94-1.69)	0.127
HIV infection	186 (22)	85 (36)	<b>1.90 (1.45;2.47)</b>	<b>&lt;0.001</b>	<b>1.85 (1.40;2.45)</b>	<b>&lt;0.001</b>
HCV infection	801 (94)	223 (94)	1.03 (0.59;1.78)	0.926		
HCV genotype 1	512 (73)	149 (75)	1.06 (0.77;1.46)	0.706		
Undetectable RNA HCV at LT	153 (18)	34 (14)	0.78 (0.54;1.13)	0.190		
MELD score at enlisting (1-unit increase)*	15 (11;18)	15 (12;17)	0.99 (0.96;1.01)	0.319		
MELD score at LT (1-unit increase)*	15 (12;19)	15 (11;18)	0.98 (0.96;1.00)	0.129		
Hepatocellular carcinoma	252 (30)	63 (27)	0.87 (0.65;1.16)	0.352		
<b>Transplant characteristics</b>						
Calendar period of LT 2002-2007	376 (43)	122 (52)	<b>1.34 (1.04;1.73)</b>	<b>0.025</b>	<b>1.33 (1.02;1.72)</b>	<b>0.035</b>
Donor Age (1-unit increase)*	52 (38;64)	52 (39;65)	1.00 (0.98;1.01)	0.219		
Cyclosporine-based initial immunosuppressive regimen	201 (24)	64 (27)	1.11 (0.83;1.48)	0.476		

\*Median and interquartile range  
HR, hazard ratio; aHR, adjusted hazard ratio; CI, confidence interval; LT, Liver transplantation

**573 Retreatment of HCV/HIV-Coinfected Patients Who Failed 12 Weeks of LDV/SOF**

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**Background:** Ledipasvir/sofosbuvir (LDV/SOF) fixed-dose combination is highly effective and safe for genotype 1 HCV-infected patients with HIV co-infection. Of the 335 HCV/HIV coinfecting patients enrolled in the ION-4 Phase 3 study, 3% relapsed (n=10) after 12 weeks of LDV/SOF treatment. These patients were eligible for a retreatment substudy that evaluated the efficacy and safety of LDV/SOF (90 mg/400 mg) plus weight-based RBV for 24 weeks.

**Methods:** Eligible patients were enrolled within 60 days from the time of confirmed virologic failure. NS5A and NS5B resistance associated variants (RAVs) were evaluated by deep sequencing prior to retreatment and at the time of virologic failure post-retreatment. The primary endpoint was SVR12.

**Results:** Nine of 10 patients were enrolled and completed treatment. All patients were black, IL28B non-CC, HIV suppressed on ARV regimens with a median baseline CD4 count of 785 cells/uL (Q1, Q3 = 404, 971). The mean age was 57 years (range 35-65) and most were male (n=7), without cirrhosis (n=7), and had genotype 1a infection (n=7). The mean baseline HCV RNA was 6.2 log10 IU/mL (range 4.4-7.1). HIV ARV regimens included tenofovir+emtricitabine (TDF+FTC) with either efavirenz (n=7) or raltegravir (n=2). Prior to retreatment, 2 patients had no NS5A RAVs and 7 patients had high-level NS5A RAVs detected (see Table). The SOF-specific NS5B RAV S282T was not detected in any patients; 1 patient had L159F. Overall SVR12 rate was 89% (8/9): 1 patient relapsed. There were no treatment-emergent SAEs. Fatigue (n=6), cough (n=4), anemia (n=2) and arthralgia (n=2) were the most common adverse events. No significant lab abnormalities were observed and creatinine clearance was stable on treatment. No patient had confirmed HIV virologic rebound (HIV-1 RNA ≥ 400 copies/mL).

**Conclusions:** Ledipasvir/sofosbuvir with ribavirin for 24 weeks was well tolerated and demonstrated that successful retreatment is possible in the majority of these genotype 1-infected, NS5A-experienced HCV/HIV co-infected patients.

Table: Baseline NS5A RAVs for 9 Patients Prior to Retreatment with LDV/SOF + RBV for 24 weeks and Virologic Outcome

GT	NS5A RAVs	SVR12
1a	None	Yes
1a	None	Yes
1a	L31M (>99%), H58D (>99%)	Yes
1a	Y93N (>99%)	Yes
1a	L31M (>99%), Y93N (>99%)	Yes
1a*	Y93N (>99%)	Yes
1a	L31M (>99%)	No
1b	L31I (11.12%), Y93H (>99%)	Yes
1b	L31V (>99%)	Yes

\*Patient also had NS5B L159F (9.86%) prior to retreatment

**574 TURQUOISE-I Part 1b: Ombitasvir/Paritaprevir/r+Dasabuvir+RBV for HCV/HIV Coinfection**

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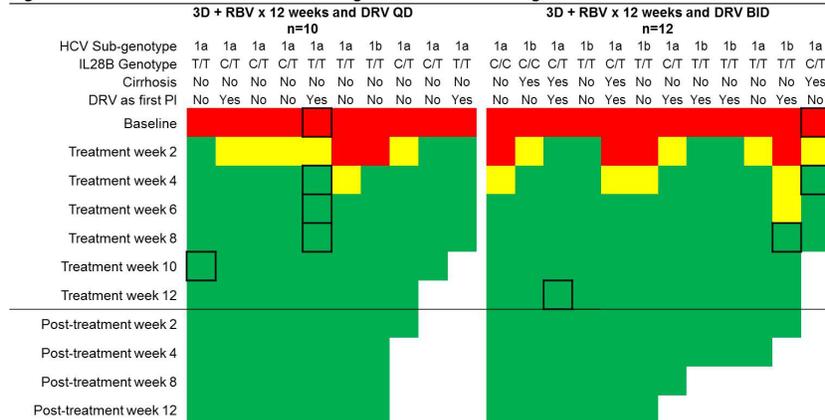
**Background:** The 3 direct-acting antiviral (DAA; 3D) regimen of ombitasvir/paritaprevir/ritonavir (OBV/PTV/r; PTV identified by AbbVie and Enanta) with dasabuvir (DSV) ± ribavirin (RBV) is approved in the US and EU for hepatitis C virus (HCV) genotype 1 (GT1) in patients with HIV-1 co-infection. In healthy volunteers, co-administration of 3D+dasabuvir (DRV) did not significantly change DAA exposures; however, DRV C<sub>trough</sub> levels were 48% and 43% lower with once and twice daily (QD and BID) DRV co-administration, respectively. To assess the clinical significance of co-administration, Part 1b of TURQUOISE-I evaluates efficacy and safety of 3D+RBV in patients with HCV GT1/HIV-1 co-infection on stable DRV-containing antiretroviral therapy (ART).

**Methods:** Eligible patients were HCV treatment-naïve/interferon-experienced, had CD4+ count ≥200cells/mm<sup>3</sup> or ≥14%, and plasma HIV-1 RNA suppression on stable QD DRV-containing ART at screening. Patients were randomized to maintain DRV 800mg QD or switch to DRV 600mg BID during a pre-treatment period. All patients received 3D+RBV for 12 weeks. Virologic response and safety from a data cut on 23Sept2015 are reported in this abstract.

**Results:** Twenty-two patients were enrolled: 77% male, 41% identified as black race, 27% as Hispanic ethnicity, 68% HCV GT1a-infected, and 18% with cirrhosis. All patients with available data achieved sustained virologic response (HCV RNA <25IU/mL) at post-treatment weeks 4 (17/17) and 12 (13/13). No DAA-related severe or serious adverse events (AEs) or AEs leading to discontinuation were reported. The most common AEs (>15% of patients) were fatigue (36%), hemoglobin decreased (23%), irritability (23%), and nausea (18%). Intensive pharmacokinetic evaluations revealed that co-administration of DRV and 3D+RBV resulted in minimal impact on DRV C<sub>max</sub> and AUC; however, DRV C<sub>trough</sub> levels were 53% and 29% lower with DRV QD and BID, respectively. All but one patient with available data (DRV BID arm) had HIV-1 RNA suppression at end of treatment based on the FDA snapshot algorithm (single HIV-1 RNA of 64copies/mL), though this patient maintained HIV-1 RNA <40copies/mL at all other visits. No patient experienced plasma HIV-1 RNA >200 copies/mL during the treatment period.

**Conclusions:** Patients with HCV GT1/HIV-1 co-infection on stable DRV-containing ART achieve high SVR rates while maintaining plasma HIV-1 RNA suppression. Despite changes in DRV exposures, episodes of intermittent HIV-1 viremia were infrequent during the study.

**Figure. HCV RNA Levels for Patients Receiving 3D+RBV for HCV Along With DRV Dosed Either QD or BID**



**Figure legend:** HCV RNA levels at each study visit are shown for patients who received 3D+RBV and DRV (QD or BID). The first four rows describe patient characteristics. Each column represents an individual patient. Red indicates an HCV RNA level ≥25 IU/mL (lower level of quantitation), yellow indicates an HCV RNA level <25 IU/mL but ≥15 IU/mL (lower level of detection), and green indicates an HCV RNA level <15 IU/mL. Black boxes indicate that plasma HIV-1 RNA was ≥40 but <200 copies/mL. HCV, hepatitis C virus; 3D, ombitasvir/paritaprevir/ritonavir+dasabuvir; RBV, ribavirin; DRV, darunavir; QD, once daily; BID twice daily; PI, protease inhibitor.

**575 NSSA and NSSB Minor Variant Analyses in HCV/HIV Patients Failing Treatment With DCV/SOF**

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**Background:** ALLY-2 was a phase 3 study of daclatasvir (DCV) plus sofosbuvir (SOF) for 12 or 8 weeks in patients with HIV/HCV coinfection. Thirteen patients in ALLY-2 experienced relapse; 2 in the 12-week and 11 in the 8-week group. Emergent drug-resistant variants were mostly not detected in the 8-week group by standard sequencing methods. We assessed the presence of minor variants among the 13 patients who experienced relapse.

**Methods:** Plasma samples drawn at baseline and relapse from the 13 HCV/HIV coinfecting patients who relapsed were prepared for next-generation sequencing (NGS) of the HCV NSSA and NSSB regions using the Illumina MiSeq sequencing platform at DDL Diagnostic Laboratories (NL). NSSA amino acids 28, 29, 30, 31, 32, 58, 62, 92, and 93 and NSSB amino acids 61, 112, 159, 237, 282, 320, 321, and 473 were monitored using NGS (sensitivity cut-off 1%) and results compared with standard direct sequencing (DS; sensitivity cut-off 25%).

**Results:** NSSA NGS and DS results were concordant at failure for both patients (both genotype [GT]1a) who relapsed after 12 weeks of DCV/SOF treatment (Table). Of these 2 patients, 1 had minor variants (M28V [1.1%], E62D [16.9%]) at baseline that were not observed at failure with emergent NS5A-Q30R. The other patient had NSSA-Y93N at baseline that was enriched with emergent NSSB-L159F and the minor variant NSSB-E237G. Eight of the 11 patients failing 8 weeks of treatment (5/8 GT1a, 1/1 GT1b, 1/1 GT2, 1/1 GT3) had no pre-existing or emergent minor variants to NSSA, however 1 of the GT1a patients had NSSB-D61G (1.6%) emerge (Table). Three patients (all GT1a) had minor NSSA variants emerge (Q30R [2.1%] or M28T [5.4%] and L31M [1.1%] or Y93C [1.0%]). The patient with Y93C also had M28L (1.9%) at baseline that was not detected at failure. No NSSB RAVs were detected in these 3 patients. For the single patient in the 8-week group with previously reported emergent NSSA-Q30E by DS, no minor variants to NSSA or NSSB emerged. For patients with emergent minor variants to NSSA, these will be further monitored to assess decay.

**Conclusions:** In ALLY-2, there was no impact of baseline NSSA or NSSB minor variants on response as these variants were not enriched at failure.

Table 1: NSSA and NSSB Variants Detected at Baseline and Virologic Escape in ALLY-2 by Deep Sequencing

Patient	GT	Weeks of Treatment	Visit	NSSA Variants Present by NGS (% Variant)	NSSB Variants Present by NGS (% Variant)
1	1a	12	Baseline	<b>M28V (1.1), E62D (16.9)</b>	<b>D61G (2.5)</b>
			FUWK4	Q30R (99.8)	None
2	1a	12	Baseline	Y93N (40.9)	None
			FUWK4	Y93N (99.4)	L159F(34.5), <b>E237G(11.8)</b>
3	1a	8	Baseline	<b>H58Y (3.6), E62D (99.4)</b>	None
			FUWK4	<b>Q30R (2.1), E62D (99.6)</b>	None
4	1a	8	Baseline	E62D (88.8)	None
			FUWK12	<b>M28T(5.4), L31M (1.1), E62D(98.8)</b>	None
5	1a	8	Baseline	None	<b>D61G (1.6)</b>
			FUWK24	None	None
6	1a	8	Baseline	<b>M28L (1.9)</b>	None
			FUWK4	<b>Y93C (1.0)</b>	None
7	1a	8	Baseline	None	<b>D61G (1.1)</b>
			FUWK4	Q30E (99.6)	<b>D61G (1.1)</b>
8	1a	8	Baseline	None	None
			FUWK4	None	<b>D61G (1.6)</b>

## 576 Safety and Tolerability of Elbasvir/Grazoprevir in Chronic Hepatitis C Infection

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**Background:** In phase 2-3 studies, treatment with elbasvir (EBR) 50 mg/grazoprevir (GZR) 100 mg ± ribavirin (RBV) resulted in high rates of sustained virologic response (SVR) in hepatitis C virus (HCV)-infected patients, including those with compensated cirrhosis. The purpose of this analysis was to define the overall safety profile of EBR/GZR given for 8, 12, 16, or 18 weeks in these studies.

**Methods:** Clinical adverse events (AEs) and laboratory abnormalities reported on therapy, or within 14 days of end of treatment, were compared in 1795 patients, of whom 1033 received EBR/GZR without RBV, 657 received EBR/GZR with RBV, and 105 received placebo.

**Results:** A diverse population was enrolled: 61% were male and 13% were black/African American; mean age was 52.6 years (11% ≥65 years); 27% had compensated cirrhosis; and 18% were HIV/HCV coinfecting. The overall safety profiles of EBR/GZR (without RBV) and placebo were comparable (Table). The addition of RBV was associated with more AEs. Among patients who received EBR/GZR, there were 3 deaths (ventricular arrhythmia, strangulated hernia, motor vehicle accident); all 3 were unrelated to study medication. No patients who received placebo died. The frequency of adverse events was not associated with sex, age, presence of cirrhosis, or HIV/HCV coinfection. Late serum alanine aminotransferase (ALT) elevations (defined as >5× upper limit of normal, in patients who had normal ALT values between treatment week [TW] 2-4) were noted in 0.8% of patients, generally at/after TW8. These were typically asymptomatic, resolving with continued therapy, scheduled end of therapy, or (in 3/1690, 0.18%) a protocol-mandated stop of therapy, and they were not associated with hyperbilirubinemia.

**Conclusions:** EBR/GZR ± RBV was generally well tolerated in a large, diverse patient population with a low serious AE rate. Fewer discontinuations due to AEs and overall improved tolerability with lower reductions in hemoglobin and lower elevations in total bilirubin were demonstrated in the RBV-free cohort. ALT elevations occurring late in the course of therapy were infrequent and not clinically significant.

Parameter	EBR/GZR (no RBV) n=1033	EBR/GZR + RBV n=657	Placebo n=105
≥1 adverse event (AE)	71.4%	83.6%	68.6%
≥1 treatment-related AE	40.1%	67.6%	39.0%
Specific treatment-related AEs in >5% of patients	Fatigue (12.0%) Headache (11.5%)	Fatigue (24.7%) Headache (16.3%) Nausea (12.6%) Asthenia (9.3%) Anemia (9.1%) Insomnia (8.8%) Pruritus (8.8%) Rash (6.8%) Dyspnea (6.4%)	Fatigue (9.5%) Headache (8.6%) Pruritus (6.7%)
Serious AEs (SAEs)	2.4%	2.6%	2.9%
Treatment-related SAEs	0.1%	0.5%	0.0%
Discontinuation due to AE	0.5%	1.7%	1.0%
Discontinuation due to treatment-related AE	0.3%	0.8%	1.0%
Grade 3 <sup>+</sup> ↓hemoglobin (Hgb)	0.0%	2.7%	0.0%
Mean Hgb decline (mg/dL) at TW8	-0.3	-2.2	-0.1
Grade 3 <sup>+</sup> ↑alanine aminotransferase	1.6%	0.6%	8.6%
Grade 3 <sup>+</sup> ↑total bilirubin	0.3%	5.9%	0.0%

**577 HCV Resistance to Daclatasvir/Sofosbuvir Across Different Genotypes in the Real Life**

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**Background:** The impact of preexisting HCV NS5A and/or NS5B resistance-associated variants (RAVs) on the response to daclatasvir (DCV) in combination with sofosbuvir (SOF) is unknown in the "real life" clinical setting. We studied the impact of preexisting substitutions at RAV positions on treatment outcomes in a cohort of patients infected with HCV genotypes (GT) 1a, 1b, 3 and 4 receiving SOF plus DCV without ribavirin for 12 weeks.

**Methods:** Population sequencing of the NS5A and NS5B genes was performed prior to SOF/DCV treatment in 177 patients (GT 1a, n=44; GT1b, n=63; GT3, n=29; GT4, n=41) and at treatment failure (relapse) in 8 patients who did not achieve SVR12. NS5A sequences were examined at 9 known RAV positions in domain I (residues 28, 29, 30, 31, 32, 58, 62, 92 and 93), while NS5B sequences were examined at 5 known RAV positions (159, 282, 316, 320, 321).

**Results:** Prior to treatment, NS5A RAVs were detected at a frequency of 9% in GT1a-, 32% in GT1b-, 21% in GT3- and 10% in GT4-infected patients. The most frequent RAVs were Y93H in GT1b (11%) and GT3 (3.5%), and L28M in GT4 (10%). No NS5B S282T, L320F or V321A variants were detected, while L159F was found in 12% of GT1b patients at baseline. C316N and C316H were detected in GT1b patients (20% and 2%, respectively) and in GT4 patients (5% and 2%, respectively). RAVs frequency at baseline did not differ between patients with and without cirrhosis. Post-treatment relapse occurred in 8 patients, including 5 infected with GT3. Virological failure tended to be more frequent when an NS5A Y93H substitution was present at baseline (p=0.067). All relapse patients harbored dominant NS5A RAVs post-treatment, with Y93H present in all GT3-infected subjects failing treatment. In addition, a so far undescribed NS5A amino-acid substitution (H85Y) was detected in a GT3-infected patient at relapse.

**Conclusions:** The prevalence of NS5A RAVs at baseline varied considerably across genotypes 1a, 1b, 3 and 4. Depending on the genotype, natural polymorphisms in NS5A (e.g. Y93H) differentially influence virological outcomes. A new amino acid substitution, H85Y, was selected in the NS5A region by SOF/DCV treatment. Real-life assessment will be key to better understand the mechanisms of HCV resistance and its role in interferon-free treatment failures.

**578 Polymorphisms at Codon 28 of HCV NS5A Impact NS5A Inhibitor Susceptibility**

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**Background:** Amino acid substitutions at position 28 of NS5A in HCV genotype 1a (GT1a) represent a major resistance pathway to ledipasvir (LDV), ombitasvir (OBV) and daclatasvir (DCV). GT1a and GT1b viruses are polymorphic at position 28: methionine (M) is most common in GT1a whereas leucine (L) predominates in GT1b. To investigate the impact of amino acid 28 polymorphisms on NS5A drug susceptibility, we evaluated replicons containing M28 or L28 substitutions alone and in combination with NS5A drug resistance associated mutations (RAMs) at positions 30, 31 and 93.

**Methods:** A panel of replicons containing M28 or L28 substitutions alone, and in combination with RAMs at positions 30, 31 and 93 was constructed using H77 (GT1a) and Con1 (GT1b) NS5A sequences. NS5A drug susceptibility (fold change in  $IC_{50}$ , FC) relative to the parental reference replicon was determined using a luciferase-reporter replicon assay.

**Results:** The introduction of an L28M substitution to the GT1b NS5A sequence conferred subtle or no reductions in susceptibility to LDV, OBV and DCV (FC=1.0 to 2.5). L28M in combination with one additional NS5A RAM notably reduced susceptibility relative to the RAM alone: L28M+L31M vs. L31M (FC: LDV=165, 4.5; OBV=2.4, 0.9; DCV=6.7, 1.5), L28M+Y93H vs. Y93H (FC: LDV=12597, 368; OBV=1371, 125; DCV=188, 8). The introduction of an M28L substitution to the GT1a NS5A sequence subtly increases susceptibility to LDV, OBV and DCV (FC=0.2 to 0.8). M28L in combination with one additional RAM notably increased susceptibility compared to the RAM alone: M28L+Q30R vs. Q30R (FC: LDV=1.4, 477; OBV=14, 4115; DCV=1.2, 298), M28L+Q30H vs. Q30H (FC: LDV=0.7, 269; OBV=0.5, 4.8; DCV=1.3, 567), M28L+L31M vs. L31M (FC: LDV=2.1, 187; OBV=0.4, 2.3; DCV=1.4, 132), M28L+Y93H vs. Y93H (FC: LDV=250, 813; OBV=1770, 47582; DCV=74, 1059).

**Conclusions:** This study demonstrated that the M/L polymorphism at position 28 of NS5A differentially impacts the susceptibility of HCV GT1a and GT1b to NS5A inhibitors. The introduction of RAMs at positions 30, 31 or 93 conferred larger reductions in NS5A drug susceptibility in the context of the M28 substitution in GT1a compared to the L28 substitution in GT1b. These findings explain, in part, why GT1a viruses containing NS5A RAMs display larger reductions in NS5A drug susceptibility versus comparable GT1b viruses, which in turn may explain the less favorable treatment responses of GT1a HCV infected individuals treated with NS5A inhibitors.

**579 Genotypic and Phenotypic Characterization of Clinical HCV NS5A Drug Resistance**

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**Background:** Treatment regimens containing the NS5A inhibitors (NS5AI) ledipasvir (LDV) and ombitasvir (OBV) are approved for the treatment of HCV genotype 1 (GT1) infection. Overall, GT1b infected individuals have better treatment responses to NS5AI-containing regimens than GT1a infected individuals. Previous studies have demonstrated that the presence of NS5AI resistance-associated variants (RAVs) may impact treatment outcome. We characterized the prevalence of NS5A RAVs and NS5AI susceptibility of samples submitted for routine NS5AI resistance testing.

**Methods:** The entire NS5A region was amplified from 564 GT1 (1a=442, 1b=122) samples submitted for routine drug resistance testing. NS5A sequencing was performed using the Illumina MiSeq platform. NS5AI RAVs at positions 28, 30, 31, 58 and 93, detected at  $\geq 10\%$ , were included in the analysis. Replicons containing clinical NS5A sequences, or site-directed mutations (SDMs), were evaluated for susceptibility to LDV and OBV using a luciferase-reporter assay.

**Results:** NS5AI RAVs were identified in 113/442 (25.6%) GT1a viruses: M28A/T/V=34 (7.7%), Q30E/H/K/L/N/R/S/T/Y=53 (12.0%), L31M/V=24 (5.4%), H58D=7 (1.6%), and Y93C/F/H/N/S=38 (8.6%). Of these, 76/113 (67.2%) harbored a single RAV and 37 (32.7%) had >1 RAV. NS5A RAVs were identified in 41/122 (33.6%) GT1b viruses: L31I/M/V=18 (14.8%), Y93H=33 (27.0%). Of these, 31/41 (75.6%) harbored a single RAV and 10 (24.3%) had >1 RAV. Replicons with SDMs at position 28, 30, 31, 58 and 93, or NS5A regions from clinical samples with RAVs, exhibited large reductions in susceptibility to LDV and/or OBV. Reductions in susceptibility varied by subtype, RAV position, RAV amino acid substitution and NS5A inhibitor. GT1a viruses with RAVs generally exhibited larger reductions in NS5AI susceptibility than GT1b viruses. The GT1b Y93H mutation had a large impact on NS5AI susceptibility, conferring 367- and 125-fold reductions in LDV and OBV susceptibility, respectively.

**Conclusions:** We observed a similar prevalence of NS5AI RAVs among GT1a and GT1b viruses submitted for routine resistance testing. GT1a viruses had a broader array of RAVs and generally exhibited larger reductions in NS5AI susceptibility than GT1b viruses. RAVs among GT1b viruses were limited to positions 31 and 93. Y93H was the most common GT1b variant observed and conferred marked resistance to NS5AI. This study illustrates the potential utility of profiling NS5AI resistance in clinical treatment settings.

**580 Resistance Associated Variants: Data From the NIAID SYNERGY Trial**

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**Background:** Treatment of chronic hepatitis C (HCV) has rapidly evolved from modestly-effective interferon-based regimens to highly effective, all oral, interferon-free directly acting antiviral (DAA) therapy. While DAAs are increasingly used for treatment of HCV worldwide, the impact of emerging resistance is largely unknown. In this study, we evaluated the role of baseline, treatment-emergent and treatment-enriched resistance associated variants of HCV on sustained virologic response (SVR) at initial, short-duration, all oral DAA treatment and at re-treatment with a second DAA only regimen for patients who failed initial therapy.

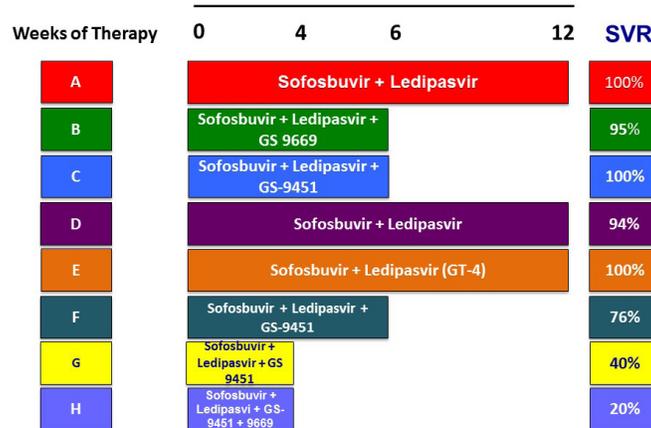
**Methods:** NIAID SYNERGY is an innovative 8 arm clinical trial that treated over 200 hepatitis C patients with varying sofosbuvir(SOF)-based DAA regimens (Figure 1). Deep sequencing was performed on plasma at baseline on all patients, at the time of relapse in patients failing therapy, and prior to retreatment, when appropriate. RAVs were classified

as baseline, treatment-enriched (those increasing in proportion to wild type from baseline to relapse) and treatment-emergent (those which were not present at baseline). Correlation analysis was performed between RAVs and sustained virologic response (SVR).

**Results:** For treatment naïve patients, pre-treatment RAVs did not predict SVR in patients who received at least 6 weeks of DAA therapy (SYNERGY A, B, C, E, F). Similarly, no association was observed between baseline RAVs and SVR among treatment-experienced patients and those with advanced liver fibrosis (SYNERGY F) treated for 6 weeks (P=0.69). For patients receiving ultra short duration therapy (4 weeks), regardless of liver fibrosis, high level RAVs (conferring > 20 fold resistance) present prior to treatment were associated with relapse (SYNERGY G/H, P=0.03). However, when these relapsed patients were retreated with SOF and ledipasvir (LDV) for 12 weeks (85% of those previously treated with LDV/SOF had high level NSSA RAVs prior to retreatment), 91% achieved SVR (SYNERGY D).

**Conclusions:** HCV NSSA RAVs do not predict relapse when using sofosbuvir-based treatment for at least 6 weeks or during re-treatment with standard 12 week regimens of LDV/SOF. RAVs do appear to impact treatment response when treating with ultra-short, 4 week duration therapies.

## SYNERGY TRIAL Design



### 581 Efficacy and Safety of Sofosbuvir-Based Regimens in Clinical Practice

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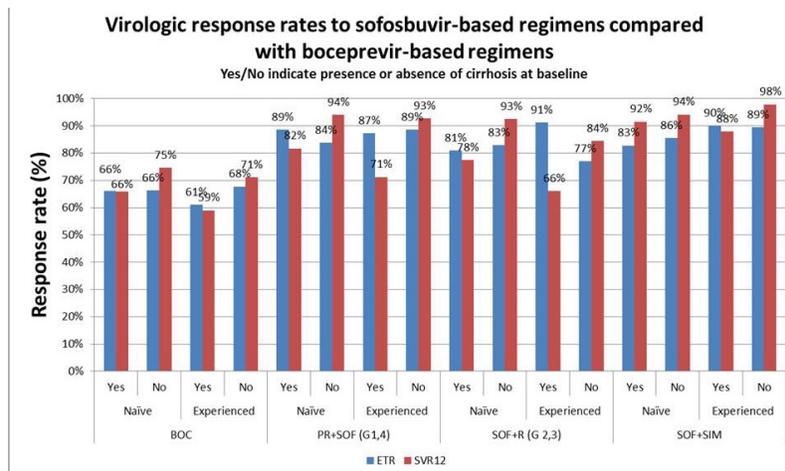
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**Background:** With recent approvals of multiple directing acting antiviral agents for treatment of HCV, it is important to validate and compare their efficacy in real-world settings. We compared the sustained virologic response rates (SVR), and hematologic toxicity among sofosbuvir vs. boceprevir treated persons.

**Methods:** We used ERCHIVES (Electronically Retrieved Cohort of HCV infected Veterans) to identify HCV infected persons initiated on sofosbuvir- or boceprevir-based regimens. We excluded persons with HIV, positive hepatitis B surface antigen, hepatocellular carcinoma and missing HCV RNA.

**Results:** Among 2,282 sofosbuvir and 1,688 boceprevir treated persons meeting the inclusion criteria, sofosbuvir-treated persons were older, more likely to be White, had higher body mass index, were more likely to have cirrhosis, diabetes, chronic kidney disease and higher HCV RNA at baseline. Overall SVR rates were 89% for the sofosbuvir group vs. 71% for the boceprevir group (P<0.001). Sofosbuvir-treated persons had higher SVR rates regardless of prior treatment status or presence of cirrhosis (P<0.001). (Table) Black race, diabetes, higher baseline HCV RNA and anemia predicted a lower SVR in boceprevir treated persons but were not associated with SVR in sofosbuvir treated persons. Presence of cirrhosis at baseline was associated with a lower SVR in both groups. Statin use was associated with a higher SVR in both groups, though the effect size was twice in the sofosbuvir group. On treatment grade 3/4 anemia (1.8% vs. 6.1%), thrombocytopenia (6.7% vs. 13.3%) and neutropenia (2.8% vs. 23.3%) were significantly less frequent among sofosbuvir- vs. boceprevir-treated persons (P<0.01 for all comparisons).

**Conclusions:** Sofosbuvir-based regimens are more often being prescribed in persons traditionally considered to have clinical factors associated with lower SVR. Despite that, sofosbuvir-based regimens in clinical practice are associated with higher SVR rates and lower rates of grade3/4 hematologic adverse events compared with boceprevir-based regimens.



**582 Response to DAA-Based Regimens in HIV-HCV Coinfected Patients in Real Life, France**

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**Background:** Several new oral direct active agent (DAA)-based regimens are available in France for HCV-HIV co-infected patients. We report on efficacy and safety of DAA-based regimen in real-life settings.

**Methods:** HIV-HCV co-infected patients enrolled in the French nationwide ANRS CO13 HEPAVIH cohort were included in this analysis if an oral DAA-based regimen without peg-interferon was initiated before March 1<sup>st</sup> 2015 (3-month regimen) or before December 1<sup>st</sup> 2014 (6-month regimen) and if the patients had sufficient follow-up data to evaluate DAA efficacy. Treatment success was defined as an undetectable HCV-RNA (<15 UI/mL) at 12 weeks or thereafter (SVR12). Patients with premature treatment stops, detectable HCV-RNA and those who died during treatment were considered as treatment failures.

**Results:** We included 171 patients in 23 clinics with a median age of 53 years (IQR: 50-56), 78% men and 98% on antiretroviral therapy. HIV viral load was <50 copies/mL in 86% and median CD4 was 520 cells/mm<sup>3</sup> (IQR: 319-730). Seventy-three percent of the patients were cirrhotic, and 70% had failed previous anti-HCV treatment. HCV genotype (Gt) distribution was as follows: Gt1, 62%; Gt2, 2%; Gt3, 14%; Gt4, 22%. Overall, we observed SVR12 in 92% of patients (95% CI: 86-97): 92% (CI: 87-97) in cirrhotic and 91% (CI: 83-99) in non-cirrhotic patients. Frequencies of DAA regimen prescribed and corresponding SVR12 are presented in table 1. In a subgroup analysis of 90 cirrhotic patients receiving a regimen without ribavirin, SVR12 for 12 or 24 weeks of treatment were 91% (CI: 71-99) and 93% (CI: 84-98), respectively. Furthermore, in cirrhotic patients receiving a DAA regimen with ribavirin for 12 and 24 weeks, SVR12 rates were 83 (CI: 36-99) and 93% (CI: 77-99), respectively. Of 14 patients with treatment failure, there were 12 relapses, one premature stop for adverse event and one death. Patients with treatment failure had a median age of 55 years (IQR: 53-58), were mainly men (86%); nine of them were Gt1, three Gt3 and two Gt4; 71% were cirrhotic. Treatment duration was 24 weeks in 8 of these patients and 12 weeks for the remaining 6 patients.

**Conclusions:** In this real-life prospective French nationwide cohort of HIV-HCV co-infected patients, oral-DAA based regimens showed high efficacy and excellent tolerability. In cirrhotic patients neither a longer duration of treatment nor the addition of ribavirin seemed to have an impact on treatment response.

**Table 1: Frequencies of prescribed DAA regimens and SVR12, ANRS CO13 HEPAVIH**

	n (%)	SVR12, % (95% CI)
SOF+DCV+/-Riba	117 (68)	93 (87-96)
SOF+LDV+/-Riba	15 (9)	87 (62-96)
SOF+Riba	26 (15)	88 (74-96)
SOF+SMV+/-Riba	13 (8)	92 (67-99)

*Legend: DCV: daclatasvir; LDV: ledipasvir; RIBA: ribavirin; SOF: sofosbuvir; SMV: simeprevir; SVR12: sustained virological response 12 weeks after treatment stop; CI: confidence interval.*

**583 Multidisciplinary Approach for the Treatment of 1155 HCV/HCV-HIV Coinfected Patients**

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**Background:** The cost of the new direct-acting antivirals (DAA) against chronic hepatitis C viral infection led the French government to condition their reimbursement to the validation of treatment by a multidisciplinary committee serving in one of the 34 national hepatitis reference centers. This organization allows data concentration on regional scales. We report data and real life efficacy of a large cohort of patients treated by DAAs in south of France.

**Methods:** Analysis included all treatments by DAAs since their approval. Clinical and biological data were collected from March 2014 to July 2015, using a computerized form respecting patient's anonymity. Physicians were to fill the form to get authorization from hepatitis reference center to start DAA-based treatment.

**Results:** 113 physicians filled out a total of 1155 forms corresponding to 809 men (70%) and 346 women (30%), aging in average 57 ± 10 and 64 ± 12 years, respectively.

508 patients (44%) were treatment-naïve and 647 (56%) were pre-treated (76% Peg-IFN+ribavirin, 16% Peg-IFN+ribavirine+protease inhibitor, and 8% DAA)

266 patients (23%) were HIV-coinfected, and 104 patients (9%) had received or were waiting for liver transplantation.

In HIV-coinfected patients, 59% had genotype (G) 1 (36% 1a, 8% 1b) and 23% had G4. In HCV mono infected patients, 68% had G1 (27% 1a, 26% 1b) and 9% had G4. G3 repartition was comparable in both populations (17% and 16%, respectively)

HIV-coinfected patients were treated at earlier fibrosis stages compared to HCV mono infected (F0-F2: 9% vs 23%; F3: 35% vs 23%; F4: 56% vs 54%, for mono and coinfected patients, respectively).

95% of genotypes 1 and 4 were treated during 12 weeks with sofosbuvir+ledipasvir (63%), sofosbuvir+simeprevir (12%), ombitasvir+paritaprevir/r+dasabuvir (10%), or sofosbuvir+ledipasvir+ribavirin (8%).

In G3, duration of treatment was 12 weeks for 75%, and 24 weeks for 25%. Among 12-week treatment, sofosbuvir+daclatasvir and sofosbuvir+ledipasvir+ribavirin accounted for 56% and 26%, respectively. In 24-week treatment, sofosbuvir+daclatasvir with or without ribavirin represented 74% of the associations.

Overall sustained virologic response (SVR) rates 4 and 12 weeks after treatment completion were 92% and 92%, respectively.

**Conclusions:** This organization permitted harmonization of treatment by DAAs, in accordance with expert's recommendations and financial issues. It also permitted to follow a cohort of patients in real life reaching SVR rates comparable to those of clinical studies.

**584 Directly Acting Agents Against HCV Results From the German Hepatitis C Cohort (GECCO)**

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**Background:** Since 2014, several directly acting agents (DAA) have been approved for therapy of chronic hepatitis C virus (HCV) infection in Europe. Henceforward, interferon-free regimens have become standard-of-care in Germany. Recently shortening therapy to 8 weeks was introduced for sofosbuvir (SOF) and ledipasvir (LDV). Here, we report data from the German hepatitis C cohort (GECCO).

**Methods:** The GECCO cohort is a multicenter cohort from 8 sites in Germany. All patients starting on the following DAAs were included in the analysis: SOF/pegylated interferon (PegIFN)/ribavirin (RBV); SOF/RBV; SOF/simeprevir (SMV); SOF/daclatasvir (DCV) +/- RBV; SOF/ledipasvir (LDV) +/-RBV; paritaprevir/ritonavir/ombitasvir/dasabuvir (3D) +/-RBV. A particular interest was given to patients treated for 8 weeks with SOF/LDV. All GECCO patients are part of the German hepatitis C registry.

**Results:** Up to now, 1157 patients were included into the cohort, 65% were male, and the mean age was 53 years. 282 (24.4%) were HIV-HCV coinfecting with a median CD4 cell count of 608/mm<sup>3</sup> (IQR 403-765). The HCV genotype (GT) distribution was as follows: 841 (73%) GT 1, 49 (4.4%) GT 2, 188 (16%) GT 3, 74 (6.3%) GT4.

The SVR12 (ITT) rates were: GT1: 89%, GT2: 87%, GT3: 81%, GT4: 79%, but only 6% of failures were related to relapse. HIVHCV-coinfecting patients responded as well as HCV mono-infected patients (83% vs 81%, p=ns).

In patients with genotype 1 and 4, SVR12 rates were significantly lower in patients with liver cirrhosis than without cirrhosis (75% vs 89%, p=0.02). 3 hepatic decompensations and 3 deaths occurred. 3 reinfections after SVR were documented within the observational period.

132 (127 GT 1 and 5 GT 4) patients were treated with SOF/LDV for 8 weeks: 19% of the patients had been unsuccessfully treated before, mainly with dual therapy consisting of pegylated interferon and ribavirin. 17% were HIV coinfecting. Post-treatment week 12 has been reached by 92 patients so far, only 2 patients relapsed (SVR=98%).

**Conclusions:** DAA-based treatments are highly effective in real-life in HCV-mono- and HIV-HCV-coinfecting patients. Relapse occurs only in 6%, and more often in patients with liver cirrhosis. All DAA combinations were generally well tolerated. In particular SOF/LDV for 8 weeks seems highly effective in selected patients in this population.

**585 High HCV Cure Rates for Drug Users Treated With DAAs at an Urban Primary Care Clinic**

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**Background:** New direct-acting antivirals promise high cure rates for the majority of patients with chronic HCV; however, it is unknown whether high cure rates will be obtained in clinical practice, particularly among persons who use drugs (PWUDs). We investigated the effectiveness of onsite treatment with care coordination for patients that access primary care at an urban federally qualified health center (FQHC), and we explored differences in HCV cure rates for PWUD versus non-PWUD.

**Methods:** Onsite HCV treatment occurred once weekly by an HCV specialist at an FQHC in the Bronx, NY. An HCV care coordinator, funded by the NYC Department of Health's Check Hep C Program, was responsible for patient scheduling, reminder calls, health education, and obtaining prior authorizations. We identified 114 patients with an HCV evaluation from January 2014- February 2015, and reviewed medical records for patients who initiated HCV treatment. Patients were categorized as PWUD if they were receiving opioid agonist therapy (OAT) or noted to have active drug use in the medical chart or urine toxicology. Chi-square testing was performed to determine differences in HCV cure between PWUD and non-PWUD.

**Results:** 114 patients were evaluated for HCV and 67 (59%) initiated HCV treatment during the study time frame. Treatment patients were mostly male (64%), Latino or African American (82%), with a median age of 60. 21% were HCV treatment experienced, 22% were HIV/HCV co-infected, and 24% had cirrhosis. Over half of the patients were PWUD (52%). 28 patients were on OAT (15 on methadone, 13 on buprenorphine) and 24 patients were actively using drugs during HCV care. The majority of the patients were genotype 1 (93%) and all were treated with sofosbuvir-based regimens. The overall HCV cure rate was 94% (63/67), and there were no differences in cure rates for PWUD (94%, 33/35) versus non-PWUD (94%, 30/32, p=0.5).

**Conclusions:** Suboptimal HCV treatment of PWUD contributes to growing HCV-related morbidity and mortality, and maintains a continued reservoir for HCV infection. Among PWUD who received care coordinator assisted sofosbuvir-based therapy at an urban FQHC, HCV cure rates were high, and no different than for non-PWUD. On-site treatment with care coordination may help to mitigate barriers to specialty care and improve HCV cure rates for PWUD. Similar treatment models should be replicated and tested throughout the 1200 FQHCs in the United States, settings that are known to serve high numbers of PWUD.

Patient Characteristics	N=67
Age (median, IQR)	60 (55, 65)
Sex	
Male	64% (43)
Race/Ethnicity	
Latino/a	48% (32)
Black/African American	34% (23)
Multiracial	9% (6)
White/Other	9% (6)
Insurance Type	
Public Insurance	94% (63)
Private	6% (4)
People Who Use Drugs	52% (35)
HIV	22% (15)
Psychiatric Disorder	43% (29)
HCV Genotypes	
1	93% (62)
2	5% (3)
3	1% (1)
4	1% (1)
Prior Treatment	
IFN/RBV	21% (14)
IFN/RBV/Telaprevir	3% (2)
Cirrhosis	24% (16)
Treatment Regimens	
Sofosbuvir/Ledipasvir	34% (23)
Sofosbuvir/Simeprevir	33% (22)
Sofosbuvir/IFN/Ribavirin	27% (18)
Sofosbuvir/Ribavirin	6% (4)
HCV Cure Rate	94% (63)
Treatment Failures	
Relapse	1
Lost Insurance, no SVR12	1
Incarcerated, no SVR12	1
SVR12 results pending	1

**586 Impact of Liver Stiffness on Response to DAA-Based HCV Therapy In Cirrhotic Patients**

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**Background:** Specific clinical trials or substudies within clinical trials in cirrhotic patients are commonly conducted, including patients with a liver stiffness (LS) above a specific threshold (usually above 12.5-14 kPa). However, the median levels of LS differ considerably between studies. In addition, rates of sustained virologic response (SVR) according to the level of LS are scarcely analyzed in trials including cirrhotic subjects, in spite of the fact that LS might have an impact on the likelihood of SVR within this subset. Therefore, the aim of this study was to evaluate the impact of the grade of liver stiffness on SVR to DAA-based therapy in patients with cirrhosis in real-life practice.

Poster Abstracts

**Methods:** This is a retrospective analysis of all patients included in two prospective cohorts (clinicaltrials.gov ID NCT02057003 and NCT02333292) who harbored cirrhosis at treatment initiation and who completed a course of therapy against HCV infection including at least one DAA. Cirrhosis was defined as a LS equal to or above 12.5 kPa.

**Results:** A total of 322 patients were included, 188 (58.4%) were HIV-coinfected and 211 (65.5%) individuals received an interferon-free regimen. The overall median liver stiffness (interquartile range) at baseline was 24 (17.3-35.1) kPa. SVR twelve weeks after the scheduled end-of-therapy (SVR12) was achieved by 101 (91%) subjects treated with interferon-free regimens and by 118 (55.9%) subjects treated with interferon-based combinations. The rates of patients who achieved SVR12 according to baseline LS and treatment regimen in an intention-to-treat analysis are shown in Figure 1. In an on-treatment analysis, 3/105 (2.9%) patients with a LS below 21 kPa versus 10/106 (9.4%) with a LS equal or above 21 kPa relapsed ( $p=0.047$ ). In a multivariate analysis adjusted for age, sex, response to previous therapy, HIV coinfection, HCV genotype and baseline viral load, a baseline LS equal or above 40 kPa was the only factor that showed a trend to a significant association with SVR12 [adjusted odds ratio (95% confidence interval): 0.457 (0.187-1.119);  $p=0.086$ ] in the subpopulation treated with interferon-based therapy.

**Conclusions:** Cirrhotic patients with higher LS respond more poorly to DAA-based therapy against chronic HCV infection in clinical practice, at least when interferon-based regimens are used. This should be considered when designing clinical trials, which should stratify cirrhotic patients according to their level of baseline LS.

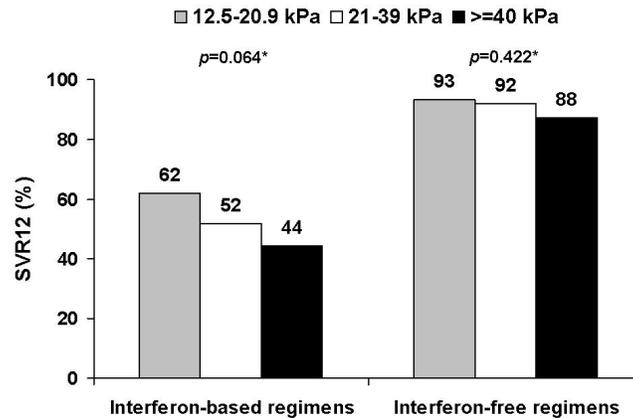


Figure 1: rates of patients who achieved sustained virologic response 12 weeks after scheduled end of therapy (SVR12) according to baseline liver stiffness and therapy; \* $p$  for linear trend.

### 587 Success of Direct-Acting Antivirals for Hepatitis C in an Indigent Population

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**Background:** Direct-acting antivirals (DAAs) have revolutionized the management of chronic hepatitis C (HCV). However, high cost of drugs and limited clinic capacities remain significant barriers to treatment. The aim of our study was to examine the impact of coordinating minimal available resources to treat HCV-infected patients at a large urban safety-net hospital.

**Methods:** Our cohort included all patients who started DAA-based HCV treatment between April 2014 and August 2015 at Parkland Memorial Hospital. Available clinic resources and staff included 0.4 FTE infectious disease/hepatology physician champions, 0.5 FTE nurse navigator, and 0.25 FTE mid-level provider that met once weekly for liver clinic.

**Results:** During a 16-month period, 184 patients were started on DAAs, representing a 280% increase in the volume of HCV treatment initiation from historical rates at Parkland. Median age was 58 [IQR 54-61], 55% were male, and our cohort was racially diverse (43% White, 37% Black, 20% Hispanic, 1% Asian). 60% of patients had cirrhosis, 22% were treatment-experienced, 13% had HIV co-infection and 5% were liver transplant recipients. Nearly half (49%) of the patients had no insurance, 30% had Medicare, 20% Medicaid, and 1% commercial insurance; uninsured and Medicaid patients initiating treatment prior to January 2015 received medications through patient assistance programs. HCV regimens included 23% sofosbuvir (SOF) ± ribavirin (RBV), 38% simeprevir (SMV) and SOF ± RBV, 38% ledipasvir (LDV) and SOF ± RBV, and 2% daclatasvir (DCV) and SOF. 56% of patients reported medication side effects including fatigue (35%), gastrointestinal upset (20%), rash (20%), mood disturbances (8%) and headache (6%). Treatment response was assessed in patients at 12 weeks after treatment completion (data available for 90 patients at time of submission). Sustained viral response (SVR12) was achieved in 76% (n=68), 7% (n=6) had viral relapse, 4% (n=4) stopped treatment due to serious adverse events and 13% (n=12) were lost to follow-up. Per protocol analysis, SVR12 was achieved in 87%.

**Conclusions:** Our model of care increased treatment initiation and achieved exceptional effectiveness in a safety-net health system using only part-time staff. Loss to follow-up remained a problem in our indigent population and additional interventions may be needed to improve outcomes. Nevertheless, access to and effectiveness of HCV DAAs is high in our safety-net hospital, suggesting favorable outcomes can be achieved in limited resource settings.

### 588 Ledipasvir/Sofosbuvir Failures in the Real World: What Patients Are at Risk?

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**Background:** Ledipasvir/sofosbuvir (LDV/SOF) fixed-dose combination resulted in sustained virologic response (SVR) rates of 93-97% for both treatment naïve and experienced genotype 1 HCV-infected patients with or without cirrhosis. As more patients are treated with this regimen, a population of treatment failures is emerging. We describe 13 patients who failed LDV/SOF despite having an undetectable viral load at the end of therapy.

**Methods:** This observational cohort study was conducted on patients who failed LDV/SOF therapy at the Mount Sinai Medical Center during the interval between March and September 2015. Data were collected on age, sex, viral genotype, presence or absence of cirrhosis, prior history of hepatocellular carcinoma (HCC), co-infection with HIV, previous therapy regimen, and duration of LDV/SOF.

**Results:** Thirteen patients failed treatment, and only one had HIV co-infection (Table). Ten (77%) were male with a mean age of 60 years (range 34-69). Most (n=11) had genotype 1 HCV, six had 1a, four had 1b, one had 1i. Eight (62%) had liver cirrhosis; among them, five had a history of hepatocellular carcinoma (HCC) that was treated by liver transplantation (n=2), by chemoembolization (n=2), or resection (n=1). Six of the 13 (46%) had a history of prior HCV treatment failure. All prior regimens were IFN/RBV based; one included telaprevir. Regarding LDV/SOF treatment, 11 (85%) had 12 weeks of therapy, one had 24 weeks, and one had eight weeks. Six relapsed by post treatment week (PTW) four, and all relapsed by PTW 13. NS3 and NS5A resistance panels have been sent on all patients and will be reported.

**Conclusions:** In clinical practice, although LDV/SOF treatment failures are rare, they are growing in number. Liver cirrhosis and HCC may be risk factors. Information about patients who fail treatment may identify groups of patients who would benefit from a longer duration of therapy or a triple-drug regimen.

Sex	Age	Genotype	Cirrhosis	HCC	HIV	Previous Rx	LDV/SOF Course (weeks)	Relapse Date
M	67	1b	Yes	No	No	IFN/RBV/TVR*2	24	PTW 8
M	69	1b	No	No	No	IFN based	12	PTW 4
M	67	1a	Yes	Yes	No	None	12	PTW 4
F	64	1b	Yes	No	No	None	12	PTW4
M	62	1b	No	No	No	IFN, and IFN/RBV	12	PTW10
M	52	1a	No	No	Yes	IFN/RBV	8	PTW 7
M	68	1a	Yes	Yes	No	None	12	PTW 4
M	56	6e	Yes	No	No	None	12	PTW 4
M	59	1l	Yes	Yes	No	None	12	PTW 6
M	34	1a	No	No	No	None	12	PTW13
F	58	1a	Yes	Yes	No	IFN/RBV*2	12	PTW 7
M	65	1a	Yes	Yes	No	None	12	PTW 2
F	56	4r	No	No	No	IFN/RBV	12	PTW 12

### 589 Dolutegravir and Outcome of HCV Therapy With Direct-Acting Antiviral Agents

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**Background:** Treatment with direct acting antivirals (DAA) is standard of care for HCV therapy in HIV-coinfected patients. Drug drug interactions are an issue with some of the antiretrovirals. Dolutegravir (DTG) is a widely used HIV integrase inhibitor with a promising drug drug interaction profile. However, use of DTG has been excluded from all phase 2 or 3 HCV/HIV-coinfection trials with DAAs.

**Methods:** Analysis of the effect of antiretroviral drug class on outcome of HCV therapy and HIV suppression in a prospective cohort of chronic hepatitis C patients with and without HIV coinfection in Germany (GECCO). Patients were treated with sofosbuvir (SOF)/PegInterferon (PegIFN)/ribavirin (RBV), SOF/daclatasvir (DCV), SOF/ledipasvir (LDV) and paritaprevir/ritonavir/ombitasvir +/- dasabuvir. Fishers exact test was used for pairwise comparison ( $p < 0.05$ ).

**Results:** For the analysis a total of 282 patients with HCV/HIV-coinfection were included. Most patients had a NRTI backbone as part of antiretroviral regimen ( $n=234$ ). Distribution of drug classes were as follows: HIV-protease inhibitor (PI)  $n=83$ , raltegravir (RAL)  $n=86$ , DTG  $n=51$ , NNRTI  $n=92$ . 33 patients had no antiretroviral therapy. SVR12 data are available from 127 patients to date. There was no difference in SVR12 comparing patients with DTG as the third agent ( $n=26/28$ , SVR=93%) to RAL ( $n=35/40$ , SVR=88%), PI ( $n=26/29$ , SVR=90%) or NNRTIs ( $n=30/30$ , SVR=100%) ( $p=n.s.$ ). No patient experienced a loss of control of HIV replication so far. No patient discontinued therapy due to adverse events.

**Conclusions:** In this prospective cohort response to HCV therapy was excellent. DTG had no effect on the outcome of HCV therapy compared to other third agents used in antiretroviral therapy. Control of HIV therapy was maintained in all antiretroviral subgroups. An update from the cohort will be presented at the meeting.

### 590 Incidence/Deaths Related to Acute Hepatitis C in Spain: Impact of HIV/HCV Coinfection

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**Background:** To estimate the incidence of acute hepatitis C (AHC)-related hospital admissions and mortality, with particular attention to human immunodeficiency virus (HIV)/hepatitis C virus (HCV) coinfection patients, as well as to analyze its trend along combination antiretroviral therapy (cART) era (1997-2012) in Spain.

**Methods:** We carried out a retrospective study on patients with an AHC diagnosis in the Spanish Minimum Basic Data Set. Patients were classified as HCV-monoinfected patients and HIV/HCV-coinfected patients. The outcome variables were: i) AHC-related hospital admission (incidence); ii) AHC-related mortality (intra-hospital mortality).

**Results:** Overall, 22684 patients were diagnosed with AHC during the study period, of which 18990 subjects were HCV-monoinfected and 3694 individuals were HIV/HCV-coinfected. The overall incidence of AHC-related hospital admission over 16 years of follow-up in HIV/HCV-coinfected patients was 1.9 per 1,000 person-year (p-y) while in HCV-monoinfected patients was 3.2 per 100,000 p-y ( $p < 0.001$ ). Besides, both groups showed a dramatic decrease in AHC-related hospital admissions (approximately 6-7 times) during study period ( $p < 0.001$ ). The overall mortality over whole follow-up in HIV/HCV-coinfected group was 1.0 per 10,000 p-y while in HCV-monoinfected group was 1.7 per 100,000 p-y ( $p < 0.001$ ). Additionally, AHC-related mortality diminished significantly in both groups (approximately 4-5 times) during study period ( $p < 0.001$ ). Overall, the adjusted likelihood of death for AHC was 2.50 (95%CI=2.07-3.02) times higher in HIV/HCV-coinfected patients than in HCV-monoinfected patients.

**Conclusions:** HIV/HCV-coinfected individuals were at higher risk for hospital admissions and deaths related to AHC during the cART era, with higher incidence and mortality than HCV-monoinfected subjects.

### 591 Hepatitis A Virus Vaccination and Immunity Among At-Risk HIV-Infected Adults

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**Background:** U.S. guidelines recommend Hepatitis A virus (HAV) vaccination for HIV-infected men who have sex with men (MSM) and persons who inject drugs (PWID) due to sexual and needle-sharing practices, respectively, that may lead to fecal-oral or percutaneous HAV transmission. However, nationally representative estimates of vaccine coverage and immunity for this population are lacking.

**Methods:** We used medical record and interview data from the 2009–2012 cycles of the Medical Monitoring Project, a nationally representative surveillance system of U.S. HIV-infected adults in care, to estimate the prevalence of HAV immunity based on documentation of vaccination (defined as receipt of at least one vaccine dose) and anti-HAV antibodies among MSM and PWID. Interviews are conducted from June–April during each MMP cycle. Medical records are abstracted 12 months back from the data of interview. We used medical records and laboratory data available since the time of HIV diagnosis to estimate prevalence of HAV immunity 1) at the end of the 12 month follow-up period (ever), 2)

at the beginning of the 12 month follow up period (baseline), and 3) among those without vaccination and/or immunity at baseline, during the 12 month follow-up period (recent). We examined factors associated with recent immunity during the 12 months of follow-up using Rao-Scott chi-square tests.

**Results:** Among 8444 MSM and PWID, 64% (95% confidence interval [CI] 59-65) overall had ever been vaccinated against or demonstrated immunity to HAV, including 64% (CI 61-68) of MSM and 72% (CI 66-78) of PWID. At baseline, 59% (CI 56-62) of MSM and 65% (CI 59-71) of PWID had evidence of immunity. Among those without baseline evidence of immunity, 14% (CI 12-16) of MSM and 19% (CI 12-25) of PWID were either vaccinated or developed immunity during 12 months of follow-up. Factors associated with not being immune during follow-up included older age, white race, HIV diagnosis > 5 years, undetectable HIV viral load, not having condomless sex and not being tested for sexually transmitted diseases (Table).

**Conclusions:** Over one third of HIV-infected MSM or PWID in care during 2009-2012 were not vaccinated and demonstrated no immunity to HAV, despite inclusion in the Advisory Committee on Immunization Practices recommendations. This analysis suggests that a sizeable portion of HIV-infected MSM and PWID are at risk and susceptible for HAV infection despite current HAV vaccination recommendations, suggesting a substantial unmet need for this population.

Characteristic	HAV vaccinated or immune during 12-month follow-up (n=485)		Not HAV vaccinated or immune during 12-month follow up (n=2983)		
	n	Weighted % (95% CI)	n	Weighted % (95% CI)	P-value
Age 18-29 years (vs. ≥30 years)	91	19.1 (15.5-22.7)	213	7.3 (5.8-8.6)	<0.0001
White, non-Hispanic (vs. any other race/ethnicity)	210	44.5 (38.9-50.2)	1542	53.6 (48.3-59.0)	0.0002
HIV viral load ≥200 copies/mL	207	42.9 (38.3-47.6)	805	29.8 (27.6-32.1)	<0.0001
HIV diagnosis within 5 years of interview	201	44.3 (39.6-48.9)	546	19.2 (17.3-21.2)	<0.0001
Engaged in condomless anal/vaginal sex	159	34.6 (29.5-39.8)	848	29.8 (25.6-32.1)	0.0424
Screened for gonorrhea, chlamydia or syphilis	372	75.5 (71.1-79.9)	1582	51.3 (48.2-54.3)	<0.001

## 592 Antiretroviral Therapy and Hepatitis Delta Replication in HIV-Coinfected Patients

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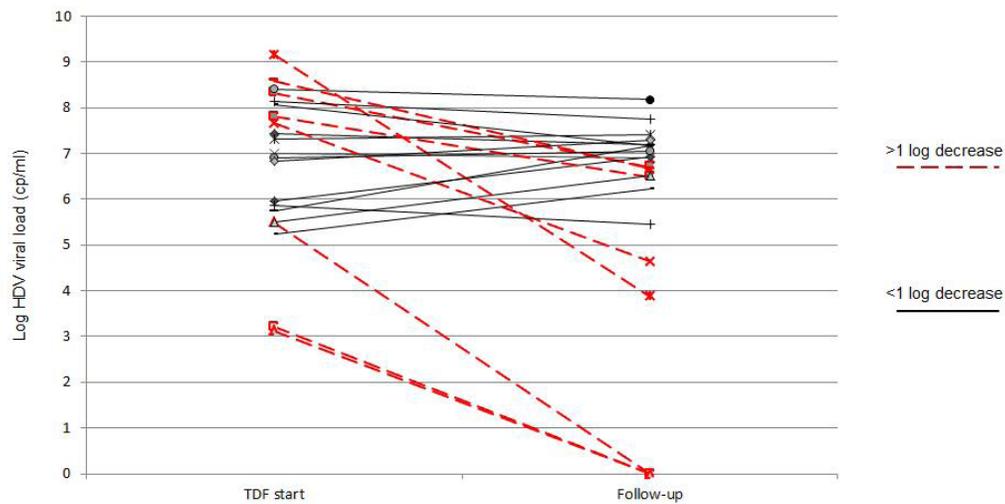
**Background:** Hepatitis delta virus (HDV) infection is present in 5-15% of HIV/hepatitis B virus (HBV)-coinfected individuals and can lead to severe liver disease. Although treatment options remain limited, tenofovir (TDF) has been associated with a reduction in HDV viral load (VL) in recent studies. We explored the virological characteristics of HDV infection in the Swiss HIV Cohort Study and describe treatment outcomes in those treated with a TDF-containing antiretroviral therapy.

**Methods:** We quantitatively assessed HDV RNA in all HIV/HBV-coinfected patients with a positive anti-HDV serology (Diasorin<sup>®</sup>) using real-time polymerase chain reaction. Demographic and clinical characteristics of individuals with and without HDV replication were compared using Chi-square and Mann-Whitney tests. Individuals with an HDV VL > limit of detection (300 cp/ml) at TDF initiation and who had at least one follow-up stored sample were included in the prospective study. HDV VL trajectories were described and the proportion of patients who reached virological suppression on TDF was evaluated.

**Results:** Among 771 HIV/HBV-coinfected patients, 139 (18%) were anti-HDV-positive. Of 122 with a sample available for amplification, 72 (59%) had a detectable HDV RNA. Median HDV VL was 4.7 log cp/ml (interquartile range [IQR]: 0-7.3 log). At time of measurement, 50% (15/30) of individuals on lamivudine, 54% (19/35) of those on TDF and 56% (38/57) of those with other or no antiretroviral treatment had detectable HDV RNA (p=0.26). Age and sex distribution, as well as CD4 cell counts, stage of HIV disease, hepatitis c antibody-positivity and ethnicity were similar between patients with detectable HDV RNA and those with undetectable levels. In 21 patients included in the prospective study, mean HDV RNA decreased from 8.1 log cp/ml before TDF initiation to 7.2 log cp/ml at the follow-up visit, with a median time of 34 months (IQR 23-35) between the two tests. Eight (38%) patients had a decrease of the HDV VL >1 log during follow-up and HDV RNA became negative in three of them (14%) (Figure).

**Conclusions:** In this nationwide cohort of HIV/HBV-coinfected individuals with positive anti-HDV serology, approximately 60% had detectable HDV RNA. TDF-containing regimens reduced HDV VL in 38% of individuals within three years of treatment initiation.

Figure: Hepatitis delta viral load trajectories during tenofovir-containing therapy

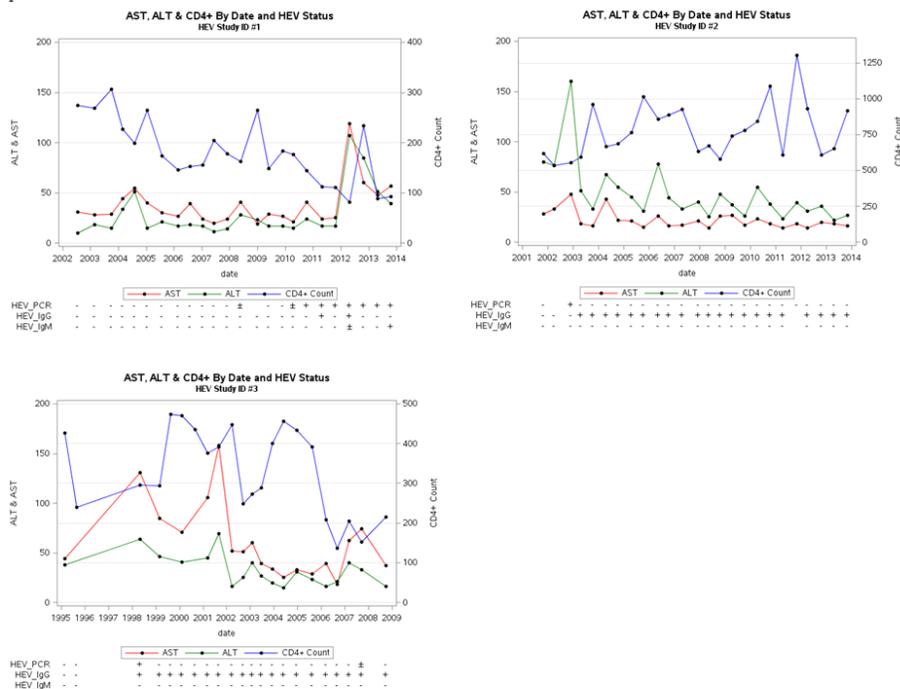


593 Acute and Chronic Hepatitis E Virus Infection in HIV-Infected United States Women

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**Background:** Exposure to hepatitis E virus (HEV) is common in the United States (US) but there are few data on prevalence of HEV/HIV co-infection in US populations.  
**Methods:** We tested 2,919 plasma samples collected from HIV-infected (HIV+) women and men enrolled in US cohort studies for HEV viremia using a high-throughput nucleic acid testing (NAT) platform. NAT+ samples were confirmed by real-time polymerase chain reaction (PCR). Samples were selected for testing primarily on the basis of biomarkers of liver disease and immune suppression.  
**Results:** Prevalence of HEV viremia was 3/2,606 and 0/313 in tested plasma samples collected from HIV+ women and men, respectively. All HEV isolates were genotype 3a. Based on follow-up testing of stored samples, one woman had chronic HEV infection for >4 years while 2 women had acute HEV detectable at only a single study visit.  
**Conclusions:** To our knowledge this is the first reported case of chronic HEV infection in an HIV+ US individual. We also confirm that chronic HEV infection can persist despite a CD4+ count >200 cells/mm<sup>3</sup>. These data suggest that HEV infection is rare in the HIV+ US population and that widespread screening for HEV in HIV+ US populations is not warranted.

Figure. Patterns of ALT, AST, CD4+ count, HEV viremia and antibodies over time for three women with confirmed HEV viremia. The ± symbol represents an inconclusive or borderline test.



## 594 High Prevalence of Anti-HEV IgG Is Not Associated With HIV Infection in Rakai, Uganda

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**Background:** Hepatitis E virus (HEV) infections are generally acute self-limiting infections, however, in select populations such as pregnant women, it can cause severe disease with a high mortality. There is limited information on the prevalence of HEV in sub-Saharan Africa, particularly in the context of HIV-infection and liver disease. We evaluated correlates of prior exposure to HEV in a rural Ugandan population with a high burden of HIV associated liver fibrosis.

**Methods:** The study population was comprised of 985 subjects from the Rakai Community Cohort Study, sampled between 2008-2009, with a mean age of 37.9 (range 18.5 to 83.0), 33% were male, and 50% HIV+. All participants were tested for HEV Immunoglobulin G (IgG) serology performed on serum or plasma using an in-house sandwich based enzyme immunoassay (EIA). All samples testing positive for anti-HEV IgG as well as all anti-HEV IgG negative samples with a CD4 cell count  $\leq$  200 cells/ $\mu$ L were tested for anti-HEV Immunoglobulin M (IgM) using an in-house class capture EIA. All samples positive for anti-HEV IgM along with a random subset of samples with a high titer of IgG anti-HEV, or CD4 count  $<$  200 cells/ $\mu$ L were tested for HEV RNA. Hepatitis B surface antigen (HBsAg), Schistosomiasis, and HIV-1 were determined using standard assays. Liver stiffness was determined using transient elastography. Prevalence risk ratios (PRR) with robust variance were estimated using modified Poisson regression to assess correlates of anti-HEV IgG seropositivity.

**Results:** The seroprevalence for anti-HEV IgG was 47%. Male gender (PRR=1.265; 95% CI: 1.088, 1.470;  $p=0.002$ ) and chronic HBV infection (PRR=1.395; 95% CI: 1.109, 1.756;  $p=0.005$ ) were associated with an increased prevalence of anti-HEV IgG (Table 1). HIV status (PRR=0.973; 95% CI: 0.852, 1.111;  $p=0.683$ ) was not associated with anti-HEV IgG. Genotype 3 HEV RNA was detected in serum from a single individual who worked in agriculture and was HIV infected.

**Conclusions:** These data suggest a large burden of exposure to HEV among HIV-infected and HIV-uninfected individuals in rural Uganda. Prior exposure to HEV was associated with male gender and chronic HBV infection. There was little evidence of active or chronic HEV infection.

**TABLE 1. Correlates of HEV IgG seropositive status**

Characteristic	Univariable PRR (95% CI)	p-value	Multivariable adjPRR (95% CI)	p-value
Male gender	1.297 (1.138, 1.479)	<0.001	1.265 (1.088, 1.470)	0.002
Age (years)	1.002 (0.995, 1.010)	0.538	1.001 (0.994, 1.009)	0.708
Fibroscan liver elastography score	0.997 (0.981, 1.014)	0.747		
Agricultural Occupation	0.995 (0.846, 1.170)	0.948		
Lifetime Occupational Fisherman	0.708 (0.228, 2.200)	0.550		
Schistosomias	1.095 (0.907, 1.323)	0.345		
Current alcohol use	1.220 (1.050, 1.418)	0.009	1.046 (0.879, 1.245)	0.613
HBsAg	1.443 (1.134, 1.836)	0.003	1.395 (1.109, 1.756)	0.005
HIV positive	0.973 (0.852, 1.111)	0.683		

PRR estimates, 95% CIs, and p-values determined using Poisson logistic regression with robust variance

## 595 Oxylinp Metabolites As Markers of Inflammation and Liver Disease in HCV Infection

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**Background:** Chronic hepatitis C virus (HCV) infection is associated with metabolic effects including dyslipidemia and insulin resistance (IR), which contributes to disease progression and affects response to therapy. We have previously shown that sofosbuvir (SOF)/ribavirin (RBV) mediated clearance of HCV results in changes in lipid metabolic pathways implicating a direct effect of HCV replication on lipid homeostasis. Oxylinps are bioactive metabolites derived from the oxygenation of polyunsaturated fatty acids and contribute to inflammatory responses associated with IR and cardiovascular disease. In this study, we investigated changes in oxylinps associated with the stage of liver disease and treatment outcomes in chronic HCV patients.

**Methods:** Twenty HCV GT1 subjects (n=10 SVR, n=10 relapse) treated with SOF/RBV for 24 weeks (SPARE HCV trial), were selected for this analysis. A targeted plasma oxylinp analysis (N=111 metabolites, high performance liquid chromatography-tandem mass spectrometry) was conducted on plasma samples at baseline and weeks 4 and 20 on therapy. Clinical data (outcome and histology) were available from the SPARE trial. Paired and unpaired t-tests were used to compare between groups.

**Results:** Our cohort was predominantly male (70%), mean age 52 years, and 80% black. There were no differences in baseline characteristics between SVR and relapsers. Several oxylinp metabolites differed by study outcome result (SVR vs relapse), and fibrosis stage (Table). Pooling all timepoints, subjects with relapse had higher oxylinp levels, whether pro- or anti-inflammatory, suggesting greater immune dysregulation. Treatment related clearance (SVR) resulted in a decrease in the pro-/anti-inflammatory ratio of key oxylinp metabolites (5-HETE/(11,12-DiHETE+14,15-DiHETE) ( $p=0.034$ ).

**Conclusions:** For the first time, we demonstrate differential expression of pro- and anti-inflammatory lipid metabolites in HCV-infected subjects treated with SOF/RBV, which differ by treatment response and fibrosis stage. Furthermore, clearance of HCV appears to result in improved immune dysregulation. In a clinical cohort of HIV/HCV patients we have identified oxylinp metabolites are associated with stage of liver disease and correlated these metabolites with serum markers of chronic inflammation. These preliminary data further support the role of HCV in modulation of host lipid homeostasis and identifies intermediary lipidomic pathways leading to the chronic inflammation seen with chronic hepatitis C infection.

**Table: Eicosanoid Lipid Mediators Associated with Treatment Outcome and Stage of Liver Fibrosis**

Inflammatory Activity	Lipid Mediator	Biogenesis Pathway	P-value	FDR Q-value
SVR versus relapse				
Anti	11-HETE	LOX	0.0183	0.1314
Pro	5-HETE	LOX	0.0239	0.1314
Pro	9-HETE	LOX	0.0158	0.1314
Anti	11, 12-DiHETrE	CYP-450	0.0073	0.1314
ISHAK histopathology fibrosis score (0-1 versus 2-4)				
Pro	5-HETE	LOX	0.0003	0.0072
Anti	11, 12-DiHETrE	CYP-450	0.0023	0.0184
Anti	14, 15-DiHETrE	CYP-450	0.0020	0.0184

596 WITHDRAWN

**597 Understanding the Relative Contributions of IDU and HCV on Systemic Immune Activation**

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**Background:** Persistent immune activation is associated with a variety of adverse clinical outcomes. People who inject drugs (PWID) have high levels of immune activation in blood and mucosal tissues; however, the relative contributions of chronic HCV infection, highly prevalent among PWIDs, and the non-sterile injection of illicit drugs have remained obscure.

**Methods:** We recruited (N=48 for each group): 1) active injectors of heroin 2) individuals who ceased injecting heroin for 1-2 months 3) individuals who ceased injecting heroin for 3-4 months 4) healthy non-injecting volunteers. Soluble (including sCD14, hs-CRP, TNF-a, IFN-g, IL-10, MIP-1a) and cell associated (CD38+HLA-DR+ CD4 and CD8+ T cells) markers of immune activation were quantified. Mixed-effects regression models with random intercepts to account for participation in more than one group were used to compare groups on markers of immune activation.

**Results:** Participant characteristics are shown in Table 1 below.

Levels of IL-12p70, IL-15, IL-1b, IL-2, IL-4, and IL-6 determined by multiplex ELISA were at or below the level of detection in 50% or more of the active injectors and were not analyzed. Mean levels of selected markers of systemic and cellular immune activation are shown in Table 2 below.

Participants in Groups 2 and 3 had statistically significantly lower levels of TNF-a and % CD4+ and CD8+ CD38+/HLA-DR+ T cells compared to actively injecting Group 1 subjects only if HCV infection was spontaneously controlled or if subjects were HCV uninfected (HCV-aviremic). sCD14 levels in HCV-aviremic Group 3 subjects were significantly lower than in aviremic Group 1 subjects and comparable to Group 4. Additionally, hs-CRP levels were significantly lower in Group 2 but not in Group 3 compared to Group 1 subjects. In contrast, in HCV-viremic subjects, the above parameters were not significantly different between the groups and were significantly higher than in the healthy non-injecting volunteers. Levels of IFN-g, IL-10, and MIP-1a were comparable across Groups 1, 2, and 3 independent of the presence/absence of viremia.

**Conclusions:** Active IDU and HCV viremia are associated with persistent immune activation. Select markers of immune activation are significantly lower among the HCV-aviremic who cease injecting but not in those who are HCV viremic. These findings may have public health consequences. Aggressive treatment of HCV infection as well as enhanced harm reduction efforts should converge to optimize long-term outcomes.

Week 48 Efficacy, including subgroup responses by screening HIV RNA			
Endpoint	DOR <sup>†</sup> (N=108)	EFV <sup>†</sup> (N=108)	Difference in % Response [DOR-EFV] (95% CI)
% with HIV RNA <40 copies/mL			
Overall <sup>‡</sup>	77.8	78.7	-1.1 (-12.2, 10.0)
Screening HIV RNA ≤100K <sup>§</sup> (n=67, 62)	86.6	87.1	-0.5 (-12.7, 11.9)
Screening HIV RNA >100K <sup>§</sup> (n=35, 37)	74.3	83.8	-9.5 (-28.7, 9.7)
% with HIV RNA <200 copies/mL			
Overall <sup>‡</sup>	85.2	84.3	0.9 (-8.9, 10.8)
Screening HIV RNA ≤100K <sup>§</sup> (n=67, 62)	89.6	91.9	-2.4 (-13.2, 8.6)
Screening HIV RNA >100K <sup>§</sup> (n=35, 37)	91.4	91.9	-0.5 (-15.6, 14.2)
Mean change in CD4 count (cells/mm <sup>3</sup> ) <sup>§</sup>	+192	+195	-3 (-47, 41)
Week 48 Clinical Adverse Event (AE) Summary			
% of Patients with:	DOR <sup>†</sup> (N=108)	EFV <sup>†</sup> (N=108)	Difference in % Response [DOR-EFV] (95% CI)
One or more AEs	87.0	88.9	-1.9 (-10.9, 7.1)
Drug-related AEs	31.5	56.5	-25.0 (-37.3, -11.8)
Serious AE	6.5	8.3	-1.9 (-9.5, 5.6)
Serious and drug-related AEs	0.0	1.9	-1.9 (-6.5, 1.6)
Discontinuation due to AEs	2.8	5.6	-2.8 (-9.2, 3.0)
<sup>†</sup> with TDF/FTC. <sup>‡</sup> Non-completer=Failure (NC=F) approach to missing data; 95% CI based on Miettinen and Nurminen's method with weights proportional to the size of each stratum. <sup>§</sup> Observed Failure (OF) approach to missing data.			

### 598 Mucosal-Associated Invariant T (MAIT) Cells in HIV/HCV Coinfection

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**Background:** MAIT cells play a crucial role in the innate immunity. A substantial reduction in MAIT cells have been described in untreated HIV, that is not restored by cART, but little is known about the relationship of MAIT cells and HCV. We investigated the role of MAIT cells in the setting of HIV/HCV co-infection and the impact of anti-HCV therapy in a cohort of cART-treated HCV/HIV patients (pts).

**Methods:** We enrolled 23 HIV/HCV+ cART treated pts with HIV-RNA<40cp/ml and median CD4 T-cell count of 527 cell/mmc (IQR 409-780); 15/23 started interferon-based anti-HCV regimen and 13 healthy controls (HC). Pts were stratified according to anti-HCV therapy outcome in sustained virological response (SVR) and non response (NR-relapser/non responder). Flow cytometry analyses were performed (CD3/CD4/CD8/CD161/TCRVa7.2/CD39/CD69/IL18R/PD1/Granzyme B/Perforin). Severity of liver fibrosis was measured by FIB-4 score. Statistical analyses: Mann-Whitney, Wilcoxon, Spearman tests.

**Results:** HIV/HCV pts displayed a significant contraction of total, CD8 and double-negative (DN) MAIT cells, compared to HC (Table 1). All MAIT cell subsets of HIV/HCV pts showed a trend towards higher CD39 expression, especially within the CD8 subset, and a lower CD69 expression (Table 1). No differences in PD-1, granzyme B and perforin expression were found.

Following anti-HCV therapy, we found a non-significant increase in total (0.48vs0.63%), CD8+ (0.59vs0.76%) and DN (0.61vs1.19%) MAIT cells. Therapy and treatment outcome did not impact on MAIT cell frequency nor function. Interestingly, HIV/HCV pts displayed a positive correlation between CD8+MAIT frequency and AST (p=.004;r=0.62) and ALT (p=.01;r=0.56). In addition, CD69+CD8+MAIT directly correlated with HCV-RNA (p=.001;r=0.75) and inversely with the duration of both HIV (p=.002;r=-0.72) and HCV (p=.004;r=-0.7) infection. No significant correlation between the frequency of MAIT cells and severity of liver fibrosis was found.

**Conclusions:** In HIV/HCV co-infected patients we show a profound contraction of the circulating MAIT cell compartment that is not recovered by anti-HCV treatment and virologically suppressive cART. The correlation between MAIT, HCV and hepatic necrosis enzymes, suggests an association between HCV-driven liver damage and the homeostasis of circulating MAIT cells via mechanisms that still need to be elucidated.

**Table 1. Phenotypes of circulating Mucosal-Associated Invariant T (MAIT) cells in healthy controls and in HIV/HCV co-infected subjects**

	Healthy Controls (n=11)	HIV/HCV+ patients (n=23)	P
MAIT CD3+ %	1.88 (0.41-2.64)	0.48 (0.19-1.95)	.027
MAITCD8+ %	1.93 (0.98-4.82)	0.59 (0.22-1.06)	.003
MAITCD4-CD8- %	10.50 (1.84-19.85)	0.61 (0.44-2.09)	.003
MAITCD3+CD69+ %	11.80 (9.82-24)	7 (2.91-14.90)	.008
MAITCD8+ CD69+%	11.10 (9.63-22.95)	5.19 (1.02-12.27)	.009
MAITCD4-CD8-CD69+ %	17.40 (11.30-28.60)	10 (2.52-31.50)	.027
MAITCD8+ CD39+%	0.99 (0.53-2.17)	1.83 (0.63-10.80)	.057

Note: All data are presented as median (Interquartile range –IQR). Statistical analysis: Mann-Whitney U test  
MAIT cells are defined as CD3++Va7.2+

**599 Hepatic & Peripheral Responses to 2 Different DAA Regimens in HIV/HCV Coinfection**

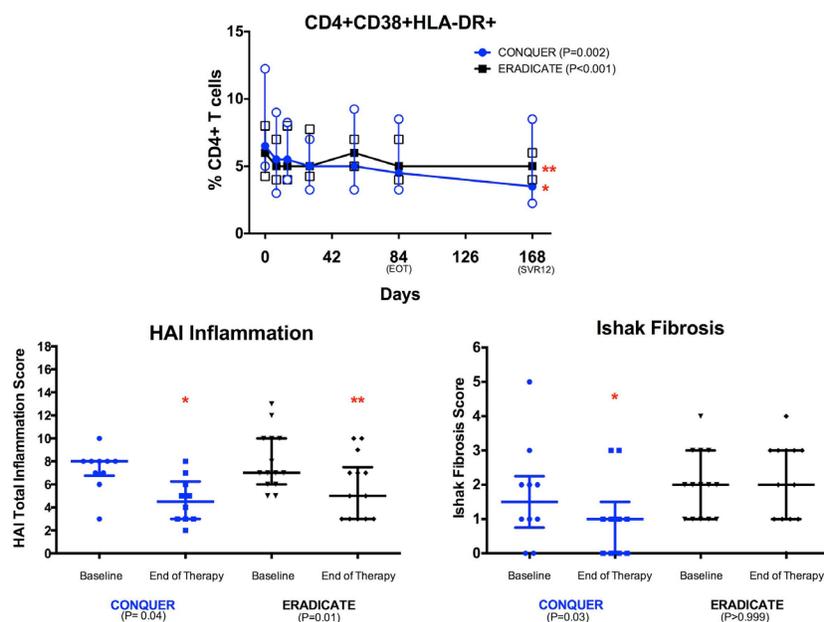
**Louisa C. Howard<sup>1</sup>**; Anita Kohli<sup>2</sup>; Julia Purdy<sup>1</sup>; Elana S. Rosenthal<sup>1</sup>; Shikha Shrivastava<sup>3</sup>; Bhawna Poonia<sup>3</sup>; Henry Masur<sup>1</sup>; Shyam Kottlil<sup>4</sup>; Eleanor M. Wilson<sup>5</sup>  
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**Background:** While new combination directly acting antiviral (DAA) regimens for treatment of HCV are equally effective in HIV/HCV co-infected and HCV mono-infected patients, HIV/HCV co-infected subjects are known to have higher levels of immune activation and accelerated fibrogenesis. We compare hepatic and peripheral cellular response in HIV/HCV co-infected subjects successfully treated for HCV with one of two DAA-only regimens.

**Methods:** Two prospective, single center, phase II studies were conducted at the NIH Clinical Center using DAA only, 12-week treatment regimens (either sofosbuvir/ledipasvir in ERADICATE (NCT01878799) or daclatasvir/asunaprevir/BMS-791325 in CONQUER (NCT02124044)) in HIV/HCV genotype 1 co-infected subjects. Paired pre/post-treatment liver biopsies were obtained on a subset of patients and evaluated by a single pathologist using Knodell-HAI and Ishak scoring systems. We also assessed changes in peripheral immune activation markers on T cell subsets (CD38, HLA DR, CD25) before, during, and after therapy. Median values are reported and comparisons were made using Wilcoxon Rank tests.

**Results:** Subjects in ERADICATE (n=36) and CONQUER (n=12) were predominately male (83%, 58%) and black (86%, 50%), with median age of 58 and 52 years, respectively. Both cohorts demonstrated early liver disease with an HAI fibrosis score <2 in 75% and 67%, respectively. While all ERADICATE subjects were treatment naïve, 58% of CONQUER subjects were treatment experienced. In both studies, there was a significant decline in both the percentage of peripheral T cells with activated profile (CD4+ CD38+ HLA DR+, 17% in ERADICATE, P<0.001, and 42% in CONQUER, P=0.002) and HAI inflammation score in the liver (median decline in CONQUER patients was 3.5 ± 2.7 (P=0.04), compared to 1.5 ± 2.8 in ERADICATE (P=0.01)) by the end of therapy. Interestingly, we also observed a statistically significant decline in Ishak fibrosis score in CONQUER (P=0.03), not observed in ERADICATE (P>0.99).

**Conclusions:** Effective therapy with either DAA regimen is associated with a substantial decline in peripheral immune activation markers and intrahepatic cellular response in HIV/HCV co-infected subjects. In this analysis, sustained virologic response after treatment with daclatasvir/asunaprevir/BMS-791325, a three DAA regimen, was also associated with regression of liver fibrosis within 3 months, which is encouraging for better long-term outcomes in this patient population.



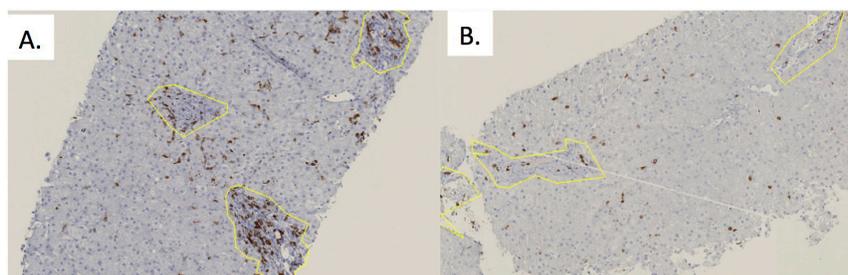
**600 Intrahepatic Immune Composition Changes Markedly With Interferon-Free HCV Treatment**Cody Orr<sup>1</sup>; Johannes Aartun<sup>1</sup>; Henry Masur<sup>2</sup>; Shyam Kottilil<sup>3</sup>; **Eric G. Meissner**<sup>1</sup><sup>1</sup>Med Univ of South Carolina, Charleston, SC, USA; <sup>2</sup>NIH, Bethesda, MD, USA; <sup>3</sup>Univ of Maryland Med Cntr, Baltimore, MD, USA

**Background:** Approximately 150 million people worldwide are chronically infected with hepatitis C virus (HCV), which can progress to end-stage liver disease and hepatocellular carcinoma. All oral, interferon-free treatments composed of directly activating antiviral (DAA) agents are now available. Rates of sustained virologic response (SVR) are markedly improved with DAA-based treatment, although virologic relapse after treatment still occurs in a subset of patients. Mechanisms of treatment relapse and any role of host immunity in modulating relapse risk are unclear.

**Methods:** To understand how host immunity is impacted by treatment, and to explore immune differences that may reflect or impact outcome, we performed immunohistochemistry (IHC) on paired pre- and post-treatment liver biopsies from subjects treated in the NIH SPARE trial with sofosbuvir and ribavirin for 24 weeks who achieved SVR (n=9) or relapsed (n=4). Single-color IHC was performed with antibodies specific for CD4, CD8, CD56, CD68, CD20, TIA-1, and alpha-smooth muscle actin (ASMA) to delineate cellular populations. Image acquisition was performed on stained liver sections, followed by manual annotation of parenchymal and portal triad regions. Quantitative image analysis was performed using Visiopharm software to enumerate total pixel and cellular counts. Changes over the course of treatment were examined, and differences based on treatment outcome were explored.

**Results:** CD8+ cells decreased markedly after DAA therapy in both parenchymal and portal triad regions irrespective of treatment outcome, similar to total HAI inflammatory score. CD20+ signal decreased in parenchymal areas while CD4+ signal decreased in portal triads. Other markers (CD56, TIA-1, KP-1, ASMA) did not change during treatment or differ by outcome. Interestingly, we observed a trend (p=0.12) toward lower pre-treatment CD4+ signal in portal triad regions of subjects who eventually relapsed compared to subjects who achieved SVR. Further delineation of CD4+ cell subtypes (i.e. T-regs, T-helper cells, etc.) was not pursued due to restricted sample availability.

**Conclusions:** These data indicate that DAA therapy results in decreased hepatic markers of adaptive immune cells (CD8, CD4, CD20) while markers of innate immune cells (CD68, CD56), activated stellate cells (ASMA), and apoptosis (TIA-1) did not change. We describe for the first time, the nature of regression of intrahepatic chronic inflammatory response in HCV-patients receiving DAA therapy.

**Figure**

Legend: Immunohistochemistry using anti-CD8 antibody pre (A) and post (B) treatment on a representative liver section. Areas annotated as portal triads are denoted by yellow shapes.

**601 GWAS of Relapse in HIV-1/HCV Coinfected Patients Treated With LDV/SOF in ION-4**Sarah E. Kleinstejn<sup>1</sup>; Patrick R. Shea<sup>1</sup>; Luisa M. Stamm<sup>2</sup>; Jenny C. Yang<sup>2</sup>; Philip S. Pang<sup>2</sup>; Mani Subramanian<sup>2</sup>; John G. McHutchison<sup>2</sup>; Mark Sulkowski<sup>3</sup>; David V. Goldstein<sup>1</sup>; Susanna Naggie<sup>4</sup><sup>1</sup>Columbia Univ, New York, NY, USA; <sup>2</sup>Gilead Scis, Inc, Foster City, CA, USA; <sup>3</sup>Johns Hopkins Univ, Baltimore, MD, USA; <sup>4</sup>Duke Univ Sch of Med, Durham, NC, USA

**Background:** A fixed dose combination of ledipasvir (LDV)/sofosbuvir (SOF) for 12 weeks provided high rates of sustained virologic response (SVR) in patients co-infected with HIV-1 and HCV genotype 1 or 4. In the ION-4 trial (N=335), 10 subjects had HCV relapse. All 10 subjects were African American (AA), and the majority (80%) were on efavirenz (EFV). A genome-wide association study was conducted to identify common host genetic determinants of HCV relapse after LDV/SOF therapy in HIV/HCV individuals on antiretrovirals (ARVs).

**Methods:** All subjects consented for genetic testing (N=275) were genotyped on the Illumina HumanCore chip (~300,000 SNPs). After data quality control, 273 samples remained (8 relapse, 265 SVR). Of these, 87 samples were from AA subjects (8 relapse, 79 SVR), and 40 AA subjects were on EFV, including 6 relapse and 34 SVR. Three analyses were performed under a logistic regression model: (1) all subjects, (2) AA subjects only, and (3) AA subjects on EFV. Targeted analyses limited to drug metabolism genes were also performed. Population ancestry was corrected for by inclusion of significant ancestry principal components. The threshold for statistical significance was Bonferroni-corrected.

**Results:** No significant genome-wide associations were observed. Although non-significant, in the AA only analysis, two SNPs were in genes of potential biological relevance to treatment failure. One SNP, rs12040970 (OR=10.97, p=6.28e-4), is intergenic, ~5kb upstream of *EFNA1*, which has been linked to the GGT pathway. While rs17514846 (OR=17.74, p=0.001) is intronic in *FURIN*, which is induced by HCV. Both targeted analyses (see **Table**) revealed the same top 3 non-coding SNPs in *CYP3A4*, which has been reported to interact with EFV. Further, SNPs in *NR1I2*, a transcriptional regulator of *CYP3A4* expression, also dominated the drug metabolism analysis. Several of these markers have plausible minor allele frequencies that are exceedingly rare in Caucasians and several fold more common among Africans.

**Conclusions:** This study provides a comprehensive genome-wide investigation of LDV/SOF treatment relapse in HIV/HCV patients on ARVs. After accounting for multiple testing, there were no significant associations with HCV relapse. Although targeted analyses reporting on drug metabolism did show evidence of a modest association among the subset of host genes reported to interact with EFV, LDV exposure did not differ between patients who relapsed and achieved SVR.

Rank	SNP	Chr	Type	Closest Gene	Function	OR (P value)
<b>Top 5 SNPs among the Gene Ontology "Drug Metabolism" Category</b>						
1	rs28988606	7	3'-UTR	CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4; monooxygenase involved in many drug metabolism reactions	15.56 (3.776E-3)
2	rs28988603	7	3'-UTR	CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4; monooxygenase involved in many drug metabolism reactions	15.56 (3.776E-3)
3	rs7801671	7	intronic	CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4; monooxygenase involved in many drug metabolism reactions	3.91 (9.713E-3)
4	rs8330	2	3'UTR	UGT1A*	UDP glucuronosyltransferase 1 family	0.20 (0.03441)
5	rs76580593	3	3'UTR	NR12	nuclear receptor superfamily; transcriptional regulator of the cytochrome P450 gene CYP3A4	6.80 (0.05672)
<b>Top 5 SNPs among Genes in the Ingenuity Literature Database with Reported Interaction with EFV</b>						
1	rs28988606	7	3'-UTR	CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4; monooxygenase involved in many drug metabolism reactions	15.56 (3.776E-3)
2	rs28988603	7	3'-UTR	CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4; monooxygenase involved in many drug metabolism reactions	15.56 (3.776E-3)
3	rs7801671	7	intronic	CYP3A4	cytochrome P450, family 3, subfamily A, polypeptide 4; monooxygenase involved in many drug metabolism reactions	3.91 (9.713E-3)
4	rs13137622	4	intronic	ABC62	ATP-binding cassette, sub-family G (WHITE), member 2; xenobiotic transporter	0.16 (0.01376)
5	rs2904185	4	intronic	ABC62	ATP-binding cassette, sub-family G (WHITE), member 2; xenobiotic transporter	7.44 (0.0141)

## 602 Pacbio Sequencing of the HCV Envelope: From Early Acute to Chronic Infection

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**Background:** Deep sequencing has revolutionized the study of genetically variable RNA virus populations, but for phylogenetic and evolutionary analyses longer sequence length and low error rates are desired. The Pacific Biosciences Single Molecule, Real Time (SMRT) sequencing approach provides long reads and circular consensus sequences (CCS). We investigated using Pacbio sequencing the evolution of the hepatitis C virus (HCV) envelope region (E1E2, 1680 bp) in five subjects with incident infection. The four subjects who progressed to chronicity were followed for a median time of 10 years.

**Methods:** The five subjects were infected with closely related HCV genotype 4d variants and coinfecting with HIV-1. Four subjects were men who have sex with men (MSM) and one subject was the female partner of one of them. Fifty samples, collected between 2001-2013, were SMRT sequenced. Prior to phylogenetic analysis, insertions with respect to a sample-specific reference sequence were removed. Neighbor Joining trees were constructed using MEGA. Pairwise distances were calculated using Ape (R package) and the molecular clock was assessed using a root-to-tip analysis (Path-O-Gen).

**Results:** The sequencing error at 7 CCS full passes was 0.37% with insertions as the main type of error (0.24%), followed by deletions (0.11%). Mismatches were surprisingly low (0.02%). The median coverage at 7 full passes was 612 CCS reads/sample (range 149-935). NJ phylogenies revealed a close phylogenetic relationship between reads from the four MSM at early time points, and evidence for transmission from one MSM to the female subject. Intra-host phylogenies of reads sampled early during infection suggest that a single founder virus established infection in all five subjects. This finding was supported by the low genetic diversity observed at these early time points. The increase in diversity coincided with progression to chronicity and the emergence of multiple co-existing lineages. Changes in the genetic diversity during chronic infection corresponded with a non-ladder like phylogeny. A strong molecular clock signal was observed and the time to the most recent common ancestor corresponded well with the estimated HCV infection date.

**Conclusions:** SMRT sequencing is able to combine great coverage with long reads and can provide rich insights into HCV dynamics from transmission bottlenecks to long-term chronic infection.

## 603 Frequency and Predictors of Liver Function Impairment in Cirrhotic Patients Treated With DAAs

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**Background:** Improvements in biological markers of liver function have been found in clinical trials in patients with advanced liver disease receiving DAA-based therapy. However, a certain proportion of patients experience a worsening in liver function. The frequency of liver function impairment, as well as the predictors thereof in these patients remain unclear. This study was aimed to clarify these issues in subjects with cirrhosis achieving SVR after DAA-based therapy in a real life setting.

**Methods:** Patients who received therapy against HCV infection including at least one DAA were selected for this international, multicohort retrospective analysis if they met the following criteria: 1) Cirrhosis at baseline; 2) Having reached sustained virologic response 12 weeks after scheduled end of therapy (SVR12); 3) Available measurements of CPT and MELD scores at baseline and the SVR12 evaluation time point. Changes in liver function parameters from baseline to SVR12 time-points were evaluated.

**Results:** One-hundred and three patients were included. Fifty-nine (57%) patients were HIV-infected. Median (Q1-Q3) baseline LS was 22 (17-33) kPa, 88 (85%) patients were at CPT class A and 13 (13%) subjects at B. Baseline total bilirubin was 0.8 mg/dL versus 0.67 mg/dL at SVR12 ( $p=5.4 \times 10^{-7}$ ). Changes in MELD and CPT scores from baseline to the SVR12 evaluation time-point are depicted in Figure 1. Subjects in whom CPT increased  $\geq 1$  and/or MELD raised  $\geq 2$  points were categorized a progressors ( $n=14$ ). Liver function parameters

in progressors versus non-progressors were: CPT score 6 versus 5 ( $p=0.379$ ); MELD score 9 versus 8 ( $p=0.032$ ); total bilirubin 1.33 mg/dL versus 0.8 mg/dL ( $p=0.006$ ); INR 1.25 versus 1.09 ( $p=0.004$ ); albumin 3.5 g/dL versus 4 g/dL ( $p=0.002$ ) and platelets 68000/L versus 121500/L ( $p=0.001$ ). Only one out of 44 (2%) patient with  $>100000$  platelets/L and total bilirubin  $<1$  mg/dL at baseline was progressor, being the negative predictive value of the combination of these two parameters for progression 0.98.

**Conclusions:** Although most cirrhotics on DAA-based therapy experience an improvement in liver function tests in the short term, MELD and/or CPT scores worsen in around one third of them. Those with a poorer baseline liver function are more prone to suffer from impairment, and should be carefully monitored while on therapy. The combination of plasma bilirubin and platelet count may accurately predict those who will not have a worsening in liver function scores.

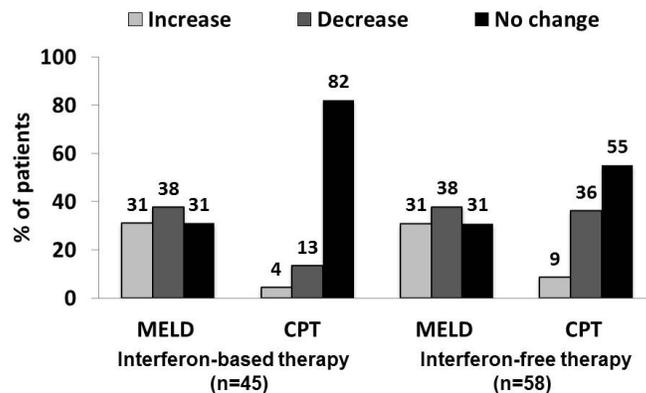


Figure 1: Changes in MELD and Child-Pugh-Turcotte (CPT) scores from baseline to week 12 after scheduled end of therapy according to treatment regimen.

#### 604 Liver Cancer After Hep C Cure: Less Cirrhosis and Less Liver Fat Than Expected

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**Background:** Patients who achieve a sustained virological response (SVR) to treatment for chronic hepatitis C (HCV) remain at risk for hepatocellular carcinoma (HCC). **Aims:** To characterize patients who developed HCC more than 12 mo after achieving SVR.

**Methods:** Demographic, laboratory, and HCC stage and treatment data on 41 cases were obtained from medical records (1/2010-12/2014). Histology of H&E stained non-tumor tissue was evaluated by blinded review using the Knodell system for necroinflammation (HAI scale, 0-18) and the Scheuer system for stage (scale, 0-4); the specimens were from 17 patients treated with resection or liver transplantation. Patients with HCC detected through surveillance were compared to those diagnosed when symptomatic.

**Results:** HCC was diagnosed a median of 6 yr post-SVR when patients were a median age of 58 yr. Only 71% of the 41 cases had cirrhosis, and only 56% had AFP  $>10$  ng/mL, 83% were male, and 88% were non-Asian. Comorbidities included diabetes (29%), HBV (2%), and HIV (12%). Median tumor size was 2.8 cm (range, 0.8-18.2), 83% had a single lesion on imaging and 51% had vascular invasion. Median laboratory values at the time of HCC diagnosis indicated that liver function was generally well-preserved: albumin 4.2 g/dL (2.1-5.0), platelets  $148 \times 10^3$  cells/ $\mu$ L (48-446), and total bilirubin 1.3 mg/dL (0.2-8.8). HCC was diagnosed via surveillance (imaging and/or AFP) in 27 patients. Among the surveillance group, 85% were within Milan criteria versus 23% in the symptomatic group ( $p<0.01$ ). Survival at 1 and 3 yr were 91% and 60% in the surveillance group and 62% and 62% in the symptomatic group ( $p=0.3$ ). The histology study yielded important insights: Only 10/17 (59%) of the (non-tumor) tissues had stage 4 cirrhosis, whereas 6/17 (35%) had stage 0-2 fibrosis. Median HAI was 4 (range, 0-8), indicating that most had mild necroinflammation. Periportal hepatitis was present in 59%, only two had (mild) steatosis and three had steatohepatitis (5/17 = 29%).

**Conclusions:** Many patients lacked traditional HCC risk factors: 44% had AFP  $<10$  ng/mL and 40% of 17 histological specimens were non-cirrhotic and 70% lacked liver fat (steatosis/steatohepatitis). Future research should seek novel indicators of persistent HCC risk after HCV cure, e.g., somatic DNA mutations and epigenetic changes. This study did not show a survival benefit to screening; however, a higher percentage of patients diagnosed through surveillance were within Milan criteria.

#### 605 Residual Risk of Disease Progression After Hepatitis C Cure in HIV-HCV Patients

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**Background:** Incidence rates of hepatic events and mortality after cure of HCV in HIV-HCV co-infected patients are not yet documented in real-life settings.

**Methods:** We included HIV-HCV co-infected patients with a sustained virological response (SVR24 – negative HCV-RNA  $\geq 6$  months after anti-HCV treatment completion) within the French nationwide ANRS CO13 HEPAVIH cohort. Hepatic events were defined as ascites, digestive hemorrhage, bilirubin level  $>2.05$  mg/mL, hepatic encephalopathy and hepatocellular carcinoma (HCC). All events were validated by a medical committee. We estimated incidence rates (95% confidence intervals [CI]) and mortality rates overall, and separately in patients with severe (F3/F4) and mild/no fibrosis (F0/F1/F2). Crude incidence risk ratios (IRR) were assessed using Poisson regression.

**Results:** We included 324 patients (36%) with SVR24 treatment with peg Interferon + Ribavirin (n=283) or triple therapy with Boceprevir/Telaprevir (n=41) and a median follow-up of 3.6 years. At SVR, median age was 45 years, median CD4 cell count was 450 (IQR 302-624)/mm<sup>3</sup>, median BMI was 22 kg/m<sup>2</sup> (IQR 20-24), 7% had an excessive alcohol consumption ( $>3$  glasses of alcohol/day for men and  $>2$  for women), 25% had a severe fibrosis (F3 n=31/F4 n=44) and 45% had a HCV genotype 1. Incidence rate of a first hepatic event was 4.2 per 1,000 person years (PY) (CI: 0.8-7.5) occurring 7 months in median after SVR. Patients with severe fibrosis tended to have a higher risk of first hepatic event compared to patients with mild/no fibrosis (IRR: 5.0 CI: 0.7; 26.3,  $P=0.10$ ). Hepatic events were: HCC (n=2) and decompensation (n=4). At the time of event, median fibrosis score was 10.3 kPa (IQR 9.0; 16.1) and 60% of these patients had an elastometry value  $>9.5$  kPa.

Death occurred 5.3 years in median after SVR. Overall mortality rate was 4.8 per 1,000 PY (CI: 1.2-8.3), 4.0 (CI: 0.2-7.2) and 5.0 (CI: 0.6-9.4) per 1,000 PY in patients with severe and mild fibrosis, respectively ( $P=0.63$ ). Causes of death were: HCC (n=2), infection (n=2), cardio-vascular disease (n=2) and unknown (n=1).

**Conclusions:** Both hepatic events and death after cure were rare after short/mid-term follow-up. A residual risk of hepatic events after hepatitis C cure in HIV-HCV co-infected patients persisted after cure and patients with severe fibrosis tended to have a higher risk for a hepatic event. Our early findings underline the need for long-term follow-up and are in favor of an early access to anti-HCV treatment.

**606 Hepatitis C Genotype 6 is Associated With Progression of Liver Fibrosis in HIV Patients**

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**Background:** Assessing liver fibrosis is critical for prioritizing candidates for HCV treatment in resource limited settings (RLS). We determined the rate of liver fibrosis progression and any factors that were associated with progression of fibrosis among untreated chronic hepatitis C patients with HIV (HIV/HCV) by using Liver stiffness measurement (LSM) by FibroScan.

**Methods:** Untreated chronic HIV/HCV co-infected patients (detectable HCV RNA for >6 months) were prospectively enrolled between January 1, 2011 and June 30, 2015. LSM was measured at first LSM (baseline) and after >6 months of follow-up. Logistic regression was used to assess factors associated with progression of liver fibrosis, defined as LSM increasing by  $\geq 1$  stage or >3 KPa if stage was F4 at baseline. Advanced liver fibrosis was defined as LSM  $\geq 9.5$  KPa (stages F3 or F4).

**Results:** Totally 133 HIV/HCV cases (85% male, 58.7% IDU, 24.8% MSM) were enrolled. The median age was 40 years. All patients were taking HAART and had undetectable HIV RNA at baseline. HCV genotype (GT) distributions were 44% GT3, 43% GT1 and 13% GT6. Median duration from baseline to last LSM was 2.1 (IQR 1.0-3.1) years. Median LSM at baseline was 8.6 (IQR 6.1-14) KPa and 41% of patients met criteria for advanced liver fibrosis. At the follow-up visit, 48/55 (87%) of subjects with advanced fibrosis at baseline remained in stage F3-F4, and 22/78 (28%) of patients with non-advanced fibrosis at baseline had LSM indicating progression to F3-F4. Liver fibrosis progression of  $\geq 1$  stage was noted in 50/133 (38%) patients. In all patients, the median rate of fibrosis progression was 1.4 (IQR 0.4-4.4) KPa/year. Patients with advanced fibrosis at baseline had a higher degree of liver fibrosis progression [median=3.6 (IQR 1.4-6.5) KPa/year] than those with less advanced disease at baseline [median=1.0 (IQR 0.3-2.4) KPa/year]. In multivariate analysis, HCV genotype 6 (OR 4.02, 95%CI=1.10-14.76, p=0.047 versus GT1) and duration of HCV infection in years (OR 1.06, 95%CI=1.00-1.11, p=0.028) were significantly associated with progression of liver fibrosis. The odds of liver fibrosis progression in patients with genotype 3 was not significantly different to patients with GT1 (OR 1.38, 95%CI=0.52-3.18, p=0.28).

**Conclusions:** Patients with untreated HIV/HCV genotype 6 had a greater rate of fibrosis progression than genotypes 1 and 3, and should be prioritized for HCV treatment where resources are constrained.

**607 Impact of Occult HBV Infection in HCC Presentation in HCV-Related Cirrhosis**

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**Background:** The role of the occult HBV infection (OBI) as an additional risk factor for hepatocellular carcinoma (HCC) has not been fully investigated. The aim of this study was to evaluate the virological and clinical characteristics of OBI (HBV DNA in liver tissue in HBsAg negative patients) in HBsAg negative subjects with HCC

**Methods:** 68 consecutive HBsAg negative patients with HCC were enrolled at two centers from June 2013 to December 2014: 57.3% males; mean age 70.2 $\pm$ 6.24 years; 58 (85.3%) with HCV-related cirrhosis and 10 (14.7%) with non-alcoholic fatty liver diseases-related cirrhosis; 85.3% with a Child-A stage cirrhosis; 69.1% at first diagnosis of HCC, 2.9% with portal thrombosis; 89.7% with a BCLC stage-A HCC. For each patient HBV DNA was sought by PCR in plasma, HCC-liver tissue and non-HCC-liver tissue, using sets of primers for core, surface and x regions of HBV genome. Occult HBV infection was defined by the presence of HBV DNA in at least two different PCRs

**Results:** OBI was identified in 3 patients (4.4%) both in HCC-liver tissue and non-HCC-liver tissue and in 10 (14.7%) only in HCC-liver tissue; no patient showed OBI in plasma. OBI was detected more frequently in the 11 anti-HBs negative/anti-HBc positive and in the 17 anti-HBs/anti-HBc positive than in the 40 anti-HBs/anti-HBc negative patients (54.5%, 29.4% and 5%, respectively; p<0.0005). The analysis of pre-S1, pre-S2 and S regions showed the presence of aa substitutions in S region (F19L, S59F, T131I, Q129H), deletions in position 4,8 an 17 in pre-S1 region and aa substitution in pre-S2 region (P41H). The demographic, biochemical and clinical (unifocal or multifocal HCC, diameter of HCC, HCC localization) were similar in the 13 patients with OBI and in the 55 without. However, the 13 patients with OBI than the 55 without showed a more severe cirrhosis (Child B or C stage: 53.9% vs. 5.5%, p<0.0001) and more advanced HCC (BCLC B or C stage: 46.1% vs. 1.8%, p<0.0001)

**Conclusions:** OBI was found in 19.1% of the HBsAg negative patients with HCC, more frequently in anti-HBc positive patients. The OBI seems to have a clinical impact in HCC presentation and HBsAg mutations correlated with HCC progression and failure in HBsAg detection

**608 CB2-63 RR Variant Is Associated With Immune-Mediated Disorders in HCV Patients**

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**Background:** Patients with HCV chronic infection frequently show immune-mediated disorders (IMDs). The Cannabinoid (CB) receptor 2, predominantly expressed in the immune cells, plays an important role on the function of the immune system. In particular, the CB2-63 variants (rs35761398) affects the ability of the CB2 receptor to exert its inhibitory function on T lymphocyte. The aim is to evaluate whether CB2 variants are associated with the presence of IMDs in patients with chronic HCV infection

**Methods:** Considering that nearly 30% of anti-HCV positive patients are affected by IMDs, we planned a 12-month recruitment period for treatment-naïve anti-HCV positive patients with signs of IMD and a 4-month period for treatment-naïve anti-HCV patients lacking these signs. The enrollment started in September 2013 and at the end of the recruitment periods 168 patients have been selected, 81 anti-HCV/HCV-RNA positive with signs of IMDs and 87 anti-HCV/HCV-RNA positive with no sign of IMDs. The presence of IMDs was defined by at least one of the following conditions: ANA positivity (titers  $\geq 1:160$ ) observed in 22 (27.2%) cases, SMA positivity (titers  $\geq 1:160$ ) in 3 (3.7%), a cryocrite  $>2\%$  in 24 (29.6%), history or active autoimmune thyroiditis in 25 (30.9%), psoriasis in 4 (4.9%), B-cells non-Hodgkin lymphoma in 2 (2.5%) and autoimmune hemolytic anemia in 1 (1.2%) case; no patient showed signs of lichen planus nor Sjogren syndrome. All patients were screened for the CNR2 rs35761398 SNP by a TaqMan Assay

**Results:** Compared with the 87 patients lacking IMDs, the 81 in the IMDs group more frequently were females (65% vs 45%, p=0.01), but not other significant difference was found in initial demographic, epidemiologic, serological, biochemical and virological data. In particular, the age (mean $\pm$ SD: 53 $\pm$ 14.1 vs. 52.9 $\pm$ 13.4 years), ALT serum levels, HCV viral load and in distribution of HCV genotypes were similar in these two groups. Instead, the prevalence of the patients with the CB2-63 RR variant was significantly higher in patients in the IMD group than in those in the non-IMD group (49.4% vs 24.1%, p=0.001). A logistic regression analysis including the CB2-63 receptor (RR vs QR or QQ), age and sex, identified the CB2-63 RR as the only independent predictor of IMDs (p=0.005).

**Conclusions:** the data suggest a significant previously unknown association between CB2-63 RR variant and IMDs in anti-HCV patients, an observation deserving further investigation on a larger series of patients to define its clinical value

**609 Prevalence and Risk Factors of Low Muscle Mass in HIV/Viral Hepatitis Coinfection**

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**Background:** Low muscle mass (LMM) in HIV is associated with reduced survival and may be mediated by systemic inflammation. Coinfection with viral hepatitis may induce additional inflammation that can contribute to LMM. We determined the prevalence of LMM in HIV/viral hepatitis patients on antiretroviral therapy (ART) compared to ART-treated HIV-monoinfected, viral hepatitis-monoinfected, and uninfected persons, and identified risk factors for LMM in coinfecting patients.

**Methods:** We performed a cross-sectional study of participants in the Women's Interagency HIV Study (n=1,957) and Multicenter AIDS Cohort Study (n=2,514) who had anthropometric data available. Viral hepatitis was defined by positive hepatitis B virus (HBV) surface antigen and/or hepatitis C virus (HCV) RNA. We evaluated the prevalence of LMM, defined as <10<sup>th</sup> percentile of age- and sex-matched reference values for mid-upper arm circumference, by HIV/viral hepatitis status. Using multivariable logistic regression, we determined adjusted odds ratios (ORs) with 95% confidence intervals (CIs) of: 1) LMM associated with viral hepatitis coinfection; and 2) factors associated with LMM in coinfecting persons.

**Results:** A total of 332 HIV/viral hepatitis-coinfecting (246 HCV; 79 HBV; 7 HBV/HCV), 1,854 HIV only, 223 viral hepatitis only, and 2,061 uninfected patients were identified. LMM was most common in coinfecting (24%), followed by HIV-monoinfecting (14%), and least common in hepatitis-monoinfecting and uninfected persons (both 10%) (all p<0.001 vs. coinfecting). After adjustment for age, alcohol use, injection drug use (IDU), and body mass index, viral hepatitis was associated with LMM in HIV-infected women (OR 3.14 [1.79-5.51]); the association did not reach significance in men (OR 1.30 [0.68-2.13]). Results were similar after exclusion of cirrhotic patients. In coinfecting women, IDU (OR 4.02 [1.11-14.53]), but not hazardous alcohol (OR 0.89 [0.43-1.82]), was associated with LMM; and, there was little association of liver aminotransferases >40 U/L (OR, 2.08 [0.93-4.65]) or glomerular filtration rate<60 ml/min (OR 1.57 [0.52-4.70]).

**Conclusions:** Low muscle mass was more common in coinfecting than HIV-monoinfecting, hepatitis-monoinfecting, and uninfected persons. In HIV, viral hepatitis was more strongly associated with low muscle mass in women than men. Future studies should determine if coinfection creates a heightened inflammatory state that promotes loss of muscle mass and explore reasons for the differential association by sex.

## 610 Rapid Improving of Glycemic Control in HCV Patients Treated With IFN-Free Regimens

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*Sapienza Univ of Rome, Rome, Italy*

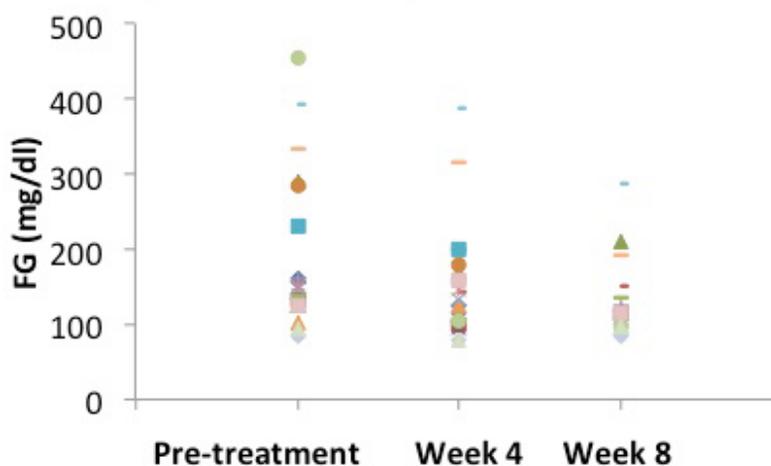
**Background:** HCV infection has been widely associated with insulin-resistance and type 2 diabetes. Improved diabetic outcomes have been demonstrated many years after the HCV eradication, but development of type 1 diabetes has been reported following IFN exposition. Little is known about the impact of new direct acting antiviral agents (DAAs) on glycemic control.

**Methods:** We retrospectively evaluated 29 HCV-infected patients (10 HIV+) with type 2 diabetes who were treated with different IFN-free regimens, including sofosbuvir, simeprevir, ledipasvir, daclatasvir, dasabuvir and ombitasvir/paritaprevir/ritonavir. To evaluate general improving of glycemic control, we used a composite endpoint given by reduction of fasting glucose (FG) (of min. 20 mg/dl) or glycated haemoglobin (A1C) (of min. 0.5%) or reduction of insulin/metformin dosing during anti-HCV treatment. Statistical analysis was performed with the paired t-test, Kruskal-Wallis test and Welch one-way ANOVA procedure (R software).

**Results:** The mean age of the patients was 59,28 years (24 M, 5 F). All the patients had HCV-RNA undetectable at end of treatment (or <15 UI/ml if still on treatment). Pre-treatment FG was reported in 27 patients, mean value 175 mg/dl (range, 85-455), pre-treatment A1C in 17 patients, mean value 7.1% (range, 5.1%-11.8%). FG during treatment was available for 21 patients and analysis showed a statistically significant reduction (p=0,007), reduction mean value was -52,86 mg/dl [Fig.1]. A1C during treatment was available for 10 patients and analysis showed a statistically significant reduction (p=0,021), reduction mean value was -1,95%. 4 patients were excluded from the analysis cause data were insufficient. The endpoint was reached by 21 of 25 patients (84%). 6 patients (23%) needed to reduce insulin dosing, 8 of 10 patients showed reduction of A1C, 14 of 21 patients (67%) showed reduced FG during treatment. FG and A1C reductions were independent from the drug used, HCV genotype and HIV. No cases of symptomatic hypoglycaemia were found. Between the 4 patients who didn't reach the endpoint 3 presented normal baseline FG (<110 mg/dl) and A1C (<6%) and the remaining 1 presented Child-Pugh B and was the only patient with persisting elevated liver enzymes at end of treatment.

**Conclusions:** HCV suppression following DAA treatment is associated with rapid improving of glycemic control. In order to avoid hypoglycaemic events, patients undergoing DAAs should be closely monitored for reduction of hypoglycaemic drugs.

**Fig.1 FG levels during DAAs treatment**



## 611 Eradication of HCV and Extrahepatic Comorbidities in HIV/HCV Coinfection

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**Background:** We studied the effect of SVR on non-liver-related (NLR) non-AIDS-related (NAR) events and mortality in HIV/HCV+ patients (Pts) after therapy with interferon plus ribavirin (IR).

**Methods:** GeSIDA 3603 is a cohort of HIV/HCV+ Pts treated with IR between 2000-2008 in 19 centers. We assessed incident NLR-NAR events from interruption of IR to the last follow-up visit, death, or loss to follow-up. Pts were classified as responders (including those who achieved SVR after retreatment) or nonresponders. We assessed NLR-NAR deaths and NLR-NAR events, including cardiovascular events (myocardial infarction, angina, stroke, peripheral artery disease, heart failure, ruptured aortic aneurism, and mesenteric

artery ischemia), renal events (chronic renal failure, dialysis, and renal transplantation), bone events (bone fractures and avascular bone necrosis), diabetes mellitus, NLR-NAR-defining cancer, and NAR sepsis requiring hospitalization. NLR-NAR events were defined according to the Cohort of the Spanish AIDS Research Network (AIDS 2013; 27:181). The censoring date was May 31, 2014. All the centers were monitored before the analysis. We calculated the aHR (95%CI) of events in responders vs nonresponders by Cox regression analysis. The variables for adjustment were age, sex, prior AIDS, HIV risk group, nadir CD4+ cell count, cART, HIV-RNA load, HCV genotype, and advanced fibrosis (FIB4≥3.25). Competing risk survival analysis was applied when analyzing NLR-NAR events (overall death) and NLR-NAR death (AR or LR death). Two sensitivity analyses were carried out: one by censoring FU in retreated patients with SVR at the date of initiation of retreatment and the other by excluding those who were retreated.

**Results:** Of 1,625 Pts, 592 (36%) had an SVR. After a median FU of 5.2 y in responders and 5.5 y in nonresponders, significantly lower frequencies and rates of renal events, diabetes mellitus, and NAR sepsis were seen in responders (Table). Cox regression analysis showed that the aHR of renal events and diabetes mellitus were significantly lower in responders (Table). These results were confirmed by the 2 sensitivity analyses.

**Conclusions:** Our findings suggest that eradication of HCV in coinfecting Pts is independently associated with a reduced hazard of renal events and diabetes mellitus. Eradication of HCV was not independently associated with a reduced hazard of NLR-NAR death, cardiovascular events, bone events, NLR-NAR cancers, or NAR sepsis.

	Events (No.)		P	Rates of events/100 person-years (95% CI)		P	aHR (95% CI) of events SVR vs. No SVR		P
	No SVR N = 992	SVR N = 633		No-SVR	SVR		SVR vs. No SVR		
<b>Mortality</b>									
Overall death	144 (14.5)	31 (4.9)	<.001	2.74 (2.31 - 3.23)	0.96 (0.65 - 1.36)	<.001	0.38 (0.26 - 0.57)	<.001	
NLR-NAR death	53 (5.3)	23 (3.6)	.141	1.01 (0.76 - 1.32)	0.71 (0.45 - 1.07)	.062	0.85 (0.50 - 1.42)	.527	
<b>Events</b>									
Cardiovascular	52 (5.2)	36 (5.7)	.699	1.00 (0.75 - 1.31)	1.23 (0.87 - 1.68)	.538	1.53 (0.98 - 2.39)	.064	
Renal	28 (2.8)	6 (0.9)	.010	0.53 (0.36 - 0.77)	0.19 (0.07 - 0.40)	.006	0.37 (0.14 - 0.97)	.042	
Bone	32 (3.2)	24 (3.8)	.542	0.62 (0.42 - 0.87)	0.78 (0.51 - 1.15)	.250	1.36 (0.75 - 2.47)	.317	
Diabetes	76 (7.7)	22 (3.5)	.001	1.48 (1.17 - 1.86)	0.72 (0.45 - 1.08)	.004	0.54 (0.34 - 0.88)	.014	
NLR-NAR cancer	66 (6.7)	32 (5.1)	.187	1.27 (0.98 - 1.61)	1.07 (0.74 - 1.49)	.466	0.94 (0.60 - 1.48)	.778	
NAR sepsis	62 (6.3)	18 (2.8)	.002	1.19 (0.92 - 1.53)	0.59 (0.36 - 0.92)	.015	0.66 (0.38 - 1.16)	.147	

612 Hepatitis C and the Risk of Non-Liver-Related Morbidity and Mortality in HIV+ Persons

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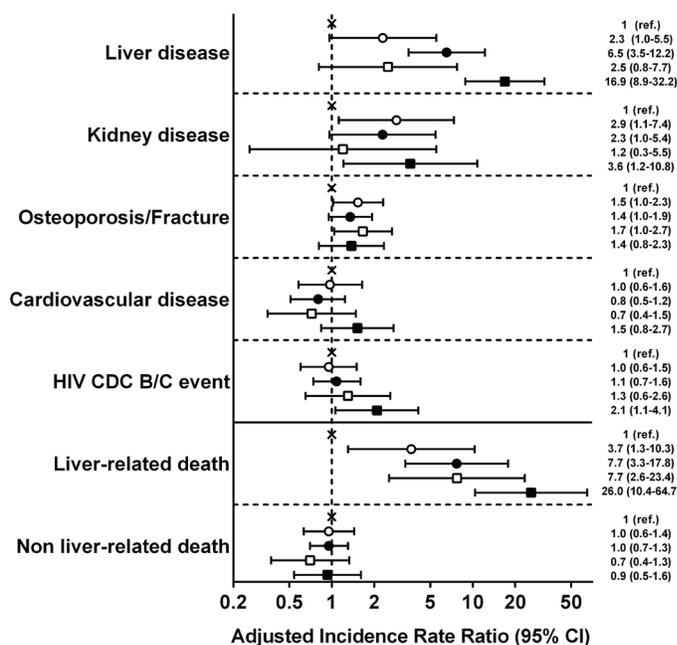
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**Background:** HCV infection has been associated with increased non-liver-related morbidity and mortality; however studies have yielded inconsistent results.

**Methods:** The incidence of clinical events in four HCV-seropositive groups (untreated spontaneously cleared/detectable HCV-RNA, treated with/without sustained virologic response (SVR)) and matched HCV-seronegative Swiss HIV Cohort Study (SHCS) participants from 08/1994 to 12/2014 were studied. We compared HCV-seropositive to HCV-seronegative patients and aviremic to replicating HCV infection. Poisson regression was used to assess differences across these groups (see footnote in Figure).

**Results:** We included 2503 HCV-seropositive individuals, 540 with cleared HCV infection, 1294 untreated viremic, 345 treated with SVR, 281 treated without SVR, and 2503 HCV-seronegative controls. After a mean follow-up of 8.2 years, we observed 107/18 (HCV-seropositive/HCV-seronegative) liver events, 41/14 kidney diseases, 230/121 osteoporosis/fracture, 114/129 cardiovascular events, 162/126 HIV CDC B/C events, 106/10 liver-related deaths and 227/218 non-liver-related deaths. Adjusted incidence rate ratios for the HCV-negative and different HCV-seropositive groups are shown in the Figure. Compared to HIV-monoinfected controls, HCV-seropositive groups combined had an increased risk of liver disease (IRR 6.29 [95% CI 3.52-11.22]), liver-related death (8.24 [3.61-18.83]), kidney events (2.43 [1.11-5.33]) and osteoporosis/fracture (1.43 [1.03-2.01]). No evidence for an association with increased risk was found for cardiovascular diseases, HIV CDC B/C events and non-liver-related death. Among HCV-seropositive individuals, those with replicating HCV infection had an increased risk of liver-related events compared to aviremic participants (untreated viremic vs cleared 2.84 [1.36-5.89]; non-SVR vs SVR 6.74 [1.36-5.89]) and liver-related death (untreated viremic vs cleared 2.10 [0.99-4.47]; non-SVR vs SVR 3.36 [1.37-8.21]). Non-liver-related diseases and death did not significantly differ between HCV viremic vs aviremic patients.

**Conclusions:** While incidence for non-liver-related death and cardiovascular events was not elevated, HCV exposure was associated with an increased risk of kidney disease and osteoporosis. This risk did not seem to be dependent of persistent HCV replication.



Nested case-control design within the Swiss HIV Cohort Study. Results from Poisson regression of incidence-density-matched HCV seropositive and -negative pairs adjusted for HIV transmission category, age, HIV-1 RNA, smoking, alcohol use, active injection drug use, duration of HIV and HCV-infection.

× HCV seronegative  
 HCV seropositive:  
 ○ No Tx, HCV-RNA neg.  
 ● No Tx, HCV-RNA pos.  
 □ Tx, SVR  
 ■ Tx, no SVR

**613 Risk of Cancer in HIV-Positive Adults on ART in South Africa: A Record Linkage Study**

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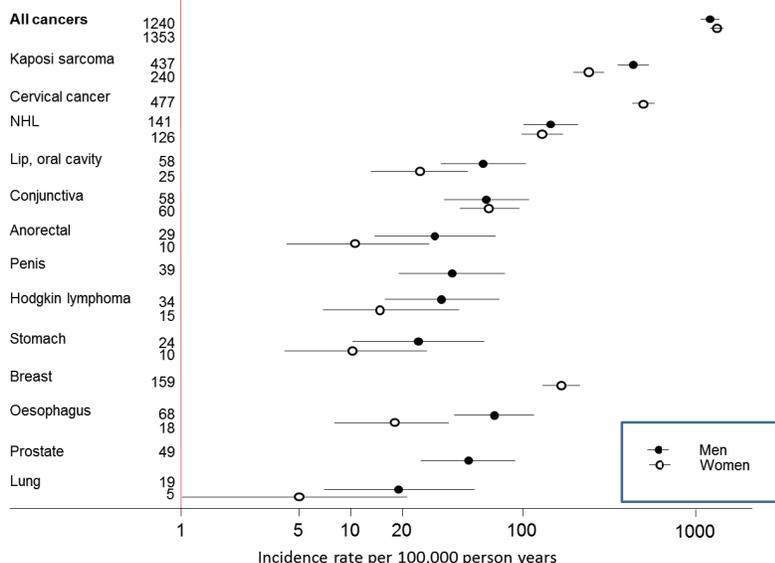
**Background:** The surveillance of HIV-related cancers in South Africa is hampered by the lack of systematic collection of cancer diagnoses in HIV cohorts and the absence of data on HIV status in cancer registries. To estimate cancer incidence and explore risk factors for developing infection-related and non-infection-related cancer, we conducted a probabilistic record linkage study of ART programs providing care for adults and the National Cancer Registry in South Africa.

**Methods:** We used data for the period 2004–2011 from two ART programs (McCord Hospital, KwaZulu-Natal and Themba Lethu Clinic, Gauteng province) and linked it to the cancer registry data for the same period. We used probabilistic record linkage methods to identify patients with both HIV and cancer. Linkage variables were names, date of birth and gender. We calculated cancer incidence rates and hazard ratios (HR) with 95% confidence intervals (CI) from multivariable Cox regression models adjusted for sex, age, CD4 cell counts and hemoglobin levels at start of ART for infection-related and non-infection-related cancers as defined by the *International Agency for Research on Cancer*.

**Results:** We included 23,120 patients, 64% were women, median age at starting ART was 36 years (IQR 31–42) and median CD4 cell count was 109 cells/μL (IQR 45–179). During 59,101 person-years (pys) of follow-up 851 patients developed incident cancers for an overall incidence rate of 1,315/100,000 pys (95% CI 1,225–1,410). Cancers with the highest incidence rates in men were Kaposi sarcoma (KS), non-Hodgkin's lymphoma (NHL), oesophagus, conjunctiva and oropharyngeal cancers. In women, cancers with the highest incidence rates were cervical cancer, KS, breast, NHL and conjunctiva (Figure). The risk of developing infection-related cancer increased with lower CD4 cell counts at start ART (<100 versus ≥350 cells/μL: adjusted HR 0.24, 0.08–0.76) and with lower hemoglobin levels (adjusted HR: 0.92, 95% CI 0.87–0.96). For cancers not associated with infections, cancer risk increased with age at ART start (>=56 versus 16–25 years: adjusted HR 2.63, 95% CI 1.08–6.39).

**Conclusions:** Incidence of cancer in HIV-positive South Africans in the era of potent ART remains high, particularly for AIDS-defining cancers and infection-related cancers. There is a need to evaluate and implement cancer-specific prevention strategies in the HIV-positive population in South Africa.

Figure: Cancer Incidence Rate in HIV-positive men and women



**614 No Difference in Stage at Cancer Diagnosis by AIDS Status Among HIV-Infected Adults**

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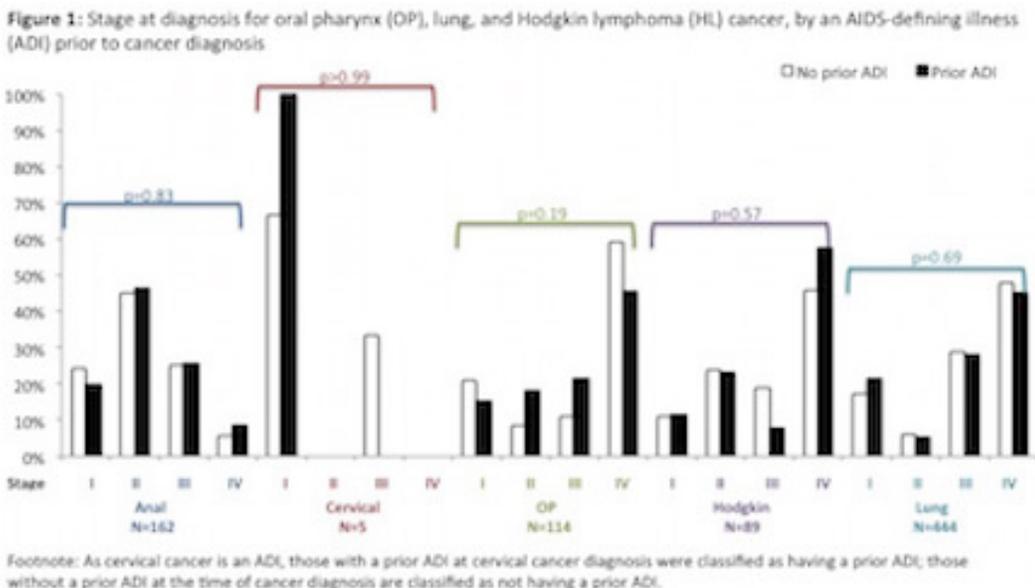
<sup>1</sup>Univ of Pennsylvania, Philadelphia, PA, USA; <sup>2</sup>Univ of Washington, Seattle, WA, USA; <sup>3</sup>Univ of Calgary, Calgary, AB, Canada; <sup>4</sup>NIH, Bethesda, MD, USA; <sup>5</sup>Johns Hopkins Univ, Baltimore, MD, USA; <sup>6</sup>Univ of North Carolina at Chapel Hill, Chapel Hill, NC, USA; <sup>7</sup>Sunnybrook Rsr Inst, Sunnybrook Hlth Scis Cntr, Toronto, ON, Canada; <sup>8</sup>Kaiser Permanente Northern California, Oakland, CA, USA; <sup>9</sup>Johns Hopkins Bloomberg Sch of PH, Baltimore, MD, USA

**Background:** HIV-associated immune suppression has been linked to an increased risk of certain cancers, but whether this risk is translated into different stages at cancer diagnosis, or risk of death after cancer diagnosis, is unclear. We estimated the effect of prior AIDS-defining illness (ADI) as a surrogate for advanced HIV disease progression on cancer stage at diagnosis and subsequent mortality risk.

**Methods:** HIV-infected adults (≥18 years of age) with validated diagnoses of anal, oropharynx (OP), cervical, lung cancer, or Hodgkin lymphoma (HL) from 1 Jan 2000 to 31 Dec 2009 in the North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) were included. NA-ACCORD participants are HIV-infected adults who successfully link into care. The distribution of stage at cancer diagnosis was compared in those with and without a prior ADI. Stage at diagnosis was identified via local review of medical records by trained medical record abstractors under the supervision of physician or via cancer registries. Analyses were stratified by type-specific cancer. Adjusted mortality rate ratios [aMRRs] and 95% confidence intervals (CI) were estimated using Poisson regression models accounting for sex, race, smoking status, CD4 count, HIV RNA, ART use, and cancer stage, all measured at cancer diagnosis; age was time-varying.

**Results:** Of the 81,865 participants, 814 had validated type-specific cancer diagnoses of interest (n=162 anal, n=5 cervical, n=444 lung, n=114 OP, n=89 HL); 642 (79%) with a prior ADI and 728 (89%) with ART use prior to cancer diagnosis. Cancer stage was comparable by prior ADI diagnosis for each cancer (Figure 1). Prior ADI diagnosis increased mortality rates after cancer diagnosis for each cancer, except cervical cancer as none died after cervical cancer diagnosis. After adjustment for age, sex, race, smoking, ART use, CD4 count, HIV RNA, and cancer stage at diagnosis, the aMRRs comparing those with vs. without a prior ADI at cancer diagnosis were as follows: anal: 1.5 [0.8, 2.6]; lung: 1.6 [1.3, 2.0]; OP: 1.9 [0.9, 3.6]; and HL: 1.9 [0.8, 4.4].

**Conclusions:** A marker of at least one prior episode of advanced HIV disease (i.e. a prior ADI) was not associated with differences in stage at diagnosis or risk of death after cancer diagnosis (except for lung cancer) in this population of HIV-infected adults with access to care.



615 Cancer Versus Tuberculosis Mortality Among HIV-Infected Individuals in Botswana

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**Background:** With declining mortality due to infections, cancer has become the leading cause of death among HIV-infected individuals in high-income countries. However, in sub-Saharan Africa where more than two-thirds of HIV infections occur, the relative contribution of infection and malignancy to HIV-associated mortality is unknown. We sought to estimate cancer mortality since availability of antiretroviral treatment (ART) in Botswana, and compare with mortality due to tuberculosis (TB), the leading infectious cause of death in sub-Saharan Africa.

**Methods:** Incidence by cancer type was estimated from 8479 incident cases from the Botswana National Cancer Registry during the period of ART expansion, 2003-2008. We utilized Poisson regression in an inverse probability weighted population with known HIV status and projected cancer incidence through 2013. Cancer mortality was estimated using parametric Weibull models from observed survival in a separate prospective cancer cohort in Botswana (2010-2015). Survival probabilities for each cancer type were assumed to be constant during the study period and all deaths were attributed to cancer. We utilized estimates from the WHO Global TB Program (derived from Botswana government data) to estimate TB-HIV deaths.

**Results:** A total of 808 patients with HIV and cancer followed for median of 12.2 months (IQR 6.1 to 24.3 months) contributed to survival estimates (1.2% loss-to-followup). Estimated 5-year survival was low: cervix 3.9%, head and neck 4.4%, breast 19.3%, non-Hodgkin lymphoma 39.7%, Kaposi sarcoma 52.1%, and combined other sites 15.1%. Mortality due Kaposi sarcoma declined over the study period (-4.2% annually, 95% CI -5.0 to -3.3%), but cervical cancer mortality increased (13.3% annually, 95% CI 11.7 to 14.9). Overall cancer mortality increased (1.2% annually, 95% CI 0.7 to 3.1%) while TB mortality declined substantially between 2003 and 2013. In 2013, projected cancer mortality (293 per 100,000, 95% CI 264-331) approximated projected TB mortality (324 per 100,000, estimate range 241-419).

**Conclusions:** With ART coverage exceeding 90%, mortality due to cancer in HIV-infected individuals has increased in Botswana and now likely exceeds mortality due to TB. Cervical cancer mortality is rising sharply. Interventions to reduce cancer risk, establish screening programs, and improve access to treatment are urgently needed for HIV-infected individuals.

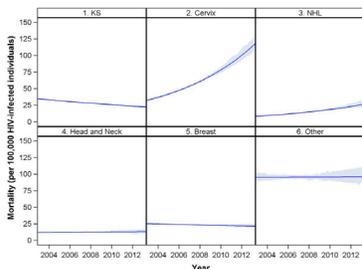


Fig 1. Estimated mortality by cancer site among HIV-infected individuals in Botswana.

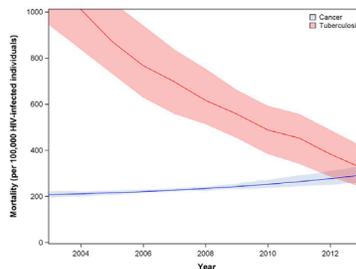


Fig 2. Estimated cancer and tuberculosis mortality among HIV-infected in Botswana.

616 Excess Mortality Rates Among HIV-Infected Cancer Patients in the United States

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**Background:** We have recently reported that HIV-infected cancer patients have a higher risk of dying from their cancer than their HIV-uninfected counterparts, providing evidence that immunosuppression plays a role in cancer progression. However, the magnitude of excess mortality resulting from this association among US HIV-infected cancer patients remains unclear. In the present study, we examine whether patients diagnosed with both HIV and cancer die at higher rates than expected based on the mortality rates for HIV and cancer individually.

**Methods:** We addressed this question using data from HIV and cancer registries in 6 states participating in the HIV/AIDS Cancer Match Study and corresponding general population mortality data from the National Center for Health Statistics (1996-2010). We compared age-stratified mortality rates in 4 groups: (1) the general population, (2) individuals diagnosed with HIV, (3) individuals diagnosed with cancer, and (4) individuals diagnosed with both HIV and cancer. We assessed associations with age-stratified overall mortality rates using a Poisson model including additive terms for HIV and cancer, and an interaction term for the combined effect of HIV and cancer. We tested the statistical significance of the interaction term to determine whether patients diagnosed with both diseases experienced significant excess mortality in addition to mortality from HIV and cancer separately.

**Results:** For 5 of the 6 cancers evaluated, HIV-infected cancer patients experienced statistically significant excess mortality rates. (Table 1) This excess mortality rate was most pronounced among younger individuals (30-49 years), with large excess rates per 10<sup>5</sup> person-years for cancers of the lung (59,004), anus (3,736), colorectum (6,364), and breast (10,537), and melanoma (6,364); there was actually a deficit in mortality for prostate cancer in this age group (2,349 per 10<sup>5</sup> person-years). For breast cancer patients ≥70 years of age, we observed a significant excess mortality rate of 32,773 per 10<sup>5</sup> person-years.

**Conclusions:** In the era of effective HIV treatment, US patients diagnosed with both HIV and cancer experienced significant excess mortality rates due to the combination of both diseases. The magnitude of this excess was substantial for non-AIDS-defining malignancies of varying etiologies, including common cancers such as lung, colorectal, and breast, and was particularly acute in younger populations who had higher baseline life expectancies.

Excess Mortality in Patients Diagnosed with both HIV and Cancer

Cancer and age	Excess Mortality Rate per 10 <sup>5</sup> person-years	p-value
<b>Lung Cancer</b>		
30-49 years	59,004	<0.0001
50-69 years	42,061	<0.0001
70+ years	--	0.15
<b>Anal Cancer</b>		
30-49 years	3,736	<0.001
50-69 years	3,246	0.02
70+ years	--	0.55
<b>Melanoma</b>		
30-49 years	6,364	<0.01
50-69 years	4,508	0.06
70+ years	--	--
<b>Colorectal Cancer</b>		
30-49 years	14,952	<0.0001
50-69 years	5,856	0.08
70+ years	--	0.66
<b>Breast Cancer</b>		
30-49 years	10,537	<0.0001
50-69 years	8,011	<0.0001
70+ years	32,773	0.01
<b>Prostate Cancer</b>		
30-49 years	-2,349	0.03
50-69 years	--	0.15
70+ years	--	0.35

**617 Global Burden of Cervical Cancer in HIV-Positive Women on Antiretroviral Therapy**

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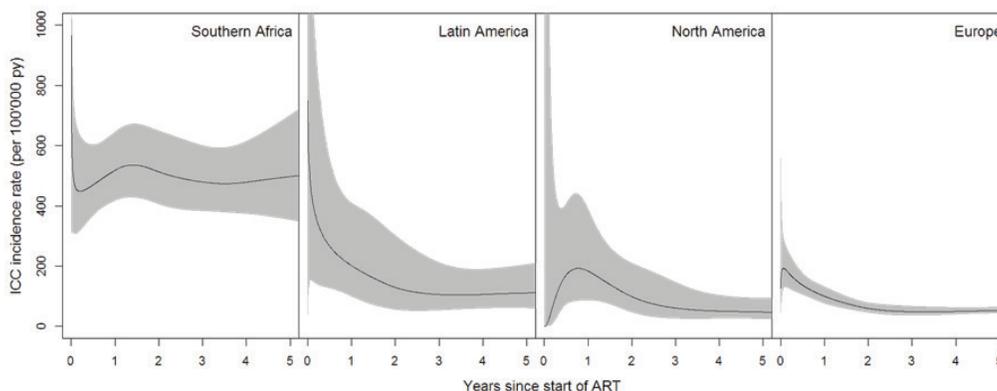
**Background:** HIV-positive women are at increased risk of human papillomavirus (HPV) infection and progression to invasive cervical cancer (ICC). HIV and HPV epidemics and access to cervical cancer screening vary between regions. We compared ICC risk in women on combination antiretroviral therapy (ART) in Asia-Pacific, North America, Latin America, Southern Africa, and Europe.

**Methods:** We included cohorts participating in the International Epidemiologic Databases to Evaluate AIDS (IeDEA) and the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) in EuroCoord. We included HIV-positive women aged ≥ 16 years who started ART after cohort enrollment from 1996 onwards. We used flexible parametric survival models with region-specific baseline hazards adjusted for time-updated CD4 cell counts, age, and year of ART start to compare regional ICC rates. We excluded Asia-Pacific from multivariable analyses due to small sample size. We present incidence rates and adjusted hazard ratios (aHR) with 95% confidence intervals (CI).

**Results:** We included 65,726 women from 55 countries. Median age at ART start was 35 years and similar across regions. Median CD4 cell count (cells/μL) at ART start was 115 in Southern Africa, 146 in Asia-Pacific, 179 in Latin America, and 241 in Europe and North America. Median follow-up time was 3.9 years (interquartile range 1.5-7.3). During 323,224 person-years (pys) 390 women developed ICC. Incidence rate per 100,000 pys was highest in Southern Africa (497, 95%CI 429-577) followed by Latin America (152, 95%CI 97-238), North America (76, 95%CI 48-119), Europe (71, 95%CI 62-83) and Asia-Pacific (42, 95%CI 6-297). With the exception of Southern Africa regional ICC risks decreased with time since ART start (see Figure). Adjusted hazard ratios comparing Europe with other regions at 2 and 5 years were 8.9 (95%CI 6.0-13.3) and 12.4 (95%CI 7.8-20.0), respectively, for Southern Africa, and 2.1 (95%CI 0.8-5.0) and 2.2 (95%CI 1.2-4.2), respectively, for Latin America. No difference was observed between North America and Europe.

**Conclusions:** HIV-positive women in Southern Africa and Latin America had a markedly higher ICC risk than women from North America and Europe, and rates did not decline with time on ART in Southern Africa. These regional differences were not explained by differences in CD4 counts, age, or year of starting ART, but could be explained by a higher prevalence and incidence of HPV infection and limited access to effective cervical cancer screening.

**Figure: Hazards and 95% CIs for invasive cervical cancer in different regions.**



**618 Risk of Kaposi Sarcoma in HIV-Positive Adults on ART: A Global Analysis**

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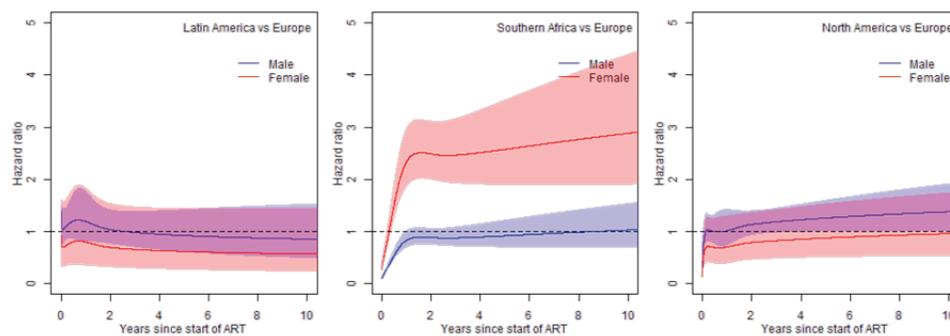
**Background:** No comparisons of Kaposi sarcoma (KS) risk are available between regions with different HIV and Human Herpesvirus 8 (HHV-8) prevalence. We examined KS risk in adults on combination antiretroviral therapy (ART) in the IeDEA and COHERE in EuroCoord cohort collaborations.

**Methods:** We included HIV-positive adults (≥ 16 years) who started ART from 1996 onwards. We compared the risk of incident KS between regions using flexible parametric survival models with region-specific baseline hazards, adjusted for age, sex and its interaction with region, time-updated CD4 counts and year of ART start. We excluded Asia-Pacific and Australia from multivariable analyses due to the small sample size. We present hazard ratios (HR) and 95% confidence intervals (CI) by time on ART and at 2 years after ART start.

**Results:** We included 352,013 patients from Asia-Pacific, Australia, Latin and North America, Southern Africa, and Europe. Median age at ART start was 36 years and similar across regions. Median CD4 count at ART start was <200 cells/μL in Asia, Southern Africa and Latin America, and >200 cells/μL in Australia, Europe and North America. The proportion of men and the subset who have sex with men (MSM) was highest in Australia, followed by North and Latin America and Europe. Over 1.3 million person-years (pys) 2,935 adults developed KS for an overall incidence rate of 199/100,000 pys (95%CI 192-207). After 2 years on ART KS incidence was higher in women from Southern Africa than in European women (adjusted HR 2.5, 95%CI 2.0-3.1), and similar to European women in women from Latin and North America. In men crude KS risk was higher in North America compared to Europe (HR 1.5, 95%CI 1.3-1.9), in multivariable analyses this risk declined to HR 1.1 (95%CI 0.9-1.4). The change was mainly explained by adjusting for time-updated CD4 counts. KS risk was similar in men from other regions (Figure).

**Conclusions:** Women in Southern Africa had a higher KS risk than women in Europe which was not explained by HIV-related risk factors. In men KS risk was similar across regions after adjusting for HIV-related risk factors. This pattern likely reflects different HHV-8 risk profiles: while men were at high risk of HHV-8 infection in most regions (MSM or resident in HHV-8 endemic regions) the main risk factor for HHV-8 infection in women was residence in endemic regions. Migration data were not available for all regions and hence not considered in the analysis.

**Figure:** Adjusted hazard ratios and 95% CIs for the risk of developing KS in different regions.



**619 Kaposi Sarcoma Risk in Children on ART From Africa, Europe, and Asia**

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**Background:** Epidemic Kaposi sarcoma (KS) is caused by human herpesvirus 8 (HHV-8) infection and HIV-induced immunosuppression. HHV-8 prevalence and access to HIV care vary between regions. HHV-8 prevalence is higher in Eastern Africa than Southern Africa, and lower in Europe and Asia. We compared the KS burden in HIV-positive children on combination antiretroviral therapy (ART) between Asia, Europe, Eastern and Southern Africa.

**Methods:** We analyzed cohort data of the International Epidemiologic Databases to Evaluate AIDS Southern Africa (IeDEA-SA), the Collaboration of Observational HIV Epidemiological Research in Europe (COHERE) in EuroCoord, and the TREAT Asia Pediatric HIV Observational Database (TAPHOD). We included HIV-positive children aged <16 years who started ART between 1996 and 2014. We calculated KS incidence rates per 100,000 person-years (pys) and hazard ratios (HR) from Cox regression adjusted for region, sex, age at ART start, ART regimen, and ART start year. We used CD4 cell counts and CD4% to define degree of immunodeficiency at ART start according to WHO criteria.

**Results:** We included 24,383 children from Asia (Cambodia, India, Indonesia, Malaysia, Vietnam, Thailand), Europe (Denmark, France, Germany, Spain, Netherlands, UK), Eastern Africa (Zambia, Zimbabwe) and Southern Africa (South Africa). Median age at ART start was 5.1 years and lower in Southern Africa than in the other regions, see Table. Most children (55%) started ART with advanced or severe immunodeficiency; 10% of children were in CDC stage C. We observed 25 incident KS cases on ART (68% boys; median age at KS diagnosis 10.0 years). KS incidence rates were 85/100,000 pys (95% confidence interval [CI] 55-132) in Eastern Africa, 26/100,000 pys (95% CI 9-82) in Europe, and 9/100,000 pys (95% CI 2-37) in Southern Africa. All KS cases in Europe were in children who originated in sub-Saharan Africa (KS incidence rate 82/100,000 pys). We observed no incident KS in Asia. KS risk increased with age (10-15 versus 0-4 years; adjusted HR 4.1; 95% CI 1.4-12.1) and with advanced stage of HIV/AIDS (CDC stage C versus A/B; adjusted HR 3.1; 95% CI 1.1-8.7) at ART start.

**Conclusions:** KS risk is substantial in HIV-positive children of sub-Saharan African origin, whether they live in Africa or Europe. In the absence of measures to prevent HHV-8 infection, ART should be initiated in a timely fashion, before advanced HIV/AIDS stage is reached, to reduce KS risk in these children.

**Table: Characteristics of included children by United Nations geographical regions and sub-regions.**

	Eastern Africa	Southern Africa	Europe	Asia
<b>Children (N)</b>	11,193	8,482	1,650	3,058
<b>Median follow-up time [years]</b>	1.6 (0.5-3.4)	2.1 (0.7-4.1)	6.7 (3.3-10.2)	4.4 (2.1-6.5)
<b>Boys</b>	50%	50%	49%	51%
<b>Median age at ART start [years]</b>	6.1 (2.3-10.3)	3.3 (0.9-7.2)	5.9 (1.6-10.6)	5.8 (3.0-8.8)
<b>CDC stage C at ART start</b>	8%	11%	17%	13%
<b>CDC stage missing</b>	10%	2%	4%	2%
<b>Advanced/severe immunodeficiency</b>	53%	54%	51%	66%
<b>Immunodeficiency level missing</b>	24%	22%	16%	17%

KS, Kaposi sarcoma; ART, combination antiretroviral therapy.

Medians are presented with interquartile ranges.

**620 Long-Term Complications of Radiotherapy For Anal Cancer in HIV**

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**Background:** Squamous cell carcinoma of the anus (SCCA) is a leading non-AIDS defining cancer in HIV infected (HIV+) persons. The standard of care for invasive SCCA often includes radiotherapy (RT), but there is limited data about the types and rates of long-term complications of RT for this tumor type. Data regarding long-term complications of RT use in HIV+ populations are needed to better understand the harms and benefits associated with this common SCCA treatment modality.

**Methods:** We identified a cohort of 436 HIV+ SCCA patients from the Surveillance, Epidemiology, and End Results (SEER) database linked to Medicare claims (1991-2011) using relevant diagnostic codes. We used claims and SEER data to identify RT use and then beginning 6 months after cancer diagnosis identified potential complications longitudinally. We ascertained multiple complication types including proctitis, anal fissure and/or bleeding, bowel obstruction, femoral or pelvic fracture, abscess and others. We evaluated the association of these long-term complications with RT use in unadjusted analyses as well as adjusted Cox regression models accounting for age, sex, race and stage of SCCA at diagnosis.

**Results:** In our cohort 76% of HIV+ SCCA patients were treated with RT. Patients receiving RT were older and more likely to have advanced cancer stage than those who did not receive RT. Patients treated with RT compared to those who were not treated with RT were more likely to experience proctitis, anal bleeding, and bowel obstruction (all  $p < 0.05$ ) but not abscesses, fractures or other major complications in unadjusted analyses. In adjusted models, RT use was associated with proctitis (hazard ratio [HR] 1.6;  $p = 0.004$ ) and bowel obstruction (HR 2.8;  $p = 0.03$ ) during long-term follow-up.

**Conclusions:** Treatment of SCCA with RT in HIV+ patients was associated with an increased risk of proctitis and bowel obstruction during long-term follow-up in a population-based cohort. More research is needed to clarify the harms and benefits associated with RT treatment for SCCA in this population.

**621 Randomized Trial Comparing HIV-1 Cervical Shedding After Cryotherapy Versus LEEP**Sharon A. Greene<sup>1</sup>; Christine J. McGrath<sup>2</sup>; Dara Lehman<sup>3</sup>; T. T. Trinh<sup>1</sup>; Nelly Yatchi<sup>1</sup>; Barbra A. Richardson<sup>1</sup>; Grace C. John-Stewart<sup>1</sup>; Hugo De Vuyst<sup>4</sup>; Nelly R. Mugso<sup>5</sup>; Michael H. Chung<sup>1</sup><sup>1</sup>Univ of Washington, Seattle, WA, USA; <sup>2</sup>Univ of Texas Med Branch at Galveston, Galveston, TX, USA; <sup>3</sup>Fred Hutchinson Cancer Rsr Cntr, Seattle, WA, USA; <sup>4</sup>Intl Agency for Rsr on Cancer, Lyon, France; <sup>5</sup>Kenya Med Rsr Inst, Thika, Kenya

**Background:** Treatment of HIV-positive women to prevent cervical cancer may increase HIV-1 RNA cervical shedding and subsequent transmission of HIV-1. We compared cervical HIV-1 RNA levels among women receiving cryotherapy versus loop electrosurgical excisional procedure (LEEP) for treatment of cervical intraepithelial neoplasia stage 2 or 3 (CIN2/3).

**Methods:** Between June 2011 and July 2014, 400 HIV-positive women diagnosed with CIN2/3 in Nairobi, Kenya were randomized to receive cryotherapy or LEEP. Cervical swabs were collected at baseline and at weekly intervals for three weeks. Samples were tested for HIV-1 RNA using Gen-Probe Aptima HIV-1 assay with a minimum detection level of 60 copies/ml and analyzed using general estimating equations with a log link, accounting for time. Wilcoxon Signed Rank Test compared post-intervention HIV-1 RNA to baseline within each arm.

**Results:** Among 400 HIV-positive women randomized, median age was 37.0 years, median CD4 was 377 copies/ml, and 392 had baseline cervical swabs collected. Median cervical HIV-1 RNA levels at baseline, 7, 14, and 21 days after treatment were below the detection limit in both arms. There was no difference in the proportion of detectable cervical HIV-1 RNA between cryotherapy and LEEP at 7 (29.2 vs. 26.0;  $p = 0.502$ ), 14 (31.0 vs. 26.1;  $p = 0.310$ ), or 21 days (25.6 vs. 25.3;  $p = 0.947$ ) after intervention. The lack of a significant difference between the cervical treatments remained after adjusting for baseline cervical HIV-1 RNA and exposure to antiretroviral therapy (ART) (adjusted Incidence Rate Ratio [aIRR] = 0.91; 95% CI: 0.74-1.11). Within the LEEP arm there was a significant increase in cervical HIV-1 RNA levels compared to baseline at 7 ( $p = 0.014$ ) and 14 ( $p = 0.011$ ) days after treatment, while there was no significant increase in cervical HIV-1 shedding at any time point within the cryotherapy arm. ART unexposed, HIV-positive women receiving LEEP were more likely to shed HIV-1 RNA from the cervix at 7 [Odds Ratio (OR), 1.52;  $p = 0.048$ ] and 14 (OR, 1.60;  $p = 0.028$ ) days after intervention compared to baseline.

**Conclusions:** There was no significant difference in cervical HIV-1 RNA shedding between cryotherapy and LEEP, supporting the use of see-and-treat cervical cancer screening approaches using cryotherapy in HIV-positive women. However, cervical HIV-1 RNA shedding appears to increase for up to 2 weeks after LEEP, particularly in those not receiving ART, and warrants further investigation.

**622 Decline in Annual Pap Testing Among HIV-Positive Women in Ontario, Canada**Ann N. Burchell<sup>1</sup>; Claire Kendall<sup>2</sup>; Stephanie Cheng<sup>3</sup>; Tony Antoniou<sup>1</sup>; Ahmed M. Bayoumi<sup>1</sup>; Rick Glazier<sup>1</sup>; Aisha Lofters<sup>1</sup>; Janet M. Raboud<sup>4</sup>; Mark Yudin<sup>1</sup>; Mona Loutfy<sup>5</sup><sup>1</sup>St Michael's Hosp, Toronto, ON, Canada; <sup>2</sup>Univ of Ottawa, Ottawa, ON, Canada; <sup>3</sup>Inst for Clinical Evaluative Scis, Toronto, ON, Canada; <sup>4</sup>Dalla Lana Sch of PH, Toronto, ON, Canada; <sup>5</sup>Women's Coll Rsr Inst, Univ of Toronto, Toronto, ON, Canada

**Background:** In 2012-13, North American cervical cancer screening guidelines were revised to recommend a lengthened 3-year screen interval for HIV-negative, average-risk women. Annual screening remains recommended for HIV-positive women due to heightened cancer risk. To determine whether the changes had unintended effects among women with HIV, we estimated annual rates of Pap testing among screen-eligible women in Ontario, Canada, by HIV status. We also identified prognostic factors for annual testing among HIV-positive women.

**Methods:** We conducted a population-based cohort study using administrative health data. We identified HIV-positive women using validated algorithms and Pap testing using laboratory and procedure codes. Annual proportions of women tested at least once from 2008 to 2013 were compared between HIV-positive and -negative women, adjusted for age, geographic region, immigration status, neighborhood income quintile and comorbidity score using John's Hopkins' Aggregated Diagnosis Groups. Among HIV-positive women, prognostic indicators were identified using generalized estimating equations with a log link to calculate adjusted ratios of proportions (ARP).

**Results:** A total of 2,600 HIV-positive and 4,731,760 HIV-negative women were included. From 2008-11, annual proportions tested were constant [2011, HIV+: 32.6% (CI 31.0-34.3); HIV-: 36.0% (CI 36.0-36.1)] then declined [2013, HIV+: 26.9% (CI 25.6-28.2); HIV-: 19.8% (CI 19.8-19.9)]. In the sub-analysis of HIV+ women, women most likely to undergo cytologic testing were younger (21-29 years cf 50-70: ARP 1.43, 95%CI 1.22-1.67), recent entries to HIV care ( $\geq 2008$  cf  $< 2000$ : ARP 1.22, 95%CI 1.04-1.42), immigrants from HIV-endemic countries (cf non-immigrants: 1.19, 95%CI 1.08-1.31), lived in highest income neighbourhoods (cf lowest: ARP 1.19, 95%CI 1.02-1.38), had a higher comorbidity score (RUB 3-5 cf 0-2: ARP 1.58, 95%CI 1.17-2.14), and a female physician (cf male: ARP 1.15, 95%CI 1.04-1.25). In all, 28.7% of HIV+ women had no Pap testing over the 6-year period.

**Conclusions:** There was a concerning drop in annual Pap testing among HIV-positive women following recommendations for less frequent screening for average risk women. A substantial proportion was underscreened even in this setting with universal access to medically-necessary services. As jurisdictions across North America aim to improve guideline adherence for less frequent screening in the general population, care must be taken to ensure that cancer prevention remains a priority among HIV-positive women.

**623 Feasibility of Post-Partum Testing for HR-HPV Among HIV-Positive and -Negative Women**Erin Dressel<sup>1</sup>; Gabriela DelBianco<sup>2</sup>; Gilhen Rodriguez<sup>2</sup>; James R. Murphy<sup>2</sup>; Gloria P. Heresi<sup>2</sup>; Laura J. Benjamins<sup>2</sup><sup>1</sup>Univ of Texas Hlth Sci Cntr at Houston, Houston, TX, USA; <sup>2</sup>Univ of Texas Med Sch at Houston, Houston, TX, USA

**Background:** More than 80% of deaths related to cervical cancer occur in developing countries. Lack of access to screening procedures delays diagnosis and contributes to increased morbidity and mortality. Women with HIV have a higher prevalence of HPV infection; HPV is also more likely to persist in HIV-positive women, which contributes to an increased risk of cervical cancer. For underserved women, childbirth may be a woman's first entry into medical care. Identifying methods of screening for High-Risk Human Papillomavirus (HR-HPV) at delivery could improve detection for high-risk groups. We therefore set out to assess the feasibility of testing a cohort of HIV-positive and negative women for HR-HPV shortly after delivery, and to evaluate associated co-morbid conditions.

**Methods:** We conducted a retrospective chart review of 37 HIV-positive and 35 HIV-negative mothers enrolled in a study evaluating vertical transmission of HR-HPV. Results from vaginal swabs collected 24-72 hours after delivery were reviewed, as well as maternal charts for sexually transmitted infections (STI) and obstetric history. Descriptive statistics and chi square analysis were used.

**Results:** 92% of the mothers were African American and 8% Hispanic. The majority of patients had Medicaid or the County District insurance. Among HIV-positive women, 16 (43%) tested positive for HR-HPV post-partum (PP) compared to 10 (29%) of women without HIV (p=0.23). In addition, among those with a positive HR-HPV PP test, 6 (38%) of HIV-positive women had history of STI during pregnancy and 5 (31%) received no prenatal care, compared to 1 (10%) and 2 (20%) of HIV-negative women, respectively. Among women with no documented history of an abnormal PAP smear, 10/24 (42%) HIV-positive women and 5/29 (17%) HIV-negative women had a positive HR-HPV PP test (p=0.07).

**Conclusions:** We found a higher prevalence HR-HPV in women infected with HIV. Having an STI during pregnancy or no prenatal care could be potential risk factors for HR-HPV PP among HIV-positive women. Of the women positive for HR-HPV PP 15/53 (28%) had not previously been identified; two thirds of these women had HIV. For underserved women where access to care is difficult or limited, testing for HR-HPV during the post-partum period may be a feasible screening tool.

**624 Role of p16 Testing in Cervical Cancer Screening Among HIV-Infected Women**

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**Background:** We evaluated the utility of p16 staining to screen for cervical intraepithelial neoplasia 2 or 3 (CIN2/3) among HIV-infected women.

**Methods:** Between June and December 2009, 500 HIV-infected women underwent Papanicolaou (Pap) smear, visual inspection for acetic acid (VIA), high-risk human papillomavirus (hrHPV) testing, and colposcopy-directed biopsy (gold standard) at the Coptic Hope Center in Nairobi, Kenya. After Pap smears were read for cytology, a portion of samples were de-stained and treated with p16 immunohistochemistry to evaluate for expression of p16 protein by a separate study pathologist. Defining disease as the detection of CIN2/3 by colposcopy-directed biopsy, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of abnormal Pap smear (low-grade squamous intraepithelial lesions or greater [LSIL+]), hrHPV, and positive VIA were compared to the positive detection of p16 protein alone and in combination.

**Results:** Of 500 HIV-infected women who were originally screened, 150 samples had p16 protein expression results available for analysis. On histology, 43 (28.7%) were normal, 65 (43.3%) CIN1, 18 (12.0%) CIN2, 11 (7.3%) CIN3, and 13 (8.7%) indeterminate. On cytology, 52 (34.7%) were normal, 33 (22.0%) ASCUS, 40 (26.7%) LSIL, 17 (11.3%) HSIL, and 8 (5.3%) indeterminate. On VIA, 43 (28.7%) were positive, 102 (68.0%) were negative, and 5 (3.3%) indeterminate. On hrHPV, 78 (52.0%) were positive and 72 (48.0%) were negative. On p16, 40 (26.7%) were positive and 110 (73.3%) were negative. The sensitivity, specificity, PPV and NPV were: 62.1%, 67.8%, 31.6%, and 88.2% for Pap smear; 51.7%, 72.7%, 31.3%, and 86.3% for VIA; 82.8%, 55.4%, 30.8%, and 93.1% for hrHPV; and 41.4%, 76.9%, 30.0%, and 84.6%, for p16. Triaging an abnormal result with a p16 test reduced sensitivity and increased specificity from 62.1% to 31.0% and 67.8% to 90.1% for Pap smear; 51.7% to 24.2% and 72.7% to 93.4% for VIA; and 82.8% to 37.9% and 55.4% to 86.0% for hrHPV.

**Conclusions:** Using cytologic p16 protein expression as an adjunctive test to triage positive results to screen for cervical cancer significantly increases specificity but also decreases the sensitivity of Pap smear, VIA, and hrHPV among HIV-infected women. Use of p16 testing alone as a screening tool has relatively high specificity (76.9%) but lower sensitivity (41.4%). Additional data and analyses are needed to assess the utility of p16 testing to detect CIN2/3 among HIV-infected women.

**625 High Prevalence of HR-HPV Infection in HIV-Infected Women From Tanzania**

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**Background:** Cervical cancer caused by high risk human papilloma virus (HR-HPV) is among the leading cancer in sub-Saharan Africa, especially in HIV infected women. Screening programs are based on a screen-and-treat approach including visual inspection with acetic acid (VIA). Alternatively HR-HPV based screening is discussed to have greater sensitivity to detect cancerous and pre-malignant lesions. The objective of our analysis was to investigate the association of HR-HPV genotypes with cervical lesions in HIV+ and HIV- women, risk factors and HPV based screening feasibility.

**Methods:** In our analysis we included HIV+ and HIV- women who received VIA based screening with available cytological/histological (Bethesda criteria) and HPV genotyping results (Linear Array HPV Test, Roche Molecular Systems) from the ongoing, prospective 2H cohort study conducted in Mbeya, Tanzania.

**Results:** Among 376 women screened 203 (54%) were HIV infected (68% on ART, 19% with CD4 counts <200 cells/μl). Cervical cancer, high grade squamous intraepithelial lesions (HSIL), low grade squamous intraepithelial lesions (LSIL) or no lesions were detected in 8.2%, 4.5%, 13.0% and 74.2% of women. HR-HPV genotypes were detected in 65.1% of HIV+ and 34.9% of HIV- women. Risk factors associated with HR-HPV were HIV infection (RR 1.6, 95%CI 1.3 to 1.9, p<0.001), CD4 count <200 cells/μl (RR 1.4, 95%CI 1.1 to 1.6, p=0.001), first sexual intercourse before 18 years of age (RR 1.3, 95%CI 1.2 to 1.6, p=0.013), and age below 30 years (RR 1.4, 95%CI 1.1 to 1.6, p=0.002). In cancer/HSIL cases HR-HPV was detected in 93.8% and HPV16/18 in 60.4%, and proportions were comparable between HIV+ and HIV- women. Of note HR-HPV or HPV 16/18 was also detected in 43.4% (HIV+ 53.7% versus HIV- 33.6, p=0.001) and 21.5% in women without cervical lesions. The sensitivity of HR-HPV as compared to VIA based screening to detect cancer/HSIL in HIV infected women was slightly higher (95.8% versus 89.5%) but low for specificity (36.9% versus 75.9%) and positive predictive values (7.4% versus 16.3).

**Conclusions:** We detected high proportions of cervical HR-HPV infections, especially in HIV infected, young women with low CD4 counts. As expected, HR-HPV infection was prevalent in cancer/HSIL cases, but also frequently detected in HIV infected women without cervical lesions. Low HR-HPV test specificity therefore impacts the feasibility of HPV based screening algorithms, the long term impact of HPV/HIV co-infection without cervical disease needs to be evaluated.

		Total	Cancer	HSIL/CIN2+	LSIL/CIN1	No lesion
Screening outcome (cytology/histology)	HIV-	173 (46%)	19 (11%)	5 (3%)	6 (3%)	143 (83%)
	HIV+	203 (54%)	12 (6%)	12 (6%)	43 (21%)	136 (67%)
VIA positive or suspicion of cancer	HIV-	31/173 (18%)	17/19 (89%)	3/5 (60%)	2/6 (33%)	9/143(6%)
	HIV+	52/203 (26%)	9/12 (75%)	8/12 (67%)	17/43 (40%)	18/136 (13%)
High risk HPV genotypes detected (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68)	HIV-	73/173 (42%)	17/19 (89%)	5/5 (100%)	3/6 (50%)	48/143 (34%)
	HIV+	136/203 (67%)	11/12 (92%)	12/12 (100%)	40/43 (93%)	73/136 (54%)
HPV 16/18 detected	HIV-	43/173 (25%)	11/19 (58%)	3/5 (60%)	1/6 (17%)	28/143 (20%)
	HIV+	70/203 (34%)	9/12 (75%)	6/12 (50%)	23/43 (53%)	32/136 (24%)

**626 HPV Type Distribution in HIV-Infected Persons With Anal HSIL and Impact on Recurrence**

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**Background:** Infection of the anal canal by high-risk human papillomavirus (HR-HPV) is associated with high-grade squamous intraepithelial lesions (HSIL), the precursor of anal carcinoma. HPV type distribution in anal HSIL and its potential impact on recurrent lesions following ablation are poorly understood.

**Methods:** 110 intraanal HSILs from 89 HIV-infected patients were analyzed. Probe-based qPCR was used to detect HR-HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 from genomic DNA extracted from biopsy specimens. HSIL was treated using electrocautery ablation (EA) and surveillance biopsies of suspicious lesions were obtained during follow-up evaluations. Clinical data were abstracted from a longitudinal database.

**Results:** 71 (80%) patients were men who have sex with men (MSM), 16 (18%) women and 2 (2%) heterosexual men (HM). 66 patients (74%) had moderate and 23 (26%) had severe dysplasia. 16% of biopsies had no HPV detected from the selected panel of 15 high-risk types; 58% had a single HPV type, 19% had two types, and 7% had ≥3 HPV types. HPV16 was the most prevalent HPV type detected in 38% of samples, followed by HPV33 (12%), HPV35 (9%) and HPV39 (8%). HPV18 was present in only 3% and HPV26 was not detected in any sample.

Anal infection with >1 HPV type was present in 16 patients (24%) with moderate and in 12 (52%) with severe dysplasia (p=0.01). Women were more likely to be concomitantly infected with >1 HR-HPV type (56%) than MSM (27%) and HM (0%; p=0.04). Neither CD4 T-cell count, smoking history, receptive anal sex nor age were significantly associated with infection by multiple HR-HPV types.

There was low correlation between HR-HPV types present in separate lesions in patients with multiple HSILs (R=0.01).

Surveillance biopsies were obtained in 52 patients within a median follow-up of 29 months (IQR 19-35). 43% had recurrent HSIL and 57% had low grade or no dysplasia on follow-up. Recurrent HSIL did not correlate significantly with number or type of HR-HPV on initial biopsy.

**Conclusions:** Anal HSIL contains a wide range of HR-HPV types and presence of multiple types per lesion is common. HPV16 was most prevalent and infection with >1 HR-HPV type was associated with more severe dysplasia. The risk of developing recurrent HSIL following EA is high and not associated with number or type of HR-HPV on initial biopsy.

**627 Multiple HPV Genotypes As a Risk Factor for High Grade AIN in HIV-1 Infected Males**

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**Background:** HIV-infected patients (pts) have an increased risk for HPV infection and related lesions. Objectives of the study were to determine prevalence of high grade anal intraepithelial neoplasia, high risk HPV (HR-HPV) genotypes and to identify factors associated with a high grade anal intraepithelial neoplasia in a cohort of HIV-infected males.

**Methods:** Cross-sectional study on HIV-infected pts followed at the Department of Infectious Diseases of San Raffaele Scientific Institute with HPV screening performed in absence of clinical symptoms. We considered in the analysis pts who were tested for multiple HPV genotypes (Abbott Real Time High Risk HPV DNA). The presence of oncogenic HPV genotypes (16,18,31,33,35,39,45,51,52,56,58,59,66,68) classified pts as having HR-HPV. Pts were categorized according to the cytological findings (AIN): no (AIN=0)/low (AIN=1) grade of anal intraepithelial neoplasia (LG-AIN) or high (AIN=2-3) grade anal intraepithelial neoplasia (HG-AIN).

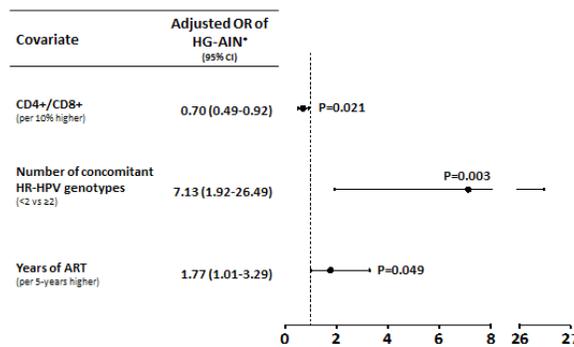
Results were described with median (IQR) or frequency (%). Logistic regression was used to identify risk factors for HG-AIN.

**Results:** 669 pts were screened and in 273/669 pts data on multiple oncogenic HPV type were available and were considered in the analyses. Pts' characteristics at HPV screening were: age 43.9(37.8-50.1) years, 75% MSM, HIV diagnosis since 9.6(3.7-17.3) years, 50% with previous syphilis, CD4+ 611(470-787) cells/μL, CD4+/CD8+ 0.62(0.42-0.93), 97% pts were treated with antiretroviral therapy (ART) since 7.2(1.8-14.1) years, 75% had HIV-RNA<50 cps/mL.

Eighteen (7%) pts had HG-AIN; 111 (41%) pts had HR-HPV infection: HPV-16 was found in 75 (32%) pts and HPV-18 in 47 (20%) pts. Both HPV types were more frequent among pts with HG-AIN [HPV-16: 72% vs 29% in HG-AIN and LG-AIN, respectively (p<0.0001); HPV-18: 44% vs 18% in HG-AIN and LG-AIN, respectively (p=0.013)]. Seventy-one pts (26%) had multiple HPV genotypes. HG-AIN was more frequent among pts with multiple HR-HPV types (≥2) rather than pts with 0-1 genotypes [12 (17%) vs 6 (3%), p<0.0001].

At multivariate logistic regression (Figure 1) we found that pts with a lower CD4+/CD8+ ratio, ≥2 HR-HPV genotypes and longer ART duration were more likely to have HG-AIN.

**Conclusions:** Infection with multiple strains of HR-HPV is a risk factor for HG-AIN, in addition to CD4+/CD8+ ratio and years of ART. These features suggest that the identification of HPV genotypes over time might improve prevention of HPV-related neoplasms.



\* Also adjusted for age, risk factor (MSM vs other), previous syphilis infection, nadir CD4+ and log<sub>10</sub>HIV-RNA

**628 Prevalence of HPV-Related Lesions in an Urban Cohort of HIV-Positive Men in Germany**

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**Background:** Human papillomaviruses (HPV) induce condylomata, anogenital cancers and their precursor lesions as anal or penile intraepithelial neoplasia (AIN/PIN). HIV-positive individuals have an increased risk for the development of anogenital HPV-induced lesions.

The aim of the male Screening-Study was to evaluate the prevalence of genital and anal condylomata, intraepithelial neoplasia, anal and penile cancer, and related HPV-types in HIV-infected men at the Interdisciplinary Immunological Outpatient Clinic of the Department of Dermatology of the St. Josef Hospital, Ruhr-University Bochum.

**Methods:** 400 HIV-positive men (98% men who have sex with men (MSM)) were enrolled in this prospective observational study from 2008 to 2011. Participants were seen every 3 to 12 months. All patients received an inspection of the anogenital region, digital rectal examination, high-resolution anoscopy (HRA), anal cytology, anal/penile histology if required, and HPV-typing of anal and penile swabs. The statistical analysis was performed in a descriptive manner; no formal statistical hypotheses were tested. Approximate 95% confidence interval was calculated with an approximate formula [Woodward]. The prevalence analyses were performed by the statistic software SAS version 9.3 TS1M0.

**Results:** At baseline only 25% of the 400 HIV-infected men had normal anal results, 75% of the men presented with abnormal cytological and histological results. 8% presented with ASCUS (atypical squamous cells of undetermined significance), 41% with low-grade (n=164) dysplasia and 24% with high-grade anal dysplasia (n=95), and two men with invasive anal cancer (0.5%). 2.3% had PIN (n=9) and one patient had penile cancer at baseline. Throughout the study period only 16.8% of the patients always had normal anal results. 75% had anal dysplasia (low-grade n=177, high-grade n=125), 3.3% (n=13) had PIN, and two further patients developed anal cancer. Within the study period 52.8% (n=211) had condylomata.

At baseline, 88.5% of anal and 39.3% of penile swabs were HPV-DNA positive, and 77.8% of anal and 26.5% of penile swabs carried high-risk HPV-types. HPV16 was the most frequent HPV-type.

**Conclusions:** HIV-positive MSM have a high risk for HPV-induced condylomata, (pre)malignant anogenital lesions and anogenital cancers. Screening for HPV-induced dysplasia is crucial to avoid progression to invasive carcinomas. Additionally, HPV-vaccination recommendations should be extended to high-risk populations.

## 629 APOBEC3 Boosters Kill Primary Effusion Lymphoma and Other Cancer Cell Lines

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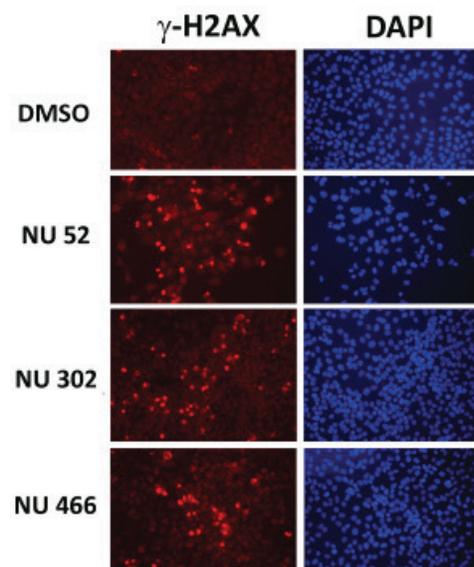
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**Background:** DNA cytosine deamination of chromosomal DNA by APOBEC3B (A3B) is a major source of mutation associated with worsening of malignant phenotypes in different types of cancer. This includes primary effusion lymphoma (PEL), a continuing treatment challenge in AIDS patients. We have identified small molecules that boost APOBEC3G (A3G) and APOBEC3F (A3F) protein levels by increasing post-translational stability of these proteins (data not shown). Here, we test effects of these compounds on APOBEC3B (A3B) levels in cancer cell lines, and whether they affect proliferation/viability of cancer cell lines.

**Methods:** 3 PEL cell lines (BC-1, BC-3, BCBL1), 5 breast cancer lines (MCF10A, MCF7, T47D, MDA-MB-157 and MDA-MB-231), and a cervical cancer cell line (HeLa) were treated with either one of our A3 boosters or DMSO for 8 days or more. BC-1, BC-3, and BCBL1 are each KSHV-infected, and BC-1 is also co-infected by EBV. MCF-10A and MCF7 breast cancer cells have low baseline A3B mRNA; all other lines studied have higher baseline A3B mRNA levels. Primary CD4+ T lymphocytes and HEK293 were controls lacking A3B expression.

**Results:** No cell line tested had higher cell counts after exposure to any of the A3 boosters than to DMSO. Each of the 3 PEL cell lines had markedly decreased cell numbers after exposure to each A3 booster. The other cell lines previously reported to have elevated baseline A3B levels (T47D, MDA-MB-157, MDA-MB-231, HeLa) also were killed after exposure to each A3 booster studied. Cell counts of HEK293 cells, MCF-10a cells, MCF7 cells, and primary lymphocytes did not differ in A3 boosters versus DMSO. Anti-A3B antibody immunofluorescence of MDA-MB-231 breast cancer cells was increased by A3 booster exposure, relative to DMSO. MDA-MB-231 cells exposed to each of 3 A3 boosters had DNA damage (Fig. 1) and increased staining for activated caspase and PARP.

**Conclusions:** The A3 boosters did not increase proliferation of any cancer cell lines studied, increased A3B protein level in MDA-MB-231 cells, and had marked cytotoxic effects against PEL and several other cancer cell lines with high baseline A3B expression. The data suggest that further increasing high cancer-associated baseline A3B protein levels may enhance DNA damage beyond a 'mutation catastrophe' threshold, cause apoptosis-mediated synthetic lethality, and have potential as a novel strategy for PEL in AIDS.



## 630 Risk Factors for Hodgkin (HL) and Non-Hodgkin Lymphoma (NHL) in Europe

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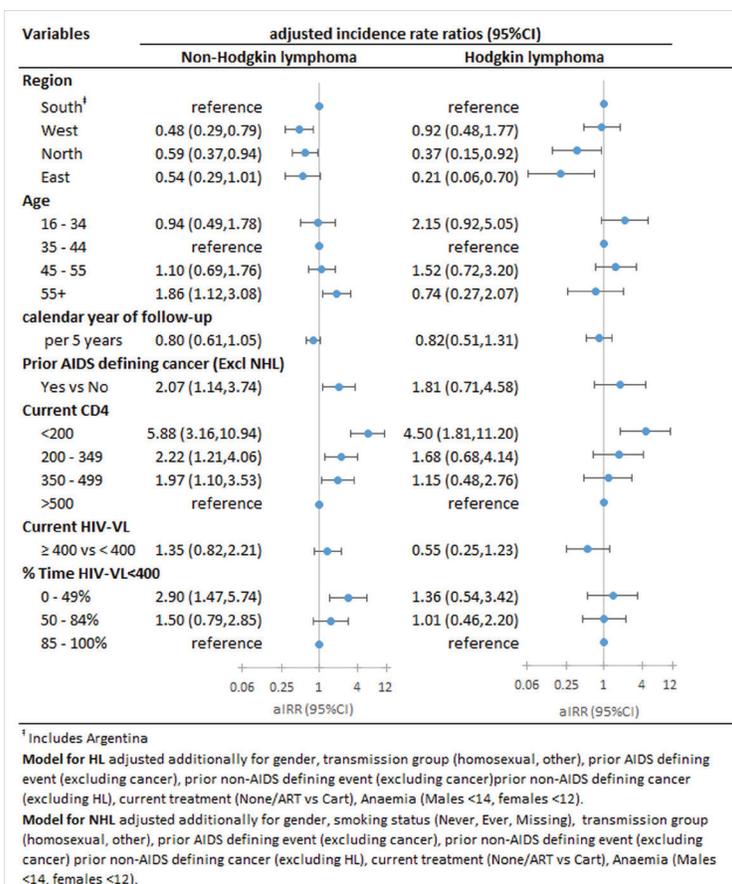
<sup>1</sup>Univ Coll London, London, UK; <sup>2</sup>CHIP, Rigshospitalet, Univ of Copenhagen, Copenhagen, Denmark; <sup>3</sup>Medizinische Poliklinik, Munich, Germany; <sup>4</sup>Hosp for Infectious Diseases in Warsaw, Med Univ of Warsaw, Warsaw, Poland; <sup>5</sup>Academician Blokhina Nizhny Novgorod Rsr Inst of Epi and Microbiology, Novgorod, Russian Federation; <sup>6</sup>Univ Hosp Basel, Basel, Switzerland; <sup>7</sup>Hosp J.M. Ramos Mejia, Buenos Aires, Argentina; <sup>8</sup>Rigshospitalet, Univ of Copenhagen, Copenhagen, Denmark

**Background:** NHL and HL are common in HIV+ people. Previous research has described significant declines in NHL and, to a lesser extent, HL after cART, but data on individual risk factors is limited. We sought to determine the role of demographic factors, and cumulative time spent with immunodeficiency (CD4 <200) and viral suppression (HIV RNA <400) on NHL and HL across Europe.

**Methods:** EuroSIDA participants with follow-up after 1/1/2001 and without NHL or HL at baseline were included and followed to first NHL or HL diagnosis, last visit or death. Risk factors for NHL and HL were assessed separately using Poisson regression including current and cumulative measures of HIV RNA (% of time with HIV RNA <400 copies/ml) and immunosuppression (% of time with CD4 <200 cells/mm<sup>3</sup>).

**Results:** 14820 people contributed 101281 person years of follow-up (PYFU), 117 developed NHL (incidence rate 1.2/1000 PYFU, 95%CI 1.0-1.5) and 45 developed HL (0.5/1000 PYFU, 95%CI 0.3-0.6). Crude incidence of NHL and HL declined by 12% (95%CI: 7-16%) and 9% (95% CI: 1-15%) per year, however, no trend remained after adjustment (figure). In adjusted analyses, NHL incidence was lower in North and West and HL incidence in North and East compared to south Europe (figure). Lower current CD4 cell count (figure), but not cumulative exposure to immunodeficiency (P>0.05), was associated with higher incidence of both NHL and HL. NHL incidence was strongly associated with current HIV RNA in univariate analyses (incidence rate ratio [IRR]: 3.35 95%CI: 2.33,4.83), but after adjustment, a history of poor control of HIV infection were more strongly associated with NHL, such as a prior diagnosis of AIDS-defining malignancies and lower % of time with controlled HIV RNA (figure). Other risk factors included older age (NHL only) and region of follow-up (both), whereas nadir CD4 was not associated with either HL or NHL (P>0.05).

**Conclusions:** Incidence of NHL and HL vary significantly by region, possibly reflecting differences in long term virological suppression through access to and availability of cART. NHL incidence was associated with lower CD4 and cumulative exposure to viral replication over time, suggesting that exposure to uncontrolled viral replication may play a part in NHL development in addition to current immunodeficiency. Conversely, HL incidence was elevated in those with current severe immunodeficiency (CD4 <200), but cumulative exposure to uncontrolled HIV replication or immunodeficiency were not found to be significant risk factors.



**631 Kaposi Sarcoma Survival in Adults: The South African Antiretroviral Treatment Era**

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**Background:** Kaposi sarcoma (KS) is the most common malignancy in HIV infected people in sub-Saharan Africa. The advent of antiretroviral treatment (ART) in western countries resulted in dramatic decreases in KS incidence, morbidity and mortality. Studies in the early ART era in South Africa have shown that KS morbidity and mortality is still high due to inadequate antiretroviral treatment coverage and late diagnosis. The South African ART national programme is well established with over a decade of rapid scale-up of ART programmes nationwide. We examined whether the wider availability of ART improved survival of KS patients receiving care from a tertiary academic hospital oncology unit in Pretoria, South Africa.

**Methods:** We did a retrospective review of electronic and paper records of HIV-positive KS patients accessing treatment at the Steve Biko Academic Hospital (SBAH) oncology unit between May 2004 and September 2012. We compared survival of KS patients admitted 2004-2008 and 2009-2012 and examined predictors of mortality. Patient characteristics were described using frequencies, medians and interquartile ranges. Kaplan Meier survival functions were used to estimate survival at one and two years of follow-up and multivariable Cox regression models were fitted to identify predictors for mortality.

**Results:** A total of 357 HIV-positive KS patients were included in the analysis. Nearly all patients (353, 99%) were Black Africans, (224, 63%) were male, the median age at cancer diagnosis was 37 (IQR 30-43 years) and median CD4 count was 242 (130-403 cells/μL). Most patients received ART (332, 93%), 101 (28%) received chemotherapy and radiotherapy, 108 (30%) received radiotherapy only, 68 (19%) received chemotherapy only, and 80 (22%) did not receive radio- or chemotherapy. One-year survival was 75% (95% CI 66-82) and 85% (95% CI 78-90) in the 2004-2008 and 2009-2012 time-periods, respectively (p<0.001). Two-year survival was 60% (95% CI 50-69) and 83% (95% CI 73-88) in the 2004-2008 and 2009-2012 time-periods, respectively (p<0.001). More recent calendar period, chemotherapy, radiotherapy and less advanced disease stage were associated with improved survival (Table).

**Conclusions:** The survival of HIV-positive patients with KS has improved in recent years in South Africa as more patients access ART. Chemotherapy and radiation therapy improves survival of HIV-positive patients with KS.

**Table: Predictors of mortality in KS patients**

	Hazard ratio 95% CI
<b>Enrolment period</b>	
2004-2008	1
2009-2012	0.22 (0.12 – 0.40)
<b>Baseline CD4 count (cells/μl)</b>	
< 100	1
100-199	0.64 (0.29 – 1.42)
200-349	0.98 (0.46 – 2.10)
350-500	1.18 (0.52 – 2.69)
≥ 500	0.94 (0.36 – 2.46)
<b>KS stage</b>	
No advanced disease	1
Advanced disease	2.35 (1.34 – 4.12)
<b>Chemotherapy</b>	
No	1
Yes	0.22 (0.12 – 0.41)
<b>Radiotherapy</b>	
No	1
Yes	0.53 (0.32 – 0.90)
<b>ART use</b>	
Not on ART at KS diagnosis	1
On ART KS at diagnosis	1.13 (0.65 – 1.95)

Model adjusted for all variables listed and age, sex and history of tuberculosis

## 632 HIV Testing, Status, and Treatment Among Patients at the Uganda Cancer Institute

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**Background:** HIV increases the incidence and mortality of cancer; knowledge of HIV status and treatment is essential for the management of patients with HIV-associated malignancies (HIVAM). In Uganda, where the prevalence of HIV infection is >7%, the incidence of AIDS-defining cancers (ADCs) -including Kaposi sarcoma, Non-Hodgkin lymphoma, and cervical cancer- is high, and Non-AIDS defining cancers (NADCs) are increasingly common among HIV-infected (HIV+) persons. We determined how often cancer providers documented the HIV status and clinical parameters of HIV infection among patients presenting for care at the Uganda Cancer Institute (UCI), the primary cancer treatment facility for a catchment area of 100 million.

**Methods:** Medical records of patients aged ≥18 who registered at the UCI between May-August 2015 were abstracted for demographics, cancer and HIV parameters. We calculated binomial proportions and used  $\chi^2$  tests and logistic regression to evaluate factors associated with HIV testing, HIV-positivity, and antiretroviral therapy (ART) usage.

**Results:** Among 556 patients, 30% had a potential ADC. 67.8% of charts documented HIV status. Of those with documented HIV status, 137 (36%) were HIV+, and 58% of HIV+ individuals had an ADC. The documented HIV prevalence in NADCs was 24%. Men were 1.75-fold more likely to be HIV positive (95% CI 1.15-2.68,  $p=0.009$ ), however, women were more likely to have undocumented HIV status (RR 1.32,  $p=0.009$ ). Women accounted for 54.6% of all patients; 36% of women lacked HIV test results, including 40% of women with cervical cancer. HIV+ patients were younger compared with those known to be HIV-negative (median age 41 vs 50,  $p<0.001$ ). Among those with documented HIV infection, 62% had a CD4 count recorded in the chart. The median CD4 count among persons with ADCs was 300 cells/ml (interquartile range 114-395) compared with NADC (median 353, IQR 185-601),  $p=0.08$ . There was no difference in the proportion of HIV patients receiving ART prior to UCI registration between those with ADC vs. NADC (86%),  $p=0.45$ .

**Conclusions:** HIV prevalence was 5 times higher in Ugandan cancer patients with documented status than the general population. Though the majority of cancer patients had HIV testing performed, gaps remained in documenting HIV status, CD4 count and ART usage, even among patients with ADCs. This study highlights opportunities to educate cancer clinicians in Africa on the burden of HIV in cancer patients and the importance of managing both diseases in patients with HIVAM.

**Table 1. Characteristics of cancer patients presenting to the Uganda Cancer Institute for cancer care during three months in 2015.**

We evaluated demographic and clinical differences in patients who had an HIV status documented in their medical record (HIV+/HIV-) or no HIV status documented.

	HIV + (N=137)	HIV - (N = 240)	HIV Unknown (N = 179)	P value
	N (%)	N(%)	N(%)	
Sex (Female)	59(43.1)	137(57.1)	108(60.3)	.006
Age				<.001
18-49	109(79.6)	131(54.6)	71(39.7)	
>50	28(20.4)	109(45.4)	108(60.3)	
Cancer Type				<.001
Possible ADC <sup>a</sup>	79(57.7)	54(22.5)	34(19.0)	
Tumor Stage				<.001
Early (I/II)	31(22.6) <sup>b</sup>	17(7.8)	17(9.5)	
Late (III/IV)	55(40.1)	89(37.1)	56(31.3)	
Not-stageable <sup>c</sup>	51(37.2)	134(55.8)	106(59.2)	
On ART	118(86.1)			

Abbreviations: ADC, AIDS-defining cancer; NADC, non-AIDS defining cancer; ART, antiretroviral therapy  
<sup>a</sup>ADCs include Kaposi's sarcoma, non-Hodgkin lymphoma, and cervical cancer, but are only defined as such in the presence of HIV infection. Here these tumor types are considered possible-ADC because in the HIV-unknown patients, these may or may not be ADCs. NADCs include all other cancer types whether or not HIV-infection is present

<sup>b</sup>27 of 31 early stage tumors in HIV+ were Kaposi's sarcoma, likely accounting for higher prevalence of early stage cancers in HIV+ than in HIV- or HIV unknown.

<sup>c</sup>Staging compiled as listed by the respective staging system for each tumor in chart or derived from medical notes and imaging when possible, otherwise listed as not-stageable by available documentation.

## 633 Low CD4/CD8 Ratio As a Predictor of Cancer Risk in HIV-Infected Persons

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**Background:** Low CD4/CD8 ratios in HIV-infected (HIV+) persons may represent dysfunctional immune activation and have been found to be associated with increased non-AIDS associated mortality. Using data from a large cohort of HIV+ Veterans, we investigated the relationship between cumulative CD4/CD8 ratio and risk of incident non-AIDS defining (NADC) and AIDS defining (ADC) cancers.

**Methods:** We linked the Veterans Aging Cohort Study (VACS) to the Veterans Affairs Central Cancer Registry to yield a cohort of 26,115 HIV+ subjects followed for a minimum of 2 years, during 1997-2012. We categorized the primary exposure of interest, longitudinal CD4/CD8 ratio, using previously identified cut points for non-AIDS event risk (<0.7 vs ≥0.7). We calculated observation time from VACS enrolment to the earliest of: pathologically confirmed incident cancer, loss to follow-up, or death. Cancers were classified as NADC or ADC and then further subclassified by viral association and anatomic site. Our CD4/CD8 ratio measure was the 18-month simple moving average (SMA), lagged by 6 months (to minimize reverse causality). These lagged SMAs were then evaluated as time-updated covariates in separate Cox proportional hazard regression models for each cancer type (ADC, NADC, virus-related NADC, and the most common constituents of these groups). Models were adjusted for demographics, smoking, drug and alcohol use disorders, recent CD4 count, and hepatitis C virus infection.

**Results:** We identified 1,259 and 229 incident NADCs and ADCs, respectively, in our cohort. Baseline median CD4/CD8 ratio and CD4 count was lower for subjects who were eventually diagnosed with both NADCs and ADCs compared to patients who never developed cancer. In Cox regression models (Table 1) cumulative CD4/CD8 ratio <0.7 was significantly associated with increased risk of NADCs (hazard ratio [HR]: 1.2; 95% CI: 1.1-1.4) but not with ADCs or the subset of NADCs specifically associated with viral co-infections after adjustment for potential confounders. Among NADCs only lung cancer (HR: 1.5; 95% CI: 1.1-2.1) and anal cancer (HR: 2.0; 95% CI: 1.1-3.8) were associated with low cumulative CD4/CD8 ratios after adjustment; there was no association with other NADCs.

**Conclusions:** In our large, antiretroviral therapy-era HIV cohort, we found that cumulative exposure to low CD4/CD8 ratio was associated with lung cancer and anal cancer risk after adjustment for potential confounders including recent CD4 count.

Table 1. Hazard Ratios and 95% CIs for Cumulative CD4/CD8 Ratio\* in Relation to Cancer Risk in Separate Adjusted Cox Models\*\*

Cancer group or type (number of incident cancers)	Cancer Incidence	
	Hazard Ratio**	95% CI
<b>All AIDS Defining Cancers (n=229)</b>		
CD4/CD8 Ratio*		
<0.7	1.4	0.9-2.2
≥0.7	REF	REF
<b>Non-Hodgkin Lymphoma (n=139)</b>		
CD4/CD8 Ratio*		
<0.7	1.4	0.9-2.4
≥0.7	REF	REF
<b>Kaposi Sarcoma (n=89)</b>		
CD4/CD8 Ratio*		
<0.7	1.6	0.7-3.8
≥0.7	REF	REF
<b>All Non-AIDS Defining Cancers (n=1259)</b>		
CD4/CD8 Ratio*		
<0.7	1.2	1.1-1.4
≥0.7	REF	REF
<b>Non-AIDS Defining Virally Associated Cancers (n=268)</b>		
CD4/CD8 Ratio*		
<0.7	1.3	0.9-1.8
≥0.7	REF	REF
<b>Prostate Cancer (n=317)</b>		
CD4/CD8 Ratio*		
<0.7	1.0	0.7-1.2
≥0.7	REF	REF
<b>Anal Cancer (n=109)</b>		
CD4/CD8 Ratio*		
<0.7	2.0	1.1-3.8
≥0.7	REF	REF
<b>Lung Cancer (n=274)</b>		
CD4/CD8 Ratio*		
<0.7	1.5	1.1-2.1
≥0.7	REF	REF
<b>Liver Cancer (n=88)</b>		
CD4/CD8 Ratio*		
<0.7	1.1	0.6-1.8
≥0.7	REF	REF
<b>Colorectal Cancer (n=74)</b>		
CD4/CD8 Ratio*		
<0.7	1.1	0.6-1.9
≥0.7	REF	REF

\*18-month simple moving average of CD4/CD8 ratio lagged by 6 months

\*\*Individual time-updated Cox regression models for risk of specified cancer types adjusted for age, sex, race, smoking status, alcohol use disorder, hepatitis C virus infection, and recent CD4 count

### 634 Real-Life Experience With Sorafenib for the Treatment of HCC in HIV-Infected Patients

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**Background:** Sorafenib is an oral multikinase inhibitor that has shown a survival benefit in patients with advanced hepatocellular carcinoma (HCC). To date, there is little information regarding the efficacy and safety of sorafenib in HIV-infected patients. Our objective was to report the experience with the use of sorafenib in a cohort of HIV-infected patients with HCC.

**Methods:** The GEHEP-002 cohort recruits HCC cases diagnosed in HIV-infected patients from 32 centers from Spain. For this analysis, HCC cases receiving at least one dose of sorafenib were included. Tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST). The overall survival after the start of treatment (OSaT) was defined as the time from sorafenib treatment initiation to the date of death from any cause or the date of the patient's last follow-up visit.

**Results:** 281 HCC cases have been diagnosed in HIV-infected patients in the participant centers. In 49 (17%) patients, treatment with sorafenib was started. Reasons for sorafenib use were: HCC recurrence with previous curative therapy (n=7), progression following transarterial chemoembolization (n=4), first treatment against HCC (n=38). Complete information regarding sorafenib therapy was available in 36 patients at the moment of the present analysis. The median (IRQ) elapsed time between HCC diagnosis and sorafenib initiation was 45 (19-218) days. BCLC stage at sorafenib initiation: A 3 (9%), B 2 (6%), C 26 (71%) and D 5 (14%). Median (IQR) duration of sorafenib was 60 (27-127) days. Any grade AE occurred in 21 (58%) patients. Diarrhea was the most common AE occurring in 12 (33%) patients. 15 (42%) patients developed a liver decompensation. The probability of decompensation during sorafenib was 12.5%, 50% and 90% in patients with CTP stage A, B and C, respectively (p=0.001). Antiretroviral therapy had to be modified before sorafenib initiation in 2 patients. The median CD4 cell count at sorafenib initiation and at the last clinical visit was 326 (199-623) and 305 (170-567) cells/mL, respectively (p=0.2). HIV viral load remained undetectable during therapy in all cases. Thirty (83%) patients have died at the end of the study. The median (RIQ) OSaT was 3.5 (2.1-7.1) months.

**Conclusions:** The efficacy and tolerability of sorafenib in HIV-infected patients in real-life conditions is significantly lower than figures reported in the registration clinical trial. On the contrary, sorafenib does not seem to interfere with antiretroviral therapy.

### 635 Decreased *Helicobacter pylori* Prevalence in HIV-Infected Subjects

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**Background:** HIV infected individuals are at increased risk of malignancies including gastric cancer (GC). Due to its indisputable role in gastric cancer development *H. pylori* was recognized as a 'definite carcinogen' by the World Health Organization in 1994. In the Western world about 30% of individuals are infected with *H. pylori*. Several studies have reported inconsistent data on the prevalence of *H. pylori* in HIV infected subjects. The transmission routes, risk factors and the prevalent time of infection for the two infections are substantially different. The aim of the study was to determine the prevalence of *H. pylori* and CagA as relevant virulence factor in HIV-infected subjects. As a control we used an age matched group from a previous study with healthy randomly assigned subjects that were treated in our emergency department.

**Methods:** We prospectively analyzed the serological *H. pylori* and CagA status of 203 HIV positive subjects (51 female) with a mean age of 40.8 years (±10.79 years SD). The control group of HIV-negative subjects consisted of 453 patients.

**Results:** The overall prevalence of *H. pylori* was 35% (71/203) in HIV-infected patients, compared to 43.5% in non-HIV-infected controls. CagA was detectable in 47.9% of the *H. pylori* positive patients. *H. pylori* prevalence decreased with increasing CDC stadium of the HIV patients. Patients in CDC stadium C (n=54) had a significant lower *H. pylori* prevalence (25.9%) compared to patients in the CDC-A (n=128) (39.1%) and B (n=21) (33.3%) (p=0.052). The current immune status of the HIV patients did not show a correlation with *H. pylori* prevalence. Patients with a low (< 200) (33.3%), medium (200-400) (37.2%) and a high (> 400) (32.1%) CD4 cell count did not differ in *H. pylori* infection status (p=0.496). In patients receiving antiretroviral therapy (84.7%), the *H. pylori* prevalence (34.3%) did not differ from patients without treatment (38.7%) (p=0.389). African, Asian and Hispanic patients (n=44) had an expected higher *H. pylori* prevalence (65.9%)

**Conclusions:** The serological prevalence of *H. pylori* infection in HIV-infected patients is significant lower than in HIV negative patients. In patients with advanced clinical stages of HIV the serological prevalence of *H. pylori* is lower than in patients at an early stage leading to the hypothesis that HIV-infection impacts on the diagnostic sensitivity of *H. pylori* serology. The chronicity of *H. pylori* infection seems to be driven by effective mucosal immunity disturbed in HIV- infected patients.

### 636 Design, Implementation, and Findings of Next Generation Stroke Adjudication in HIV

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**Background:** To address questions regarding stroke during HIV infection we developed a stroke adjudication protocol for HIV cohort research that enables comparisons with traditional cohort studies, addresses issues specific to HIV, and allowed us to examine factors associated with stroke in HIV.

**Methods:** CNICS is a U.S multisite clinical cohort of HIV-infected patients receiving longitudinal HIV care. The CNICS stroke protocol was based on a modified traditional protocol (Multi-Ethnic Study of Atherosclerosis). Potential events at 5 CNICS clinical sites through 12/2012 were identified. Case identification criteria included a range of diagnoses and procedure codes such as cerebral angiography. For each potential event, the site assembled de-identified packets with provider notes, imaging results, and ECGs. Antiretroviral medication exposure was redacted to allow blinded review. Using standardized criteria in the setting of ongoing quality control, two neurologists reviewed each packet, followed by a 3<sup>rd</sup> reviewer when discrepancies occurred, and categorized each stroke as Definite, Probable, or Possible. Stroke types and subtypes and whether the event was related to infection or illicit drug use was determined. Multivariable Cox regression analyses were used to determine traditional and HIV-specific risk factors for stroke comparing patients with and without stroke followed from enrollment at these 5 sites (N=16,924).

**Results:** Among 500 potential adjudicated events, 175 (35%) had a stroke. Ischemic strokes made up 81% of events, 10% were hemorrhagic, and in 9% the type was unidentifiable. Ischemic stroke subtypes included large vessel atheroembolic (19%); cardioembolic (28%); small vessel (29%); and other/unknown subtypes (23%). Strokes occurred in the setting of illicit drug use in 19% and infection in 20%. The case-fatality rate was 9%. In addition to traditional risk factors, HIV-specific factors (lower CD4 count and higher viral load) were associated with stroke (see Table).

**Conclusions:** Strokes were predominantly ischemic and were associated not only with traditional risk factors but with lower CD4 count and higher viral load suggesting potential additional benefits of earlier antiretroviral treatment initiation. Standard adjudication protocols facilitate cross-cohort comparisons but require modification to address issues such as identification of strokes related to infection or illicit drug use that is more common in HIV-infected patients.

Covariate	Adjusted Hazard Ratio	P-value	95% Conf. Interval
Age (per year)	1.06	<0.01	1.04-1.09
Female	Reference		
Male	0.88	0.6	0.56-1.40
White Race/Ethnicity	Reference		
Black	1.23	0.5	0.72-2.10
Hispanic	1.40	0.5	0.52-3.80
Other	1.49	0.5	0.51-4.31
Current viral load (per Log increase)	1.07	0.03	1.01-1.15
Current CD4+ count (per increase of 100 cells/mm <sup>3</sup> )	0.89	0.01	0.82-0.98
Smoker	2.20	<0.01	1.40-3.47
Diabetes	2.90	<0.01	1.67-5.04
Pharmacologically treated Hypertension	1.80	0.04	1.04-3.13
Pharmacologically treated dyslipidemia	1.92	0.07	0.96-3.86

### 637 Differences in Predictors for Ischaemic and Haemorrhagic Strokes in HIV+ Individuals

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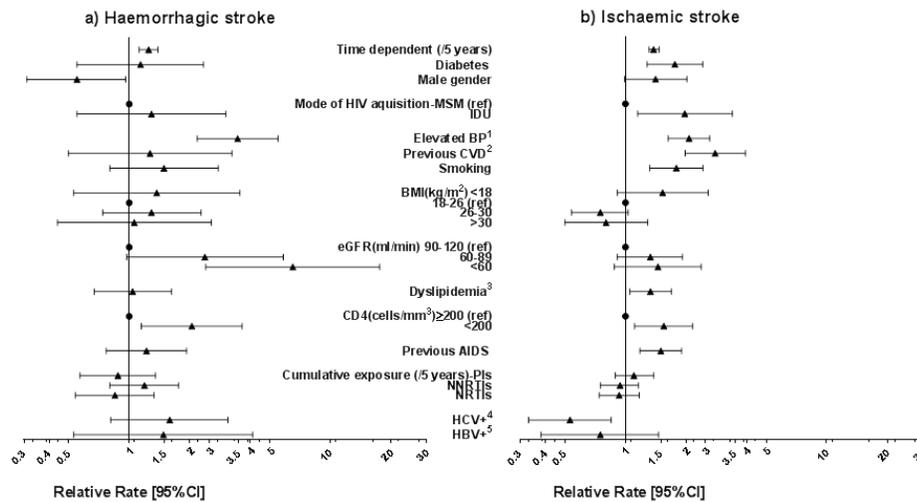
**Background:** It is unknown whether there are any differences in risk factors for haemorrhagic and ischaemic stroke in HIV+ individuals, or whether elevated blood pressure (BP) is a major risk factor for haemorrhagic stroke as known from the general population.

**Methods:** D:A:D study participants were followed from the time of the first BP measurement at/after 1/1/1999 or individual study entry and until the first of a validated stroke, 6 months after last follow up or 1/2/2014. Elevated BP during follow-up was defined as current systolic blood pressure  $\geq 140$  mm Hg and/or diastolic blood pressure  $\geq 90$  mm Hg. Poisson regression models were used to determine associations between haemorrhagic/ischaemic stroke and time-updated demographic, cardiovascular disease (CVD) -and HIV-related factors.

**Results:** Of the 43,564 included persons, 74% were men; 69% were aged 30-50 years; 42% were smokers; 26% had elevated BP and 63% had received antiretroviral therapy (ART). Of 590 strokes; 83 (14%) were haemorrhagic (incidence rate (IR)/1000 person years 0.24, 95% confidence interval [0.19, 0.30]); 296 (50%) were ischaemic (0.87 [0.77, 0.97]) and the remaining 211 strokes were of unknown etiology. IRs for both stroke subtypes were higher in those with elevated BP than in those without; haemorrhagic stroke: (0.57 [0.41, 0.73]) vs. 0.13 [0.09, 0.18]); ischaemic stroke (1.74 [1.46, 2.02]) vs. 0.58 [0.49, 0.67]). In univariable models, factors most strongly associated with increased risk for both stroke subtypes were age, elevated BP and a low estimated glomerular filtration rate (eGFR) or CD4 count. The risk factor profile for the two stroke subtypes appeared to differ in multivariable models; Ischaemic strokes were more strongly associated with metabolic CVD risk factors (dyslipidaemia, previous CVD, diabetes) and smoking than haemorrhagic stroke. Conversely, elevated BP was associated with both stroke subtypes, but the association appeared stronger for haemorrhagic strokes. Low eGFR was markedly associated with haemorrhagic stroke only. Of the HIV-related variables, only low CD4 count but not type of ART was associated with risk of both stroke subtypes; previous AIDS and HIV acquisition via injection drug use were associated with ischaemic stroke only (Figure).

**Conclusions:** Elevated BP, age and low CD4 count were the strongest predictors for both stroke subtypes. Our findings suggest that similarly to the general population, elevated BP may be a stronger predictor for haemorrhagic than ischaemic stroke in HIV+ individuals.

Factors associated with haemorrhagic and ischaemic stroke - Multivariable model



MSM = Men who have sex with men, IDU = Injection drug use, BP = Blood pressure, CVD = cardiovascular, BMI = Body mass index, eGFR = estimated glomerular filtration rate, PIs = Protease inhibitors, NRTI = Non nucleoside transcriptase inhibitors, NNRTI = Nucleoside transcriptase inhibitors, HCV+ = Hepatitis C virus positive, HBV+ = Hepatitis B virus positive

- 1: Current systolic blood pressure  $\geq 140$  mm Hg and/or diastolic blood pressure  $\geq 90$  mm Hg
- 2: Previous myocardial infarction and/or stroke
- 3: Total cholesterol  $> 6.2$  mmol/L, HDL  $< 0.9$  mmol/L, TC:HDL ratio  $> 6.5$
- 4: Seropositive and HCV-RNA positive
- 5: Positive: active infection [HB surface antigen, HB e antigen, or HBV DNA positive]; or inactive infection [HB surface antigen negative, anti-HB e antibody positive, or HBV DNA negative]

638 Persistently Increased Ischemic Stroke Risk in HIV-Infected Women

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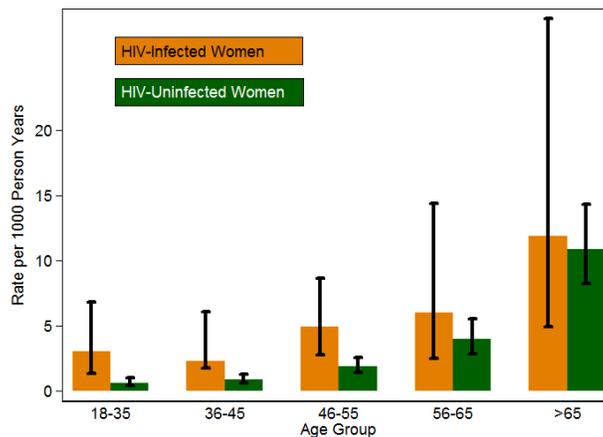
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**Background:** We have previously demonstrated that HIV infection confers a greater relative risk of ischemic stroke in women compared with men, independent of traditional stroke risk factors. The mechanism underlying this disparity, including the potential role of sex-specific stroke risk factors, is unknown. We investigated whether increased ischemic stroke risk in HIV-infected women persists after adjusting for sex-specific stroke risk factors.

**Methods:** We performed an observational cohort study of 1212 HIV-infected women and 12040 demographics-matched HIV-uninfected women followed from 1996 to 2011 in a large Boston health care system. The primary endpoint was incident ischemic stroke, defined by specific ICD-9-CM codes. Cox proportional hazard modeling was used to evaluate the association of HIV and ischemic stroke and the role of sex-specific risk factors in mediating this association.

**Results:** Among the HIV-infected women, 38 ischemic strokes occurred, compared with 167 among the HIV-uninfected women. The incidence rate ratio (IRR) for stroke comparing HIV-infected to HIV-uninfected women was 2.34 (95% confidence interval [CI] 1.60-3.34). The relative increase in stroke rates in HIV-infected women was most pronounced among younger women (18-35 years, IRR 4.96, 95% CI 1.58-13.52; 36-45 years, IRR 3.78, 95% CI 1.63-8.10; 46-55 years, IRR 2.60, 95% CI 1.25-5.03). HIV was significantly associated with increased stroke risk in univariate models (hazard ratio [HR] 2.43, 95% CI 1.78-3.61) and in models adjusting for demographics and vascular risk factors (HR 1.81, 95% CI 1.26-2.61). In a multivariable model further adjusted for sex-specific risk factors (menopause/vasomotor symptoms, premature ovarian failure, hormone replacement therapy/oral contraceptive use, pregnancy, pre-eclampsia/eclampsia, migraine, and depression/anxiety), the greater hazard of stroke associated with HIV persisted (HR 2.14, 95% CI 1.45-3.15).

**Conclusions:** HIV infection was significantly associated with an increased risk of ischemic stroke among women independent of sex-specific stroke risk factors prevalent among HIV-infected women. Investigation of additional factors to explain the differential effect of HIV on vascular risk among women, including evaluation of immunologic factors that differ by sex, will be important to further clarify the mechanism of HIV-associated vascular disease.



### 639 Stroke in HIV-Infected Patients in the Combination Antiretroviral Therapy Era

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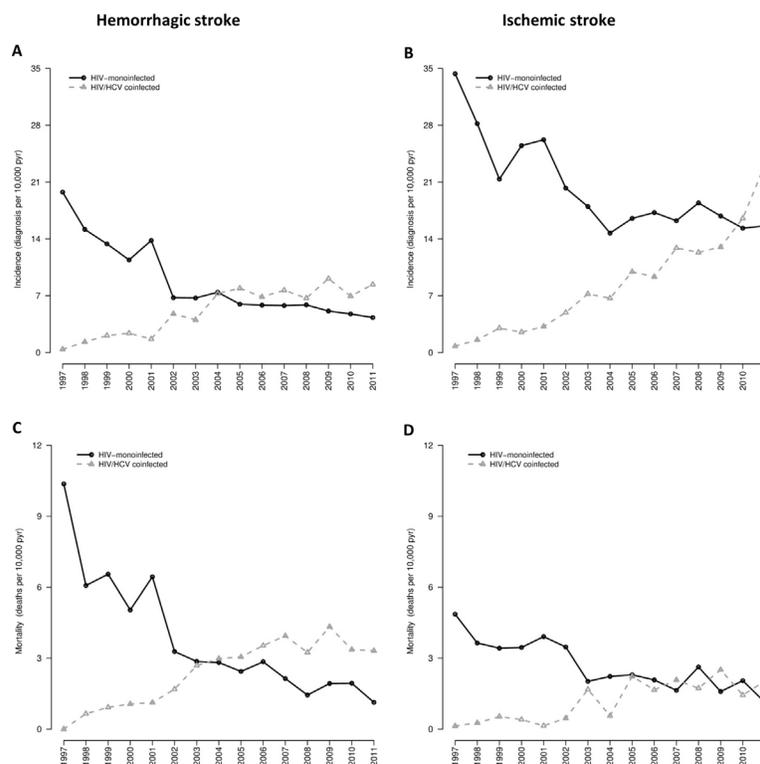
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**Background:** Both HIV and HCV infections have been associated with increased risk of stroke. We estimated incidence and mortality rates of stroke (hemorrhagic or ischemic) in HIV-infected (HIV+) patients (Pts.) in the combination antiretroviral therapy (cART) era, with particular attention to HIV/HCV-coinfected (HIV/HCV) Pts

**Methods:** We reviewed the computerized data from patients in the Spanish Minimum Basic Data Set (MBDS), that includes information from Pts. discharged in almost 300 hospitals. Pts. were identified according to the following ICD-9-CM codes: HIV infection (042 or V08) with or without HCV infection (070.44, 070.54, 070.7x, or V02.62) with hemorrhagic (h) stroke (430-432) or ischemic (i) stroke (433-437). HBV infection (070.2x, 070.3x, or V02.61) was an exclusion criterion. Pts. were classified as HIV-monoinfected (HIV-Mono) or HIV/HCV. We estimated rates (events per 10,000 patient-years) in the period 1999-2011; time interval that was broken down into three periods: 1<sup>st</sup> (1997-1999), 2<sup>nd</sup> (2000-2003) and 3<sup>rd</sup> (2004-2011). For the calculation of rates, the numerator was the number of events within each period. The denominator was the number of patient-years at risk within each period, for this purpose we estimated the number of HIV+, HIV/HCV, and HIV-Mono Pts. in each period.

**Results:** **h-stroke rates:** In the 1<sup>st</sup> period rates of h-stroke were higher for HIV-Mono Pts. than for HIV/HCV Pts. From the 1<sup>st</sup> to the 2<sup>nd</sup> period, rates of h-stroke decreased in HIV-Mono Pts. (from 16.0 to 5.5;  $P < 0.001$ ) and increased in HIV/HCV Pts. (from 1.3 to 7.6;  $P < 0.001$ ). In the 3<sup>rd</sup> period, rates of h-stroke were higher for HIV/HCV Pts. than for HIV-Mono Pts. (**Figure 1A**). **i-stroke rates:** Similar trends were found for i-stroke. Rates decreased significantly from the 1<sup>st</sup> to the 3<sup>rd</sup> period in HIV-Mono Pts. (from 27.7 to 16.4;  $P < 0.001$ ), and increased significantly in HIV/HCV Pts. (from 1.8 to 12.6;  $P < 0.001$ ) (**Figure 1B**). **Mortality rates:** mortality rates for both h-stroke and i-stroke were higher for HIV-Mono Pts. than for HIV/HCV Pts. in the 1<sup>st</sup> period; however, this trend was reversed by the 3<sup>rd</sup> period (**Figure 1C & 1D**). The adjusted likelihood of death for h-stroke in the 3<sup>rd</sup> period was higher for HIV/HCV Pts. than for HIV-Mono Pts.

**Conclusions:** In the cART era, incidence and mortality rates of stroke decreased in HIV-Mono Pts. but increased steadily in HIV/HCV Pts.



### 640 Incidental Carotid Plaque in HIV is Associated With Subsequent Cerebrovascular Events

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**Background:** Coronary atherosclerotic plaque is increased in HIV-infected individuals and associated with subsequent cardiovascular events. Cerebrovascular (CV) events including stroke and transient ischemic attack (TIA) are also increased in HIV; however there are no data characterizing the prevalence, characteristics and prognostic associations of incidental carotid plaque in HIV.

**Methods:** From a registry, we identified all HIV-infected individuals free of known CV disease who underwent a contrast neck CT from 2005 to 2014. Data collection, including CV and HIV-specific risk factors and image analysis, were performed by blinded independent teams. Image variables included the presence of carotid plaque and non-calcified plaque (NCP). The outcome of interest was a CV event (stroke, TIA) defined by ICD code and independently adjudicated. Association between plaque and events was determined using Cox proportional hazard models and compared with propensity-matched (age, gender, indication for CT, DM, HTN, HLD, smoking) HIV-uninfected controls.

**Results:** 248 HIV-infected individuals free of prior CV disease ( $43 \pm 9$  years, 24% female, 10% DM, 10% HTN, 33% smokers, 15% on statins, mean LDL  $89 \pm 38$  mg/dl) were compared to 118 matched HIV-uninfected controls. The median duration of HIV was 16 yrs (10-21) and mean nadir CD4 count was 120 cells/mm<sup>3</sup>. At time of CT, the mean CD4 count was 308 cells/mm<sup>3</sup>, 79% were on ART, 20% were co-infected with HCV and 51% had an undetectable viral load (VL). On CT, HIV-infected individuals (vs. controls) had a higher prevalence of any carotid plaque (41% vs. 25%,  $P = 0.005$ ) and NCP (56% vs. 29%,  $P = 0.03$ ). Longer duration of HIV and ART were associated with increased plaque, while lower VL was associated with decreased plaque. Over a median follow-up of 3.1 yrs, 28 events occurred in HIV-infected individuals, rate of 4%/yr, as compared to 1%/yr in uninfected controls ( $P = 0.013$ ). Within HIV, the presence of carotid plaque (adjusted HR: 3.5, 1.5-8,  $P = 0.002$ ) and NCP (adjusted HR: 2.7, 1.3-5.8,  $P = 0.01$ ) were associated with an increased risk of subsequent

CV events. CV events were compared between HIV-infected individuals with plaque and matched uninfected controls with plaque and HIV infected individuals with plaque were found to be at higher risk (P=0.03, Figure).

**Conclusions:** HIV-infected individuals without known CV disease have increased prevalence of carotid plaque and NCP, and both are associated with an increased risk of subsequent CV events.

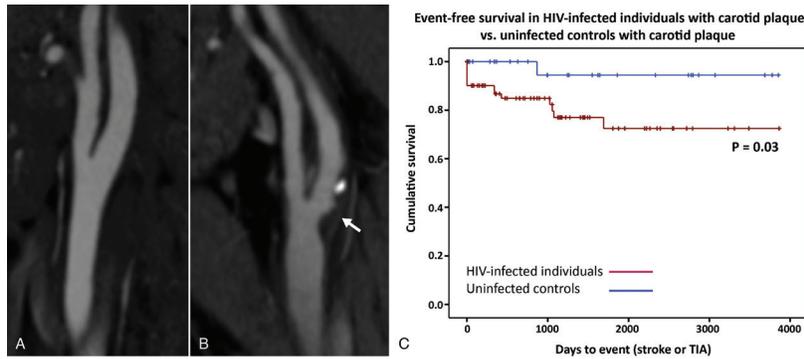


Fig 1. A neck CT image of the right carotid artery in a HIV uninfected control free of carotid plaque (A) as compared to a representative neck CT image from a HIV-infected individual with a carotid plaque with a non-calcified component (B, arrow). Kaplan-Meier event-free survival curves comparing outcomes in HIV-infected individuals with carotid plaque vs. uninfected controls with carotid plaque (C).

**641 Atherosclerotic Myocardial Infarction Risk in the NA-ACCORD Compared to MESA and ARIC**

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**Background:** HIV-infected individuals are likely at increased risk of myocardial infarction (MI) compared to uninfected individuals. However, identifying a comparable uninfected group is difficult because of differences in cardiovascular disease (CVD) risk factors. The Multi-Ethnic Study of Atherosclerosis (MESA) and the Atherosclerosis Risk in Communities (ARIC) are large, diverse general population cohorts designed to assess CVD risk. We report updated results regarding incidence of atherosclerotic (type 1) MI in the NA-ACCORD through 2013 and a comparison of rates from the NA-ACCORD to MESA and ARIC.

**Methods:** Centrally adjudicated incident type 1 MIs from 7 NA-ACCORD clinical cohorts between 1996-2013 contributed to this analysis. We excluded type 2 MIs from the NA-ACCORD analysis in order to focus on atherosclerotic events and because of the low frequency of type 2 events in the general population. MESA events occurred between 2000-2008 and ARIC events from 1987-2010. Incidence rates (IRs) per 1,000 person-years (PY), adjusted rate ratios (aIRRs) and 95% confidence intervals (CI) were estimated using Poisson regression models; rate ratios were adjusted for age, sex, race, and smoking.

**Results:** 29,212 HIV-infected NA-ACCORD participants contributed 338 type 1 MI events and 131,802 PYs. MESA and ARIC participants contributed 156 and 1,448 events, and 47,713 and 281,284 PYs, respectively. NA-ACCORD participants were younger and more likely to be of black race and male than MESA and ARIC participants. Rates of MI were significantly higher in NA-ACCORD compared to MESA and ARIC (Table 1). As expected increased age, male sex, race, and smoking were all significantly associated with MI independent of HIV infection status.

**Conclusions:** We found MI rates were higher in NA-ACCORD compared to two large CVD cohorts of presumably HIV-uninfected adults after controlling for demographic risk factors and smoking. While prevalent MIs were excluded from all cohorts, MESA more stringently excluded individuals with any baseline CVD risk factors. As a result, the magnitude of the increased risk varied between MESA and ARIC highlighting the importance of selecting an appropriate uninfected control group. A limitation of our study was the lack of controlling for other important CVD risk factors including hypertension and hyperlipidemia. Future analysis will focus on examining changes in MI incidence over calendar time and adjusting for additional traditional CVD risk factors.

**Table 1. Adjusted incidence rate ratios (aIRR) and 95% confidence intervals comparing NA-ACCORD to MESA and ARIC**

Variable	aIRR [95% CI]	
	MESA	ARIC
<b>Cohort</b>		
NA-ACCORD	2.40 [1.79, 3.20]	1.33 [1.10, 1.61]
<b>Age</b>		
40-49	1.00	1.00
50-59	1.94 [1.52, 2.49]	2.02 [1.62, 2.50]
>=60	3.83 [2.81, 5.23]	3.75 [2.99, 4.70]
<b>Sex</b>		
Male	1.00	1.00
Female	0.52 [0.40, 0.67]	0.62 [0.56, 0.68]
<b>Race</b>		
Non-black	1.00	1.00
Black	0.77 [0.62, 0.96]	1.13 [1.01, 1.26]
<b>Smoking</b>		
Never	1.00	1.00
Ever	1.56 [1.22, 1.98]	1.54 [1.38, 1.72]
Missing	1.32 [0.92, 1.89]	1.22 [0.89, 1.66]

**642 Cardiovascular Disease Risk Model Comparison and Development in HIV-Infected Veterans**

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**Background:** Persons infected with HIV have a higher risk of cardiovascular disease (CVD) after adjustment for traditional risk factors. Despite this increased risk, HIV is not accounted for in traditional CVD risk calculations or cholesterol guidelines.

**Methods:** We assessed 10-year CVD events in veterans infected with HIV using the Veterans Affairs (VA) Clinical Case Registry (CCR) from 2001-2010. Baseline (1998-2000) laboratory, comorbidity, and medication data were used to determine patient risk scores according to both the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) model

and the American College of Cardiology/American Heart Association 2013 guidelines using the Pooled Cohort Equations (PCE). Veterans with prior history of CVD, low density lipid-cholesterol (LDL-C) <70 or >190, diabetes with LDL-C>70, receiving statins, and all women were excluded. Events were defined per respective risk model (myocardial infarction [MI], bypass/angioplasty, stroke, carotid artery endarterectomy or death from coronary heart disease for D:A:D; acute coronary syndrome, MI, stable/unstable angina, revascularization, stroke, transient ischemic attack, or peripheral arterial disease for PCE). Kaplan-Meier analyses were used to compare PCE and D:A:D risk models. We also developed our own model specific to the HIV population using proportional hazards modelling of CCR data and PCE event definitions.

**Results:** In 3171 male veterans infected with HIV, observed ten-year events numbered 1165 (36.7%) by PCE criteria and 1088 (34.3%) using D:A:D criteria. As shown in the figure (by quintiles of risk score), the D:A:D model performed better than the PCE model for risk of outcome. In our new model, Hepatitis C (HCV) coinfection was associated with 50% increased hazard (adjusted HR 1.495, CI 1.275-1.752) of PCE event. HIV viral load (aHR 1.062, CI 1.033-1.092) was significantly associated with risk of outcome while CD4 count and CD4 nadir were not. Traditional risk factors were also incorporated into the model, with older age and systolic blood pressure demonstrating significant association with increased hazard of outcome.

**Conclusions:** There was a high rate of ten-year observed CVD events in HIV-infected veterans. The D:A:D model had better discrimination than the PCE for risk of outcome. Our new model additionally takes into account viral load and HCV-coinfection, which were important risk factors for PCE events.

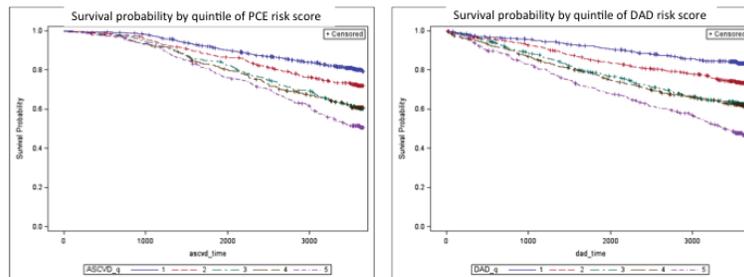


Figure 1: Kaplan-Meier survival probabilities by strata of risk score for PCE (1a) and D:A:D (1b)

#### 643 2013 ACC/AHA Guideline Undertreats HIV-Infected Adults With Atherosclerosis

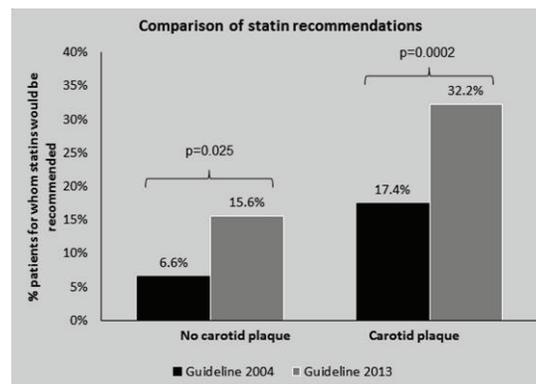
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**Background:** While HIV infection is associated with increased risk of ASCVD (atherosclerotic cardiovascular disease), it is unknown whether cholesterol guidelines can identify HIV-infected adults who may benefit from statins. The purpose of our study was to compare the 2013 ACC/AHA and 2004 ATP III recommendations in a HIV population, and to evaluate associations with carotid artery intima-media thickness (cIMT) and plaque.

**Methods:** We used ultrasound to measure cIMT at baseline and 3 years later in 352 HIV-infected adults with no ASCVD and not on statins. Plaque was defined as IMT > 1.5mm. We compared 2013 ACC/AHA and 2004 ATP III recommendations, and evaluated associations with cIMT and plaque.

**Results:** At baseline, the median age was 43 (IQR 39-49), 85% were male, 74% were on antiretroviral medication, and 50% had plaque. At followup, the median IMT progression was 0.052 mm/year (IQR: 0.025-0.094), and 66% had plaque. The 2013 guideline was more likely to recommend statins compared with the 2004 guideline, both overall (26% vs. 14%,  $p<0.001$ ), in those with plaque (32% vs. 17%,  $p=0.0002$ ), and in those without plaque (16% vs. 7%,  $p=0.025$ ). In unadjusted linear regression, the 2004 and 2013 risk scores were both strongly associated with cIMT levels (0.010 mm per 10% increase in risk,  $p<0.001$ ) and with cIMT progression (0.010mm/year per 10% increase in risk,  $p<0.001$ ). In multivariate analysis, older age, higher LDL-C, pack-years of smoking, and history of opportunistic infection were associated with baseline plaque.

**Conclusions:** While the 2013 ACC/AHA guideline recommends statins to a greater number of HIV-infected adults compared to the 2004 ATP III guideline, both failed to recommend therapy in the majority of HIV-affected adults with carotid plaque. Both the 2004 and 2013 guidelines predicted higher levels of baseline cIMT and faster progression; although associations were stronger for the 2004 guideline. HIV-specific cholesterol guidelines that include detection of subclinical atherosclerosis may help to identify HIV-infected adults who are at increased ASCVD risk and may benefit from statins.



#### 644 Differences by HIV Serostatus in Coronary Artery Disease Following Stress Testing

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**Background:** HIV-infected persons develop coronary artery disease (CAD) more commonly and earlier than uninfected patients. The role of noninvasive cardiovascular testing to stratify CAD risk in this population is not well-defined; no prior studies have evaluated cardiovascular stress testing in this group. We hypothesized that, among persons with abnormal stress tests, HIV-infected patients have a greater burden of CAD on coronary angiography than uninfected matched controls.

**Methods:** Cohort study using a cohort created in the Northwestern Medicine Enterprise Data Warehouse (NMEDW) of HIV-infected patients and age, sex, and race-matched controls who underwent cardiovascular stress testing and subsequent coronary angiography from 2000 to 2015. Persons with incomplete data for either stress tests or coronary angiography were excluded, as were persons whose stress tests did not precede coronary angiography. Relative risks were used to assess differences in CAD burden by HIV serostatus for persons with abnormal stress tests. The cutoffs for moderate and severe CAD on angiography were at least one coronary artery stenosis of  $\geq 50\%$  and  $\geq 70\%$ , respectively.

**Results:** There were 239 HIV-infected patients (mean age at stress test 53.5 years, 44.6% black, 43.6% white, 18.5% women) and 346 uninfected matched patients (mean age at stress test 53.0 years, 42.2% black, 43.9% white, 20.1% women) with abnormal stress tests (73% nuclear, 23% echocardiographic, 4% other) that prompted coronary angiography. HIV infection was associated with significantly greater risk for moderate or severe CAD on angiography (RR 1.18, 95% CI 1.01-1.38,  $P=0.03$ ) and subsequent percutaneous coronary intervention (RR 1.59, 95% CI 1.17-2.18,  $P<0.01$ ) following abnormal stress testing (Table). Among HIV-infected patients with abnormal stress tests, those with a nadir HDL cholesterol level under 30 mg/dl were at significantly elevated risk for severe CAD (RR 1.53, 95% CI 1.05-2.23,  $P=0.02$ ); this remained true when analyses were restricted to men only (RR 1.75, 95% CI 1.12-2.77,  $P=0.01$ ). Risks for severe CAD among HIV-infected persons with abnormal stress tests did not significantly differ by peak total cholesterol level, CD4 nadir, and plasma HIV RNA nadir.

**Conclusions:** HIV-infected patients with abnormal cardiovascular stress tests had significantly greater CAD burden and were more likely to undergo PCI compared with uninfected controls. The implications of these findings for CAD screening for HIV-infected persons require further study.

Table. Risks for Coronary Artery Disease following Abnormal Stress Test: HIV-Infected Persons Versus Uninfected Controls

	HIV-Infected Cases	Uninfected Controls	Relative Risk (95% CI)	P Value
Abnormal Stress Test Results: All Modalities (Mean Age = 53.3 years)				
Number Abnormal (Total Performed)	239 (314)	346 (453)		
$\geq 50\%$ Stenosis (% of Abnormal)	139 (58.2%)	170 (49.1%)	1.18 (1.01-1.38)	0.03
$\geq 70\%$ Stenosis	114 (47.7%)	141 (40.8%)	1.17 (.97-1.41)	0.09
PCI Performed	65 (27.2%)	59 (17.1%)	1.59 (1.17-2.18)	<0.01
3 Vessel CAD or L Main	39 (16.3%)	56 (16.2%)	1.01 (.69-1.46)	0.97

\* 3 Vessel CAD or L Main disease defined as lesions  $\geq 50\%$  in each epicardial coronary artery or left main disease  $\geq 50\%$

645 HIV & Obesity Synergistically Increase Interleukin 6 but Not Soluble CD14 or D-dimer

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**Background:** Obesity prevalence among people living with HIV (HIV+) is rising. HIV and obesity are associated with atherosclerosis, which is mediated by inflammation. Both are pro-inflammatory states, but their combined effect on inflammation (measured by interleukin 6, IL-6), altered coagulation (D-dimer), and monocyte activation (soluble CD14, sCD14) is unknown. We hypothesized that HIV infection and obesity (BMI  $\geq 30$  kg/m<sup>2</sup>) synergistically increase IL-6, sCD14 and D-dimer in the Veterans Aging Cohort Study Biomarker Cohort (VACS BC).

**Methods:** VACS is a prospective, observational longitudinal study of HIV+ and HIV uninfected (HIV-) participants. VACS BC is a subset of VACS participants who provided blood samples for research. VACS BC participants with BMI < 18.5 kg/m<sup>2</sup> (underweight, N=48) were excluded since they were too few to stratify by HIV status (N=4 underweight HIV-). Dependent variables were IL-6, sCD14, and D-dimer quartiles. BMI was categorized as 18.5-24, 25-29, and  $\geq 30$  kg/m<sup>2</sup>. Covariates included demographics, smoking, diabetes, CVD, hypertension, lipids, liver and renal function, HCV and statin use. Unadjusted and fully adjusted logistic regression models were constructed.

**Results:** We analyzed data on 1495 HIV+ (66% of whom had HIV-1 RNA < 500 copies/mL) and 837 HIV- participants. The mean age was 53 years. The majority were African American (68%); half were current smokers (49%). Over a quarter were obese (27%) or had prevalent CVD (22%). HIV+ obese participants were more likely to have diabetes, dyslipidemia, statin use, and HCV, and less likely to smoke than HIV- non-obese participants ( $p < 0.05$  for all). IL-6 showed a stepwise increase from HIV- non-obese, to HIV+ non-obese, to HIV- obese, to HIV+ obese. Elevated IL-6 was more prevalent in obese HIV+ than HIV- non-obese, which persisted after adjustment for potential confounders (adjusted odds ratio, aOR [95% confidence interval, CI]: 1.69 (1.13-2.51); Table). The inverse was true for sCD14 (aOR (95% CI): 0.43 (0.27-0.66); Table). No significant differences were seen for D-dimer.

**Conclusions:** HIV-obesity comorbidity is associated with higher prevalence of elevated IL-6, lower prevalence of elevated sCD14 and no significant difference in D-dimer. These findings are clinically significant as all three biomarkers are associated with mortality. Future studies should assess 1) whether other biomarkers of monocyte activation and altered coagulation show similar trends and 2) potential mechanisms for the unanticipated sCD14 and D-dimer findings.

Table: Logistic regression models estimating the association between HIV-obesity and the highest IL-6, sCD14 or D-dimer quartile. Statistically significant estimates are in bold.

Biomarker	HIV/BMI Category	N	Biomarker Median (p25-p75)	Odds ratio (95% confidence interval)	
				Unadjusted Model	Adjusted Model <sup>1</sup>
Elevated IL-6 ( $\geq 3.33$ pg/mL)	HIV- BMI (18.5-29.9)	433	1.51 (0.99-2.79)	1 (ref)	1 (ref)
	HIV+ BMI (18.5-29.9)	1228	2.02 (1.39-3.30)	<b>1.34 (1.02-1.76)</b>	1.26 (0.94-1.69)
	HIV- BMI $\geq 30$	382	2.12 (1.37-3.47)	<b>1.55 (1.12-2.15)</b>	<b>1.59 (1.12-2.25)</b>
	HIV+ BMI $\geq 30$	245	2.26 (1.55-3.60)	<b>1.61 (1.11-2.31)</b>	<b>1.69 (1.13-2.51)</b>
Elevated sCD14 ( $\geq 2.07$ ug/mL)	HIV- BMI (18.5-29.9)	438	1.74 (1.49-2.05)	1 (ref)	1 (ref)
	HIV+ BMI (18.5-29.9)	1232	1.73 (1.46-2.11)	1.16 (0.90-1.49)	0.95 (0.72-1.25)
	HIV- BMI $\geq 30$	385	1.72 (1.45-2.03)	0.93 (0.67-1.28)	0.75 (0.53-1.07)
	HIV+ BMI $\geq 30$	245	1.61 (1.35-1.87)	<b>0.58 (0.39-0.88)</b>	<b>0.43 (0.27-0.66)</b>
Elevated D-dimer ( $\geq 0.52$ ug/mL)	HIV- BMI (18.5-29.9)	437	0.28 (0.18-0.47)	1 (ref)	1 (ref)
	HIV+ BMI (18.5-29.9)	1230	0.26 (0.15-0.48)	1.05 (0.81-1.37)	1.01 (0.76-1.34)
	HIV- BMI $\geq 30$	383	0.33 (0.24-0.60)	1.48 (1.09-2.03)	1.37 (0.98-1.92)
	HIV+ BMI $\geq 30$	245	0.28 (0.16-0.47)	0.99 (0.68-1.44)	0.90 (0.60-1.35)

<sup>1</sup> Fully adjusted model controls for: age, race/ethnicity, cardiovascular disease, hypertension, diabetes mellitus, smoking status, LDL and HDL cholesterol, triglycerides, statin prescription, HCV infection, FIB4 level, and estimated glomerular filtration rate.

<sup>2</sup> Represents biomarker median (p25-p75) for the entire distribution of the biomarker for those in each HIV/BMI category, not only the highest quartile.

**646 BNP Prediction of Cardiovascular Diseases in HIV+ Patients and the General Population**

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**Background:** B-type Natriuretic Peptide (BNP) is elevated in patients with congestive heart failure and is also an independent prognostic marker for overall mortality. HIV-positive patients (HIV+) have higher BNP-levels than general population. We investigate the association between BNP and the incidence of cardiovascular diseases (CVD) in subjects without prevalent CVD.

**Methods:** We compare BNP measured at baseline and incident CVD during the follow-up in HIV+ individuals of the HIV HEART study (HIVH) and in controls of the population-based Heinz Nixdorf Recall study (HNR), both recruited from the German Ruhr area since 2000. To assess impact of BNP ( $\geq 100$ pg/mL vs.  $< 100$ pg/mL) on incident CVD (myocardial infarction, PCI stent, Coronary Artery disease, Coronary Artery Bypass Graft, cardiac death) we used the pooled data of both studies and computed Cox proportional Hazard ratio (HR) with time to CVD or last observation and BNP and study (HIVH vs. HNR) as predictors. Also the interaction between BNP and affiliation to study cohort was tested. We controlled for age and Framingham risk score (FRS) variables. Analyses were stratified by sex. We restricted the analysis to the age range of 45-75 years and to subjects without prevalent CVD.

**Results:** Our analysis data set included 268 HIV+ of the HIVH and 3905 HNR controls (Table 1). Male HIV+ had a 3.6-fold increased risk of incident CVD (95%-CI 1.3; 9.9,  $p=0.0128$ ) compared to HNR males and independent of the affiliation to the study cohort BNP  $\geq 100$  pg/mL in male subjects was associated with a HR of 3.4 (95%-CI 2.0; 5.9,  $p= <0.0001$ ). In females, HRs were 22.9 (95%-CI 2.8; 185.5,  $p=0.0034$ ) for study cohort and 2.6 (95%-CI 1.00; 6.8,  $p=0.0513$ ) for BNP  $\geq 100$  pg/mL. In both sexes no significant interaction between study cohort and BNP was found. No other classic risk factor (HR Smoking: Male: 1.2 (95%-CI 0.8; 1.7); Female: 1.1 (95%-CI 0.6; 2.0), HR Diabetes mellitus Male: 1.8 (95%-CI 1.3; 2.7); Female: 1.5 (95%-CI 0.7; 3.1)) reached such high HRs for CVD as BNP  $\geq 100$  pg/mL and HIV-infection.

**Conclusions:** Incidence of CVD was higher in HIV+ compared to the general population controlling for differences in FRS. Additionally, BNP  $\geq 100$  pg/mL was independently associated with incident CVD in HIVH and HNR. Thus, as was shown in the general population, BNP may improve prediction of CVD also in HIV+. BNP should be measured serially in HIV+  $\geq 45$  years and elevated or increasing levels should lead to intensification of care.

	HNR			HIVH		
	male	female	Total	male	female	Total
N (%)	1881 (48.17)	2024 (51.83)	3905 (100)	237 (88.43)	31 (11.57)	268 (100)
age [Years] mean $\pm$ SD	59.28 $\pm$ 7.68	59.53 $\pm$ 7.80	59.41 $\pm$ 7.74	53.80 $\pm$ 7.13	51.77 $\pm$ 6.35	53.57 $\pm$ 7.06
BNP [pg/mL] median (IQR)	14.00 (7.50 – 26.00)	21.60 (11.95 – 36.80)	17.80 (9.50 – 32.40)	12.80 (7.5 – 26.20)	22.10 (7.45 – 7.80)	14.30 (8.00 – 27.70)
BNP > 100 pg/mL N (%)	43 (2.29)	50 (2.47)	93 (2.38)	14 (5.91)	1 (3.23)	15 (5.60)
Smoker N (%)	1324 (70.39)	881 (43.53)	2205 (56.47)	179 (75.53)	17 (54.84)	196 (73.13)
Diabetes N (%)	297 (15.79)	188 (9.29)	485 (12.42)	12 (5.06)	5 (16.13)	17 (6.34)
Deceased N (%)	260 (13.82)	173 (8.55)	433 (11.09)	39 (16.46)	1 (3.23)	40 (14.93)
incident CVD	127 (6.75)	50 (2.47)	177 (4.53)	26 (10.97)	3 (9.68)	29 (10.82)
Follow-up time [Years] median (IQR)	11.42 (9.28 – 12.29)	11.47 (9.54 – 12.32)	11.45 (9.38 – 12.30)	7.45 (7.07 – 7.78)	7.56 (7.45 – 7.80)	7.46 (7.21 – 7.80)
Framingham Risk Score mean $\pm$ SD	21.57 $\pm$ 12.65	7.75 $\pm$ 5.35	14.40 $\pm$ 11.81	15.65 $\pm$ 10.02	7.71 $\pm$ 5.51	14.73 $\pm$ 9.93

**647 9-Year Trends in Non-Lipid Cardiovascular Disease Prevention Strategies in HIV+ Women**

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**Background:** Cardiovascular disease (CVD) is increasingly prominent among women with HIV. While there is a major clinical trial of statins in people with HIV, less is known about other non-lipid prevention strategies. Evaluating trends in these strategies is important to understand how well women with HIV manage CVD risk.

**Methods:** Participants were from the Women's Interagency HIV Study, a longitudinal cohort of HIV-infected (HIV+) and uninfected (HIV-) women at 6 US sites, during 2006-2014. We examined semiannual trends in medication use and/or achievement of treatment targets by HIV status for women at risk for CVD based on hypertension (HTN), diabetes or smoking. "At-risk" was defined as: for HTN, systolic BP  $\geq 140$  mmHg, diastolic BP  $\geq 90$  mmHg, self-report of HTN or HTN medication history; for diabetes, history of fasting glucose [FG]  $\geq 126$  mg/dL or HgbA1c  $\geq 6.5\%$ , self-report of diabetes or diabetes medication history; for smoking, report of smoking at previous visit. Poisson regression with generalized estimating equations tested time trends and differences between HIV+ and HIV- women.

**Results:** During 2006-2014, prevalence was 40% (HIV+) and 38% (HIV-) for HTN, 21% and 22% for diabetes, and 37% and 48% for smoking. There were 10,546 eligible person-visits for HTN (N=1,444), 6,394 for diabetes (N=474) and 11,258 for smoking (N=1,206). Use of anti-HTN medication was higher among hypertensive HIV+ women (77% vs 67%,  $P<.001$ ) and increased over time among both HIV+ (72% in 2006 to 81% in 2014,  $P<.001$ ) and HIV- (63% to 73%,  $P<.001$ ), with no difference in trend by HIV status. HTN control ( $< 140/90$  mmHg) was higher among HIV+ than HIV- ( $P<.001$ ) and differed over time by HIV status, increasing among HIV+ (55% to 59%,  $P=.01$ ) but not HIV- (45% to 46%,  $P=.31$ ). Use of anti-diabetic medication was similar among diabetic HIV+ and HIV- women (48% vs 49%,  $P=.99$ ) and increased similarly over time (37% to 63% among HIV+ and 34% to 64% among HIV-, both  $P<.001$ ). Diabetes control (FG  $< 130$  mg/dL) was higher among HIV+ than HIV- (73% vs 64%,  $P=.03$ ) and did not change over time. Smoking cessation was similar between HIV+ and HIV- smokers (10% vs 9%,  $P=.33$ ) and did not change over time.

**Conclusions:** HIV+ women more effectively manage HTN and diabetes than HIV- women. Despite this,  $>40\%$  of hypertensive and  $>25\%$  of diabetic HIV+ women do not achieve target control levels. Providers should continue to emphasize preventive strategies (including lifestyle and pharmacologic interventions when indicated) to reduce CVD risk.

**648 HIV Patients Have More High-Risk Plaque and Cardiac Events but Less Intervention**

**James Nadel**; Eoin O'Dwyer; Sam Emmanuel; James Otton; Justyn Huang; Cameron Holloway

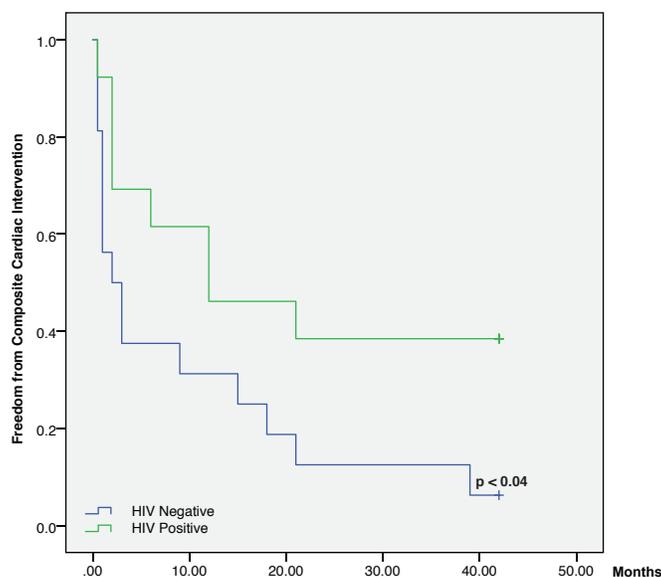
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**Background:** Human immunodeficiency virus (HIV) infection is now considered a chronic, treatable disease. Nevertheless, treatment is associated with increased rates of coronary artery disease (CAD). Screening of this at-risk population remains contentious; we therefore assessed the utility of CTCA in the screening and prediction of adverse cardiac outcomes.

**Methods:** HIV positive men (n=32) who had undergone CTCA for risk assessment were matched 2:1 for age, sex and Framingham risk with 65 HIV negative CTCA patients. CTCA data was assessed along with the occurrence of coronary events and intervention.

**Results:** Patients with HIV had higher rates of soft plaque ( $0.8 \pm 1.5$  versus  $0.3 \pm 0.7$ ,  $p=0.03$ ) and soft plaque ratios ( $0.2 \pm 0.3$  versus  $0.1 \pm 0.2$ ,  $p<0.03$ ) compared to negative controls. At a median follow-up time of 38 months, patients with HIV were at greater risk of non-ST elevation acute coronary syndrome (15.6% versus 3.2%,  $p<0.04$ ), although there was no difference in the combined endpoint of all-acute coronary syndromes despite a threefold greater risk conferred to those with HIV (18.8% versus 6.4%,  $p=0.08$ ). Conversely, on survival analysis there was a higher rate of intervention with either; invasive angiogram, percutaneous coronary intervention or coronary artery bypass grafting in patients without HIV (mean time to event  $20.6 \pm 4.9$  versus  $9.9 \pm 3.3$  months,  $p<0.04$ ).

**Conclusions:** Patients with HIV have high-risk plaque and higher rates of adverse cardiac events compared to negative controls, but lower rates of coronary angiography and intervention following CTCA. The data suggests that CTCA may have a role in predicting cardiac events in patients with HIV and further management may be suboptimal in this population group.



#### 649 Diastolic Function Correlates With Pericardial Fat and Vascular Remodeling in HIV

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**Background:** In patients with treated HIV infection, higher pericardial fat volumes are associated with coronary artery plaque and systemic inflammation, but the relationship to left ventricular (LV) structure and function is unknown.

**Methods:** We prospectively enrolled 46 patients with HIV on stable antiretroviral therapy and 23 age- and gender-matched HIV-uninfected controls (2:1), both groups without known cardiovascular disease. We measured LV volume, LV mass, systolic function (ejection fraction (EF) and global longitudinal strain) and diastolic function (graded categories, E/A, and E/E' ratio) using echocardiography. For all HIV+ subjects, pericardial fat volume and density (mean Hounsfield units) and coronary artery calcium (CAC) score were measured by CT scan; common carotid artery intima-media thickness (IMT) and distensibility were measured by ultrasound. T-tests were used to compare HIV+ and control groups. Within the HIV+ group, Spearman correlations were used to assess associations with measures of LV structure and function.

**Results:** HIV+ participants had a mean(SD) age of 55.7(5.9) years and were 87% male and 67% African American. Demographics were similar to controls (all  $p > 0.05$ ). Mean CD4 count was 628(279) cells/ $\mu$ L and all had HIV-1 RNA  $< 200$  c/ml (67%  $< 48$  c/ml). LVEF, diastolic function, and LV mass index were similar between HIV and controls ( $p > 0.05$  for all comparisons), but HIV+ participants had lower LV volume index ( $50.7 \pm 14.0$  vs  $70.3 \pm 21.7$  ml/m<sup>2</sup>,  $p < 0.001$ ). Pericardial fat volume negatively correlated with E/A ratio ( $r = -0.392$ ,  $p = 0.015$ ), and positively with diastolic dysfunction ( $r = 0.369$ ,  $p = 0.023$ ), while pericardial fat density positively correlated with E/A ratio ( $r = 0.455$ ,  $p = 0.004$ ) and negatively with diastolic dysfunction ( $r = -0.479$ ,  $p = 0.002$ ). In contrast, pericardial fat measures were not associated with EF or global longitudinal strain (all  $p > 0.05$ ). Diastolic dysfunction was also positively associated with CAC score ( $r = 0.296$ ,  $p = 0.046$ ) and carotid IMT ( $r = 0.317$ ,  $p = 0.032$ ), and negatively correlated with carotid distensibility ( $r = -0.304$ ,  $p = 0.045$ ).

**Conclusions:** Measures of LV diastolic function are associated with altered pericardial fat volume and density and measures of vascular remodeling in this population of HIV-infected subjects on antiretroviral therapy. Altered ectopic fat distribution may be a risk factor for heart failure in HIV-infected patients with and without coronary heart disease.

#### 650 Reduced Ovarian Reserve Relates to Cardiovascular Disease Risk in Women With HIV

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**Background:** HIV-infected women face a three-fold increased rate of myocardial infarction (MI) compared to non-HIV-infected women. Previous studies among HIV-infected women have explored ways in which traditional cardiovascular disease (CVD) risk factors and systemic immune activation may contribute to this heightened MI risk. To date, no study has explored how reproductive aging relates to CVD risk in this population. Here, we investigate differences in subclinical coronary atherosclerotic plaque and markers of immune activation among HIV-infected women and non-HIV-infected women categorized by degree of ovarian reserve and menopause status.

**Methods:** Seventy-four women (49 HIV-infected, 25 non-HIV) without known CVD were classified as premenopausal, premenopausal with reduced ovarian reserve, or postmenopausal based on menstrual history and levels of antimüllerian hormone (AMH) (Figure 1). Participants underwent contrast enhanced coronary computed tomography angiography and immune phenotyping. Comparisons in coronary atherosclerotic plaque burden and immune markers were made between the HIV-infected and non-HIV-infected women overall and within the HIV-infected and non-HIV-infected women by reproductive aging classification group.

**Results:** Among the overall group of HIV-infected women, the women with reduced ovarian reserve (undetectable AMH) had a higher prevalence of coronary plaque (52% versus 6%,  $p = 0.0007$ ) and noncalcified plaque (48% versus 6%,  $p = 0.002$ ), as well as higher levels of log sCD163 ( $p = 0.0004$ ) and log MCP-1 ( $p = 0.006$ ), compared with the premenopausal women with measurable AMH. Furthermore, reduced ovarian reserve in the HIV-infected group related to noncalcified plaque, controlling for traditional CVD risk factors ( $p = 0.04$ ) and sCD163 ( $p = 0.03$ ).

**Conclusions:** HIV-infected women with reduced ovarian reserve have increased subclinical coronary plaque compared with premenopausal women in whom AMH is measurable. This relationship holds when controlling for CVD risk factors (including age) and immune activation. Our findings demonstrate that reduced ovarian reserve may contribute to CVD burden in HIV-infected women and support a comprehensive assessment of CVD risk *prior* to completion of menopause in this population. Future work applying our reproductive aging classification paradigm will explore *mechanisms* through which reproductive aging influences immune activation, plaque, and overall CVD risk among women aging with HIV.

**Figure 1. Reproductive Aging Classification Scheme**

	PREMENOPAUSAL		POSTMENOPAUSAL
MENSTRUAL HISTORY	Menses within the past 12 months		No menses within the past 12 months
ANTIMULLERIAN HORMONE LEVELS (AMH)	Detectable AMH; variable by age	Undetectable AMH	Undetectable AMH
	Group 1: Premenopausal with measurable AMH	Group 2: Premenopausal with reduced ovarian reserve	Group 3: Postmenopausal

**651 Correlates and Longitudinal Implications of FGF23 Levels in HIV-Positive Individuals**

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**Background:** High plasma concentrations of fibroblast growth factor-23 (FGF23), which is crucial in phosphorus homeostasis, is an early marker of kidney dysfunction and has been associated with increased cardiovascular disease mortality. HIV-positive persons are at increased risk for cardiovascular and kidney disease, but there are few data assessing the role of FGF23 in these conditions in HIV-positive individuals.

**Methods:** We measured intact plasma FGF23 in a cohort of 100 HIV-negative and 191 HIV-positive, non-diabetic adults with normal estimated kidney function. We measured glomerular filtration rate by iohexol disappearance from plasma (iGFR) annually, albumin-creatinine ratio (ACR) every 6 months, and assessed for carotid plaque and measured carotid intima-media thickness (IMT) at baseline and at 2 years. Progressive albuminuria was defined as a follow-up ACR that was ≥2-fold higher than baseline and ≥30 mg/g. We log-transformed FGF23 levels in simple and hierarchical regression models to assess associations with baseline factors and with longitudinal changes in disease markers.

**Results:** The cohort was 29% female, 93% black, with a median age of 49 years, and 46% were infected with hepatitis C virus (HCV). FGF23 levels were similar in HIV-negative and HIV-positive subjects (median 8.9 vs. 8.4 pg/mL, P=0.53). Among HIV-positive subjects, factors independently associated with higher FGF23 levels at baseline included being female (adjusted ratio of geometric means [95% CI], 1.38 [1.13, 1.69]), serum phosphorus (1.21 [1.03, 1.42]), HCV coinfection (1.26 [1.04, 1.53]), and non-suppressed HIV RNA (1.33 [1.00, 1.76]). In cross-sectional baseline analysis of HIV-positive subjects (adjusted for demographic and cardiovascular risk factors including smoking, hypertension, and hyperlipidemia) FGF23 was not significantly associated with iGFR, albuminuria, presence of carotid plaque, or carotid IMT. However, adjusted for the same factors, higher baseline FGF23 was associated with a more rapid increase in internal carotid artery IMT (13 μm/year, 95% CI, 3, 24) and risk of progressive albuminuria (odds ratio 1.95 [95% CI]: 1.33, 2.87).

**Conclusions:** FGF23 levels in HIV-positive subjects were significantly higher in women, HCV-coinfected persons and in those with unsuppressed HIV RNA levels. Higher FGF23 levels at baseline were associated with more rapid carotid IMT progression and with progressive albuminuria. Together, these factors suggest a role for FGF23 in cardiovascular and kidney disease in HIV-positive populations.

**652 Nonclassical Monocyte, MCP-1 Predict Subclinical Atherosclerosis Progression in HIV**

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**Background:** Persistent inflammation and immune dysregulation can contribute to cardiovascular disease (CVD) risk in patients with chronic HIV infection.

**Methods:** We conducted a longitudinal analysis utilizing HIV-infected subjects on stable antiretroviral therapy (ART) in the Hawaii Aging with HIV-Cardiovascular (HAHC-CVD) study to determine the correlation of peripheral monocyte subsets and biomarkers of inflammation to progression of atherosclerosis using 2 year change in carotid artery intima-media thickness (CIMT) and coronary artery calcium (CAC). Peripheral blood mononuclear cells (PBMC) were immunophenotyped by multiparametric flow cytometry to quantify classical (CD14<sup>++</sup>CD16<sup>-</sup>), intermediate (CD14<sup>++</sup>CD16<sup>+</sup>), non-classical (CD14<sup>low/+</sup>CD16<sup>++</sup>) and transitional (CD14<sup>+</sup>CD16<sup>-</sup>) monocyte subsets. Biomarkers (sE-selectin, sVCAM-1, sICAM-1, MMP-9, tPA-1, hsCRP, IL-6, IL-8, IL-10, TNF-α, MCP-1, IFN-α) were assessed by multiplex Luminex assay. The primary outcome variables were change in CIMT (right common carotid artery (CCA) and bifurcation (BIF)) and CAC over 2 years.

**Results:** 105 subjects: 91% male, median age 51 (Q1, Q3; 47, 57) years, median CD4 count 491 (352, 660) cells/mm<sup>3</sup>, and 87% with HIV RNA ≤50 copies/mL. The rate of change in CCA, BIF, and CAC was 0.010 mm/year, 0.0097 mm/year, and 16.2 Agatston units/year, respectively. Change in CCA correlated with TNFα (r= -0.28, p=0.026) but not with any monocyte subset. Change in BIF correlated with non-classical monocytes (r=0.29, p=0.038) and MCP-1 (r=0.34, p=0.005). Change in CAC correlated with non-classical monocytes (r=0.27, p=0.015) and MCP-1 (r=0.46, p<0.001). Non-classical monocyte and MCP-1 remained significantly associated with CAC and BIF progression after adjustment for age, hypertension, diabetes mellitus, total/HDL cholesterol ratio, and smoking history.

**Conclusions:** Non-classical monocytes and MCP-1 were associated with progression of atherosclerosis. The role of monocyte subsets in atherosclerosis warrants further investigation.

Model	Variable	Coefficient	P-value
Outcome: 2 Year Change in CAC <sup>†</sup>			
1	Non-Classical Monocyte Count	0.277	0.020
2	MCP-1	0.444	< 0.001
Outcome: 2 Year Change in BIF <sup>‡</sup>			
3	Non-Classical Monocyte Count	0.491	0.046
4	MCP-1	0.829	0.010

Non-Classical Monocyte count, MCP-1, and change in BIF have been log-10 transformed to adjust for normality. A p-value < 0.05 was regarded as statistically significant. Risk factors adjusted for in all models include: age, hypertension, diabetes mellitus, total/HDL cholesterol ratio, and smoking history.

<sup>†</sup> A Spearman Partial Correlation was run to assess the correlation between 2 year change in CAC and Non-Classical Monocyte count and MCP-1, respectively, adjusted for risk factors. Coefficient is partial Spearman rho.

<sup>‡</sup> A multiple linear regression was run to assess the association between Non-Classical Monocyte count and MCP-1, respectively, on 2 year change in BIF, adjusted for risk factors. Coefficient is regression coefficient and has not been back-transformed.

**653 Association of T Cell and Macrophage Activation With Vascular Health in HIV**

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**Background:** Delineating the relationships between vascular health, inflammation, and immune activation is important to the study of cardiovascular disease (CVD) risk in HIV-infected persons on long-term antiretroviral therapy (ART). We assessed associations between T cell and macrophage activation, brachial artery flow mediated dilation (FMD; a functional measure of arterial smooth muscle response to ischemia), and circulating intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1; two markers of endothelial cell activation).

**Methods:** We enrolled 70 HIV-infected adults on efavirenz, tenofovir, and emtricitabine with sustained virologic suppression for >2 years, a CD4+ count >350 cells/μl, no known diabetes or CVD, and no statin use, and measured FMD, ICAM-1, and VCAM-1. Activated (CD38+), senescent (CD57+PD1+), and memory (CD45RO+) CD4+ and CD8+ T cells were measured by flow cytometry. Soluble markers of macrophage activation (sCD14, sCD163, and macrophage inflammatory protein-1α [MIP-1α]) were measured by ELISA and cytometric bead array. The relationships between immunologic and vascular parameters were assessed using regression models adjusted for age, sex, smoking, duration of ART, and body mass index.

**Results:** Median age was 45 years (IQR 39, 50), median CD4+ count 701 cells/μl (IQR 540, 954), 43% were female and 54% non-white. Lower brachial artery FMD was associated with high CD8+ T cell activation ( $p < 0.01$ ), but FMD was not associated with other T cell subsets or macrophage markers. In contrast, higher ICAM-1 was associated with higher sCD14, sCD163, and MIP-1α ( $p < 0.01$  for all), and higher VCAM-1 with sCD163 and MIP-1α ( $p < 0.01$  for both). Furthermore, low CD4+ activation and high CD4+ memory cells were associated with high VCAM-1 ( $p = 0.01$  for both). Results were similar when adjusted for race, hepatitis C, and CD4 count.

**Conclusions:** In HIV-infected adults with virologic suppression, cytotoxic CD8+ activation was associated with impaired brachial artery smooth muscle relaxation. In contrast, increased soluble inflammatory markers, possibly shed by vascular macrophages, were associated with endothelial cell activation. This suggests T cell and macrophage activation adversely affect vascular health via differing mechanisms, and CVD studies in HIV patients should utilize both functional and biomarker vascular assessments. A possible link between a robust CD4+ memory cell expansion on ART and endothelial activation should be investigated further.

**654 Effect of T-Cell Activation and Inflammation on Endothelial Dysfunction in HIV**

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**Background:** The underlying mechanism for endothelial dysfunction and HIV-associated cardiovascular disease (CVD) remains unknown. Immune activation and chronic inflammation persist even in the setting of effectively treated HIV. T cell activation leads to T cell dysregulation and may be one underlying cause of chronic inflammation in HIV-infected individuals. The purpose of this study was to determine whether T cell activation and inflammatory markers in HIV-infected individuals are associated with endothelial dysfunction, a predictor of future cardiovascular events.

**Methods:** We performed a cross-sectional study of immune activation and inflammatory markers with endothelial function in 359 HIV-infected adults. Endothelial function was assessed using flow-mediated vasodilation of the brachial artery (FMD) and reactive hyperemia (RH). T-cell activation including HIV and CMV-specific responses were assessed using flow cytometry in peripheral mononuclear blood cells.

**Results:** The mean age was  $49 \pm 10$  years and 84% were male, 76% were on ART, 31% smokers, and 18% with hepatitis C. The median FMD was 4.0% (IQR 2.6, 5.5) and the median RH was 57% (IQR 45, 79). After multivariable adjustment for demographics, traditional CV and HIV risk factors, CMV IgG was the only inflammatory marker associated with impaired FMD (-11.7% per doubling,  $p = 0.002$ ). In comparison, higher TNF-α (-44.5% per doubling,  $p = 0.007$ ) and higher neopterin (13.9% per doubling,  $p = 0.035$ ) were associated with lower (worsened) RH. Other inflammatory markers including hsCRP, IL-6, sCD163, and D-dimer were not associated with FMD or RH. Only CD4+IFN+ (CMV specific) T cell activation was associated with lower RH (-4.5% per doubling,  $p = 0.028$ ) and none of the other assessments of T cell activation were predictive of either FMD or RH. When the analysis was restricted to treated and suppressed individuals only, the results remained the same.

**Conclusions:** Certain inflammatory markers such as CMV IgG, TNF-α, and neopterin (a marker of plaque instability) were independently predictive of endothelial dysfunction in HIV-infected individuals. CMV-specific T cell activation was independently associated with worsened RH. Unlike FMD, RH measures microvascular function and thus could explain the different findings between the two outcomes. Interventions targeted at reducing inflammation particularly in the setting of CMV infection may improve endothelial function and decrease HIV-associated CVD.

Association of T-Cell Activation and Inflammatory/Coagulation Markers with Endothelial Function		
	Flow-Mediated Vasodilation	Reactive Hyperemia
<b>T-Cell Activation</b>		
CD4+ IFN+ (CMV Specific)	1.7% (-1.2, 4.8), $p = 0.25$	-4.5% (-8.4, -0.5), $p = 0.028$
<b>Inflammatory Markers</b>		
CMV IgG	-11.7% (-18.2, -4.6), $p = 0.002$	-3.4% (-7.3, 0.8), $p = 0.109$
TNF-α	1.1% (-9.8, 13.2), $p = 0.86$	-44.5% (-63.9, -14.6), $p = 0.007$
Neopterin	0.7% (-15.3, 19.8), $p = 0.94$	-13.9% (-25.1, -1.1), $p = 0.035$
CRP	-2.8% (-8.0, 2.6), $p = 0.31$	2.6% (-2.1, 7.6), $p = 0.28$
IL-6	-1.3% (-10.2, 8.4), $p = 0.78$	-3.4% (-11.2, 5.0), $p = 0.42$
sCD-14	19.8% (-0.8, 44.6), $p = 0.06$	-8.7% (-18.7, 2.5), $p = 0.12$
sCD-163	10.1% (-5.8, 28.8), $p = 0.23$	8.6% (-6.4, 26.0), $p = 0.28$
<b>Coagulation Markers</b>		
D-Dimer	-4.3% (-10.7, 2.6), $p = 0.22$	-3.0% (-7.8, 2.1), $p = 0.24$
Fibrinogen	-6.0% (-30.7, 27.6), $p = 0.69$	-19.4% (-37.1, 3.3), $p = 0.09$

\*Values described as Median (Interquartile Range) after multivariable analysis  
\*Not all of the studied T-Cell Activation and Inflammatory markers were included in this table

**655 CCR5+CD8+ T-Cell Levels Are Associated With Cardiovascular Events in Patients on cART**

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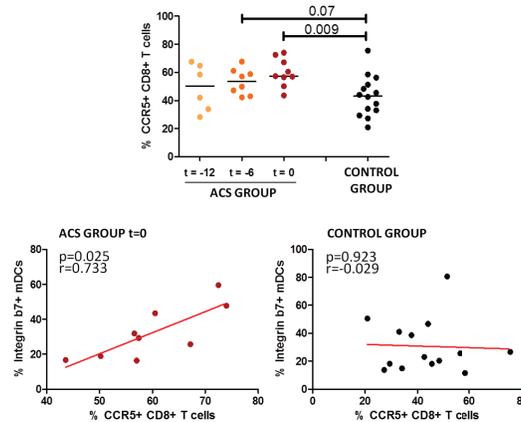
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**Background:** Acute coronary syndrome (ACS) is one of the most frequent non-AIDS-defining Event (nADEs). Previous cross-sectional studies have observed an increased incidence of atherosclerosis and higher T lymphocyte activation in HIV patients compared to healthy subjects. However, there are no longitudinal studies analyzing the characteristics of T and dendritic cells (DCs) phenotypes that precede an ischemic event in these patients. The aim of this work was to study in peripheral blood the T and DCs phenotype of HIV patients with ACS on suppressive cART along the year before the nADE.

**Methods:** HIV-infected patients on suppressive cART who suffered an ACS (myocardial infarction or angina) without other previous nADE, were included (n=15). Peripheral blood mononuclear cells were obtained from the closest time-point (t=0), and 12 (t=-12) and 6 (t=-6) months before the ACS. HIV-infected subjects on suppressive cART without previous nADEs, matched by age, sex, CD4 T-cell counts, CD4 T-cell nadir, RNA+ for HCV and time from diagnosis (n=16) were included as control group. CCR5 expression was quantified by flow cytometry in T cells (CD4 and CD8) according to the maturation profiles: T<sub>NAIVE</sub> (CD45RA+CD27+), T<sub>CM</sub> (CD45RA-CD27+), T<sub>EF</sub> (CD45RA-CD27-) and T<sub>TD</sub> (CD45RA+CD27-). CCR7 and integrin-β7 expression was quantified in myeloid DCs (mDCs; Lin2-, HLA-DR+, CD11c+) and plasmacytoid DCs (pDCs; Lin2-, HLA-DR+, CD123+).

**Results:** At t=0, patients with ACS showed a decrease in T<sub>CM</sub> CD8 (p=0.03) and an increase in T<sub>TD</sub> CD8 (p=0.04) counts compared to the control group. A progressive increase in the percentage of total CD8 T-cells expressing CCR5 was observed along the 12 months preceding the ACS. These levels were higher at t=0 (p=0.009 for total CD8 T-cells; p=0.09 for T<sub>CM</sub> CD8; p=0.03 for T<sub>NAIVE</sub> CD8; p=0.03 for T<sub>EF</sub> CD8 and p=0.05 for T<sub>TD</sub> CD8) compared to the control group. The integrin-β7 expression in mDCs and CCR7 expression in pDCs, were strongly and positively associated with the expression of CCR5 in CD8 T-cells only in the group with ACS at t=0 (p=0.025; Rho=0.733 and p=0.03; Rho=0.717 respectively).

**Conclusions:** The increase in the levels of CD8 T-cells expressing CCR5 precedes the occurrence of ACS in HIV-infected patients on suppressive cART. This increase may be due to a greater recruitment of CD8 T-cells and DCs into the inflammatory focus. These new data suggest CCR5 as a new marker of cardiovascular risk and a potential therapeutic target to prevent the development of such events.



656 Soluble TWEAK May Predict Carotid Atherosclerosis in Treated HIV Infection

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**Background:** Despite potent antiretroviral therapy (ART), HIV-infected subjects remain at higher risk of cardiovascular disease (CVD) when compared to the general population. Soluble Tumor Necrosis Factor Weak Inducer of Apoptosis (sTWEAK) is a cytokine that belongs to the tumor necrosis factor (TNF) family and has been proposed as a novel biomarker of cardiovascular disease risk, specifically in inflammatory conditions. This study compares levels of sTWEAK, sCD163 and the sCD163/sTWEAK ratio in HIV positive and negative patients and the cardiovascular and inflammatory factors associated with sTWEAK levels.

**Methods:** The data for our analysis come from 174 HIV positive adults and 59 healthy controls. HIV participants were on stable antiretroviral therapy (ART), with HIV-1 RNA < 50 copies. Markers of systemic inflammation and monocyte activation, as well as carotid intima-media thickness (IMT), were assessed. Non-parametric Wilcoxon-Mann-Whitney tests were used for comparing markers by groups, and multivariable quantile regression analyses used to assess associations of sTWEAK and sCD163 with other markers of inflammation and cIMT.

**Results:** Overall, 71% were male; 62% African Americans; median age was 39 years; median absolute CD4 was 652. HIV infected participants had reduced sTWEAK levels and increased sCD163 compared with healthy subjects (see table). In separate multivariable models, after adjusting for age, sex and race, sTWEAK and sCD163 were significantly correlated with markers of inflammation and CVD. sTWEAK was associated with IL-6 (β= 29.2, p= <0.01) and common carotid artery IMT (β= 1806.5, p= <0.01); sCD163 with IL-6 (β=6.6, p=0.01), d-dimer (β=-69.5, p=0.05), VCAM (β=72.4, p=0.05), TNF RI (β=91.1, p<0.01), TNF RII (β=87.8, p<0.01), CD14+CD16+ monocytes (β=7.9, p<0.01) and common carotid artery IMT (β=678.9, p=0.03); sCD163/sTWEAK ratio was also associated with common carotid artery IMT (β=-0.7, p=0.05).

**Conclusions:** HIV-infected participants showed an inflammatory profile as shown by increased systemic inflammatory and monocyte activation markers. Soluble CD163 and sTWEAK concentrations were independently associated with carotid intima-media.

	HIV positive (n=174)	HIV negative (n=59)	P value
sTWEAK median (IQR)	850 (541, 2539)	1707 (629, 4780)	0.0098
sCD163 median (IQR)	607 (363, 901)	449 (265; 651)	0.0054
sCD163/sTWEAK median (IQR)	0.52 (0.17, 1.24)	0.17 (0.06; 0.61)	0.0009

Table 1: sTWEAK, sCD163 and sCD163/sTWEAK ratio in HIV positive and negative patients

657 Diet, Gut Integrity Markers, and Cardiovascular Disease Risk in HIV+ Adults

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**Background:** HIV+ adults are at increased risk of cardiovascular disease (CVD), at least partially driven by monocyte activation and inflammation. The role of dietary factors on microbial translocation, inflammation and subsequent markers of CVD has not been explored in this population. Our purpose was to describe the relationships between diet, markers of gut integrity, inflammation, and subclinical CVD. We hypothesized that higher dietary intake of fat, specifically saturated fat, would be related to worse gut integrity (intestinal fatty acid binding protein (IFAB), lipopolysaccharide binding protein (LBP)), increased inflammation (CRP, IL-6, sTNFR1I), and worse markers of subclinical CVD (carotid intima-media thickness (CIMT) and coronary calcification).

**Methods:** We conducted a secondary analysis of 147 HIV+ participants in the SATURN-HIV study, a 96-week RCT testing the effect of rosuvastatin on markers of CVD. Dietary intake was assessed using a dietary recall; serum biomarkers were collected; CIMT was measured by high resolution ultrasound; and coronary calcification was assessed by cardiac CT using the Agatston method. Quantile regression and generalized estimating equations were used to analyze cross-sectional and longitudinal relationships.

**Results:** Median age was 45 years and 78% were male. On average, participants had HIV for 12 years and been on antiretroviral therapy (ART) for 7.2 years. Participants consumed an average of 2395 calories per day of which 43% was carbohydrates, 41% fat (14% saturated), and 16% protein. Diet was stable over time. IFAB and LBP were elevated at baseline and did not change over time. At baseline, saturated fat and polyunsaturated fat were both negatively associated with IFAB ( $p=0.04$  and  $p=0.08$ , respectively), but not with LBP, after controlling for age, sex, race, PI duration and BMI. During study follow up, IFAB levels were negatively associated with sTNFR1I ( $p=0.02$ ) but not the other inflammation markers, and also negatively with CIMT ( $p=0.06$ ) and coronary calcification ( $p<0.01$ ), after controlling for age, sex, race, and BMI.

**Conclusions:** We found that dietary fat, both saturated and unsaturated, were associated with IFAB in HIV+ adults. IFAB levels are elevated in HIV+ adults compared with HIV uninfected populations and did not change after statin therapy. Over time we found consistent unexpected evidence that high IFAB is associated with more favorable subclinical CVD markers.

## 658 Does Pulse-Wave Velocity Normalize With Increasing Time on ART? Evidence From CHER

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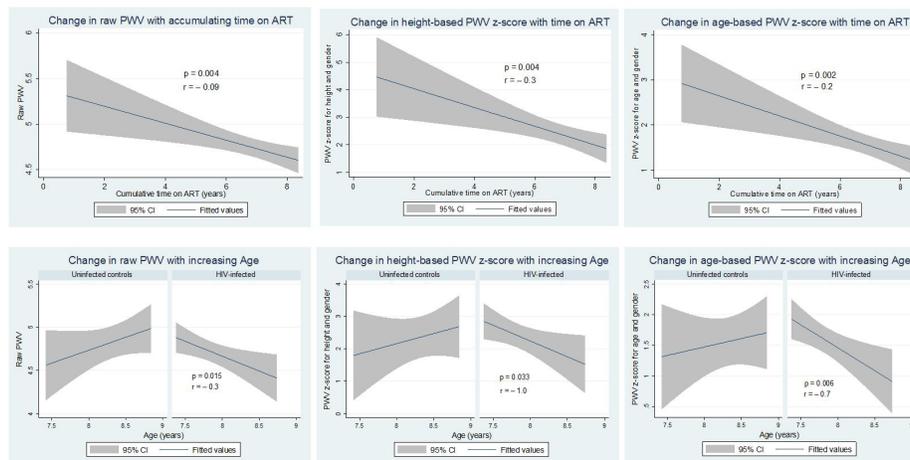
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**Background:** Cross-sectional evidence strongly suggests increased prevalence of vascular disease in HIV+ children on antiretroviral therapy (ART) after adjusting for traditional atherosclerosis risk factors. Vascular abnormalities are typically associated with advanced HIV disease and with ART, particularly lopinavir/ritonavir (LPVr). Thus far, pediatric studies have focused on children initiating ART much later than 3 months of age. Whether very early ART will prevent HIV-related vascular disease is unknown. Aorto-femoral pulse wave velocity (PWV) is a sophisticated and sensitive measure of elevated arterial wall stiffness, typically due to atherosclerosis or subclinical arteritis. Reduced arterial wall elasticity leads to progressively faster propagation of the arterial pulse wave. Early PWV elevations strongly predict subsequent incident cardiovascular events in asymptomatic adults. Aim: To determine the trajectory of PWV in HIV-infected school children who initiated LPVr-based ART very early in life with minimal HIV disease and normal CD4 counts in a well-resourced trial setting.

**Methods:** PWV measurements on primary-school-age children who initiated LPVr-based ART in the CHER trial. HIV-uninfected control group from the same communities and socio-economic background. Simple linear regressions of PWV z-scores on age and on cumulative time on ART.

**Results:** 89 HIV-infected (median age 7.7 [IQR 7.6 – 8.5] years; 54% female) who initiated ART [zidovudine, lamivudine, LPVr] at median 9 [7 – 12] weeks of age, with cumulative time on ART of median 7.1 [6.7 – 7.5] years and normal CD4 counts. 53 uninfected (median age 8.5 [7.8 – 8.7] years; 40% female) with similar weight, height, body mass index z-scores and waist circumference to height ratio ( $p>0.10$ ). In HIV-infected children, all PWV measures improved consistently with increasing cumulative time on ART and with increasing age (figure 1). In socio-economically-matched uninfected controls, PWV measures did not improve or worsened with increasing age (figure 1).

**Conclusions:** In children who initiated ART very early in life, PWV progressively normalizes with accumulating time on ART.



## 659 Changes in CVD Risk Factors With Early and Deferred ART in the START Trial

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**Background:** HIV infection and certain antiretroviral medications appear to contribute to cardiovascular disease (CVD) risk. CVD events were similar between the early (at CD4 >500cells/ $\mu$ L) and deferred (to CD4 <350cells/ $\mu$ L) antiretroviral therapy (ART) arms in START. We studied individual risk factor changes over time in START to understand the net influence on CVD risk.

**Methods:** Clinical and laboratory measures were ascertained annually among START participants. Mean change from baseline in risk factors between the early and deferred ART arms were compared over follow-up. Framingham and D:A:D 10yr CVD risk scores were calculated using published equations. Incident dyslipidemia (LDL >160mg/dL or use of lipid lowering drug), diabetes (diagnosis or fasting blood glucose  $\geq$ 126 mg/dL) and hypertension (diagnosis or use of blood pressure medication) during follow-up were assessed using unadjusted Cox proportional hazards regression.

**Results:** Characteristic among 4,685 START participants across 35 countries at entry were: median age was 36 years, CD4 count 651cells/mm<sup>3</sup>, HIV viral load 12,759 copies/mL, 73% male, 32% smokers; median CVD risk variables were SBP/DBP 120/76 mmHg, Total Cholesterol (C) 168 mg/dL, LDL-C 102 mg/dL, HDL-C 43 mg/dL, fasting glucose 85 mg/dL. At entry median 10 year risk for CVD was 2.3% and 1.8% (Framingham and D:A:D, respectively). Mean follow-up was 3.0 years. The early and deferred ART groups spent 94% and 28% of follow-up time on ART, respectively. Differences in CVD risk factors between early and deferred ART over time are shown in the table. Early ART increased fasting glucose and all lipid parameters, with a minimal decline in Total:HDL-C. Incident dyslipidemia was greater among early vs. deferred groups (hazard ratio 1.62 [95% CI: 1.33-1.94]), though incident diabetes or hypertension did not differ between groups. The use of lipid-lowering therapy increased overall during follow-up, but use did not differ between ART groups over time.

**Conclusions:** Among a diverse global population of HIV+ persons with high CD4 counts, early ART initiation had increased LDL-C and the prevalence of dyslipidemia. However, concurrent increases in HDL-C and other mixed effects resulted in no consistent differences in CVD risk scores over time. These randomized data suggest that early ART has both positive and negative influences on CVD risk among HIV+ individuals with preserved immunity.

**TABLE: Differences (Early-Deferred) in Traditional CVD Risk Factors between Early and Deferred ART in START at Annual Visits and Overall During Follow-up Among Participants in START**

CVD Risk Factors	1 Yr (n=4384) Diff.* ± SE	2 Yr (n=3690) Diff.* ± SE	3 Yr (n=2155) Diff.* ± SE	4 Yr (n=1131) Diff.* ± SE	All Visits Diff.** (p-value)
Total-C (mg/dL)	12 ± 0.9	12 ± 1.1	13 ± 1.4	11 ± 2.1	11 (<0.001)
LDL-C (mg/dL)	5 ± 0.8	7 ± 0.9	5 ± 1.2	7 ± 1.8	6 (<0.001)
HDL-C (mg/dL)	5 ± 0.3	5 ± 0.4	5 ± 0.5	5 ± 0.8	5 (<0.001)
TotalHDL-C	-0.1 ± 0.04	-0.1 ± 0.05	-0.1 ± 0.05	-0.2 ± 0.07	-0.1 (<0.001)
Non-HDL-C (mg/dL)	7 ± 0.8	7 ± 1.0	8 ± 1.4	6 ± 1.9	7 (<0.001)
Fasting glucose (mg/dL)	2 ± 0.7	2 ± 0.7	3 ± 0.9	1 ± 1.4	2 (<0.001)
SBP (mmHg)	-0.1 ± 0.4	-0.01 ± 0.4	-0.3 ± 0.6	-0.9 ± 0.8	-0.1 (0.57)
DBP (mmHg)	-0.3 ± 0.3	-0.2 ± 0.3	-0.9 ± 0.4	-0.6 ± 0.6	-0.3 (0.07)
FRS 10yr CVD (%)	-0.1 ± 0.09	-0.1 ± 0.11	-0.2 ± 0.16	-0.3 ± 0.23	-0.1 (0.07)
D.A.D 10yr CVD (%)	0.2 ± 0.05	0.2 ± 0.06	0.1 ± 0.09	-0.03 ± 0.17	0.2 (<0.001)

\*Early minus deferred mean change from baseline (± standard error) for each variable using unadjusted linear models at each visit.  
\*\*Differences over all follow-up using longitudinal mixed models adjusting for baseline levels.  
SE= Standard Error FRS=Framingham Risk Score. D.A.D=Data collection on Adverse events of Anti-HIV Drugs Cohort

**660 Cardiovascular Biomarkers After Switch to ABC/DTG/3TC: The STRIVING Study**

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**Background:** Improvements in inflammatory biomarker profiles have been documented in virologically-suppressed patients switching from PI and/or NNRTI to RAL and EVG. Because improvements in chronic inflammation may affect mortality and co-morbid disease development, we evaluated the effects of switch to ABC/DTG/3TC on markers of inflammation and immune activation.

**Methods:** Participants were randomized 1:1 to switch to ABC/DTG/3TC or continue current suppressive (HIV-1 RNA <50 copies/mL) ART for 24 weeks. C-reactive protein (hs-CRP), interleukin-6 (IL-6), D-dimer, soluble vascular cell adhesion molecule (sVCAM), soluble CD14 and CD163 (sCD14, sCD163), and intestinal fatty acid binding protein (I-FABP) were measured as secondary endpoints on cryopreserved specimens using standardized assays. Mean 24-week changes in log-transformed biomarker values were compared between participants switching ABC/DTG/3TC vs continued suppressive ART using analysis of covariance (ANCOVA). No statistical adjustment for multiple comparisons was made.

**Results:** Participants (274 switched to ABC/DTG/3TC, 277 continued current ART) were 86% male, 28% African American and had median age 45 years, CD4+ T lymphocyte count 610 cells/mm<sup>3</sup> and median time on ART 4.4 years. At entry, ART use was 42% PI, 31% NNRTI, 26% RAL or EVG, 77% tenofovir and 23% abacavir. After 24 weeks, mean changes in hs-CRP, IL-6, D-dimer, sVCAM and sCD163 were similar between participants switching to ABC/DTG/3TC and continuing current ART; however, greater declines in I-FABP and sCD14 were observed among participants switching to ABC/DTG/3TC (p<0.05), some of whom switched from RAL and EVG.

**Conclusions:** Switch to ABC/DTG/3TC was associated with greater declines in sCD14 and I-FABP levels, even among participants switching from other integrase inhibitors. These changes may suggest reduced microbial translocation and monocyte activation following switch to ABC/DTG/3TC, which could have important implications for morbidity and mortality. Additionally, no worsening of markers associated with cardiovascular disease were observed following switch to ABC/DTG/3TC.

	Baseline Value (n)	Change from baseline at Week 24 (n)	Geometric Adjusted Mean Change*100 (%)	Geometric Mean Ratio; 95% CI for Ratio	P value
D-dimer (nmol/L)					
ABC/DTG/3TC	2.54 (267)	-0.04 (206)	0%	0.00 (-0.09, 0.10)	0.98
Current ART	1.99 (271)	0.08 (217)	0%		
hs-CRP (mg/L)					
ABC/DTG/3TC	3.24 (271)	0.18 (223)	22%	0.00	1.00
Current ART	3.08 (274)	0.27 (230)	22%	(-0.17, 0.17)	
I-FABP (ng/L)					
ABC/DTG/3TC	1,817 (265)	-519 (204)	-40%	-0.44 (-0.56, -0.33)	<0.001
Current ART	1,948 (273)	-11 (220)	4%		
IL-6 (ng/L)					
ABC/DTG/3TC	3.13 (265)	-0.69 (206)	-16%	0.07 (-0.07, 0.21)	0.31
Current ART	2.92 (274)	-0.58 (221)	-23%		
sCD14 (ng/L)					
ABC/DTG/3TC	2,169,181 (266)	-532,288 (207)	-28%	-0.08 (-0.12, -0.04)	<0.001
Current ART	2,259,431 (274)	-451,744 (222)	-20%		
sCD163 (µg/L)					
ABC/DTG/3TC	627.18 (266)	47.74 (206)	9%	0.01 (-0.03, 0.06)	0.58
Current ART	627.61 (274)	48.59 (221)	7%		
sVCAM-1 (ng/L)					
ABC/DTG/3TC	1,639,099 (266)	-142,718 (206)	-15%	0.01 (-0.06, 0.08)	0.74
Current ART	1,656,664 (274)	-181,428 (221)	-16%		

Note: Cardiovascular biomarkers are analyzed based on log transformed data. Estimates were from an ANCOVA model adjusting for ART third agent class, interaction of treatment and original ART third agent class, sex, race (white, black or African American, Other), and baseline biomarker level.

**661 Abacavir (ABC) Use and Risk of Recurrent Myocardial Infarction (MI)**

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**Background:** Whilst several studies have reported that current exposure to ABC is associated with an increased risk of MI, associations with the risk of a subsequent MI have not been investigated.

**Methods:** We considered the rate of recurrent MI among 816 D:A:D participants who experienced an MI during study follow-up and who remained under follow-up at 28 days post-MI. Follow-up was considered from 28 days post-MI to the date of next MI, death, 1/2/2014 or 6 months after last clinic visit. Poisson regression models considered associations between recurrent MI and exposure to ABC (use at initial MI, current post-MI exposure and cumulative exposure), before and after adjusting for year and age.

**Results:** Included individuals were largely male (91.4%), infected with HIV through sex between men (59.6%) and had a median age of 51 years (inter-quartile range [IQR] 44-58) at initial MI. 80.1% of participants were current/ex-smokers. Median CD4 at initial MI was 503 (IQR 340-728) cells/mm<sup>3</sup> and 66.8% had a HIV RNA  $\leq 50$  cps/ml. 415 people (50.9%) had received ABC prior to initial MI for a median of 3.1 years (IQR 0.1-13.9). Of the 277 (34.0%) still on ABC at initial MI, 204 (73.7%) subsequently stopped it at a median of 337 (0-3616) days post-MI. There were 102 recurrent MIs over 3863 person-years (PY, rate 2.64/1000 PY, 95% confidence interval [95%CI] 2.13-3.15). Rates of recurrent MI were 2.75 (1.90-3.60) and 2.57 (1.93-3.21)/1000 PY in those who were and were not on ABC at initial MI, and 3.47 (2.37-4.57) and 2.31 (1.75-2.88)/1000 PY in those who were and were not currently receiving ABC post-MI. Whilst neither cumulative exposure to ABC nor receipt of ABC at initial MI were associated with recurrent MI risk, current post-MI exposure was associated with an increased risk (Table). With the exception of age (1.02 (1.00-1.04)/5 years), there were no significant associations between demographic/lifestyle factors and recurrent MI. Earlier initial MI was, however, associated with an increased risk (1999-2001 vs. 2011-2013: 12.32 (5.17-29.37)). The association between recent ABC use and recurrent MI risk was similar after controlling for age, but attenuated after controlling for calendar year (Table).

**Conclusions:** Whilst we found some evidence that use of ABC post-MI was also associated with an elevated risk of a recurrent MI, this appeared to be largely explained by greater use of ABC in those with an MI in the earlier years of the study.

**Table:** Associations between use of ABC at initial MI, current post-MI use and cumulative exposure to ABC post-MI and risk of recurrent MI

	Relative hazard (95%CI)	p-value
<i>Unadjusted</i>		
Receipt of ABC at initial MI	1.07 (0.72-1.59)	0.74
Current post-MI use of ABC	1.50 (1.00-2.24)	0.05
Cumulative exposure to ABC (/5 years)	0.88 (0.65-1.19)	0.41
<i>Association between current post-MI use of ABC after adjustment for:</i>		
Current age	1.51 (1.01-2.25)	0.04
Calendar year	1.19 (0.79-1.79)	0.40

**662 Cardiovascular Risk Profile of Abacavir and Tenofovir Independent of HIV Infection**

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**Background:** Observational and clinical studies suggest that abacavir sulphate (ABC), a component of antiretroviral therapy (ART) may be associated with a reversible increased risk of myocardial infarction (MI) and increased platelet aggregation in HIV positive patients. It is not clear whether increased cardiovascular risk is driven pharmacologically by ABC or pathophysiologically by HIV and co-morbidities. Since MI is platelet-driven, our hypothesis was that ABC increases cardiovascular risk *via* pharmacological modulation of platelet aggregation.

**Methods:** The direct effect of ART on platelets, independent of HIV infection, was determined by assessing aggregation of isolated human platelets from non-infected volunteers in the presence of approximate C<sub>max</sub> concentrations of metabolites of ABC or tenofovir disoproxil fumarate (TDF). The ability of ABC and TDF to interrupt inhibition of platelet aggregation by the endogenous negative regulator nitric oxide (NO) was also compared. In addition, platelet thromboembolism was assessed in the circulation of animals treated with ABC or TDF allowing for assessment of the effects of ART in the presence of endogenous NO.

**Results:** Tenofovir (TFV, metabolite of TDF that is converted to active metabolite intracellularly) significantly inhibited isolated platelet aggregation *in vitro*, however, no effect was detected with carbovir triphosphate (CT, active metabolite of ABC). CT blocked NO-mediated inhibition of platelet aggregation, in contrast no significant effect was observed for TFV. Administration of ABC to mice significantly enhanced platelet thromboembolism 30 mins after treatment. The effect of ABC in mice dissipated within 4 hours indicating a reversible effect. No effect was observed following treatment of mice with TDF at any time point.

**Conclusions:** The increased cardiovascular risk associated with ABC in patient studies may be mediated by reversible pharmacological modulation of platelet and endothelial function rather than by HIV infection or differences in confounding factors between patient groups. In contrast, TDF exerts effects upon platelets that would not be expected to increase the incidence of platelet-driven events such as MI.

**663 Abacavir Induces Platelet-Endothelium Interactions Through Endothelial P2X7 Receptors**

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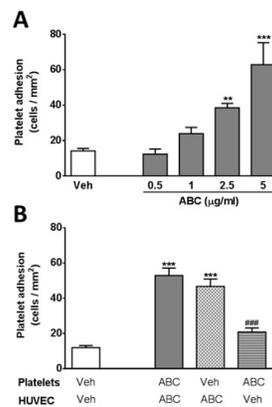
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**Background:** Controversy surrounding the epidemiological association of Abacavir (ABC) with cardiovascular diseases is fuelled by the lack of clear evidence concerning the underlying mechanisms. We have reported that ABC induces leukocyte-endothelial cell interactions. This is relevant, as thrombus formation also occurs as a result of the interplay between platelets and endothelial cells. Given the chemical similarity between then drug and purinergic mediators (ATP, ADP and AMP), we have now evaluated the role of ATP-receptors on platelet-endothelial cell interactions induced by ABC.

**Methods:** Human umbilical vein endothelial cells (HUVEC) and washed platelets were treated with clinical concentrations of ABC (0.5 - 5 µg/ml) and platelet-endothelial interactions were evaluated using an *in vitro* flow chamber system. To determine the cell type involved, each one was treated individually with ABC. To assess the implication of purinergic receptors, cells were pre-treated prior to administration of ABC (5 µg/ml) with non-selective (Suramin) or selective P2X7 (A804598) or P2X2/3 (A317491) ATP-receptor antagonists in the case of HUVEC, and with selective P2Y1 (MRS2500) or P2Y12 (Clopidogrel) or P2X1 (NF449) ADP or ATP-receptor antagonists in the case of platelets.

**Results:** ABC induced a highly significant and dose-dependent increase in platelet adhesion to the endothelium, which was observed exclusively when endothelial (and not platelets) were exposed to ABC (Figure 1). These interactions were absent when HUVEC were pre-treated with Suramin or the selective P2X7 antagonist (A804598), but were present when other P2X receptors were blocked. Platelet purinergic receptor antagonists did not modify the effects of ABC.

**Conclusions:** ABC produces adhesion of human platelets to endothelial cells and the endothelium plays an important role in these interactions. These actions of ABC seem to result from the activation of endothelial P2X7 receptors, and reproduce the drug's effects on leukocyte/endothelium interactions, thus highlighting the importance of purinergic signalling interference in the vascular effects of ABC. Our results support the relationship between ABC and cardiovascular toxicity.



**Figure 1: Effects of ABC on platelet-endothelial cell interactions.** A. Dose-response actions of ABC. B. Effects of ABC when both cell populations were incubated with the drug, or when either endothelial cells or platelets was individually in contact with ABC (5 µg/ml). Data (n=5) were mean±SEM. \*\*p<0.01 or \*\*\*p<0.001 vs. control, \*\*\*p<0.001 vs. treatment with ABC in both cell types (one-way ANOVA-Newman-Keuls). HUVEC: human umbilical venular endothelial cells. ABC: Abacavir.

#### 664 Cholesterol Efflux in Newly Diagnosed HIV and Effects of Antiretroviral Therapy

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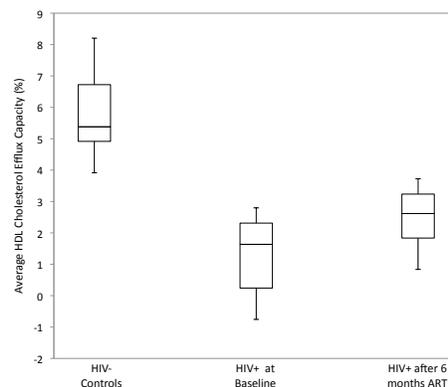
**Background:** HDL cholesterol efflux capacity (HCEC) relates inversely to incident cardiovascular events in the general population. Previous studies suggest that HCEC is decreased in HIV and data on effects of antiretroviral therapy (ART) on HCEC are conflicting. Here, we compare HCEC in ART-naïve, newly diagnosed HIV+ subjects versus that in matched HIV- controls. We further test effects of newly initiated elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (E/C/F/TDF) on HCEC. Within the HIV+ cohort, we assess relationships between ART-induced changes in metabolic/immune parameters and HCEC.

**Methods:** Baseline data from 10 ART-naïve HIV+ subjects and 12 prospectively matched HIV- controls were analyzed. In the HIV+ cohort, findings before and 6 months after E/C/F/TDF therapy were also assessed. The primary outcome was HCEC, as measured by the ability of J774 mouse macrophages under low-level LXR stimulation to efflux cholesterol to apo-B depleted sera from participants.

**Results:** In the ART-naïve HIV+ group, HIV diagnosis was established within 0.73±0.62 years, median age was 29 years, and median HDL level was 40 mg/dl. CD4 count was 440±143 cells/mm<sup>3</sup> and median viral load was 32,000 copies/mL. There were no statistically significant differences in age or HDL levels in the HIV+ versus HIV- group. HCEC was significantly lower in the HIV+ group (1.3% HIV+ vs. 5.8% HIV-, p<0.0001). In the HIV+ group, as expected, 6 months of E/C/F/TDF resulted in a rise in CD4 and suppression of viral load. E/C/F/TDF significantly increased HCEC (mean Δ 1.1%, p=0.02), although not to the level seen in controls. With E/C/F/TDF, there were trends towards an increase in HDL levels (p=0.06) and decrease in levels of the monokine CXCL10 (p=0.09). ART-induced changes in HCEC related inversely to ART-induced changes in CXCL10 (R<sup>2</sup> 0.47, p=0.03). Among the whole group, in multivariate modeling, HIV status and HDL levels remained significantly, independently related to HCEC (R<sup>2</sup> 0.84, p for overall model <0.0001, p for HIV status <0.0001, p for HDL = 0.01).

**Conclusions:** Our data suggest benefits of E/C/F/TDF on HCEC, a CVD risk surrogate linked to events. Moreover, among newly diagnosed HIV+ individuals with preserved CD4, we show a relationship between ART-induced dampening of immune activation and ART-induced improvement in HCEC. Further work is needed to characterize whether ongoing HDL dysfunction contributes to CVD risk even among ART-treated HIV+ individuals.

**Figure 1: HDL Cholesterol Efflux Capacity among Newly-Diagnosed ART-Naïve HIV+ Subjects before and after 6 months of ECF/TDF therapy, and among HIV-controls**



## 665 Dysregulated Monocyte Cholesterol Metabolism Gene Expression With ART Initiation

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**Background:** *In vitro* and *ex vivo*, untreated HIV is associated with accumulation of intracellular cholesterol in monocyte/macrophages (M/M) and impaired cholesterol efflux, both of which may impact on atherogenesis and cardiovascular disease (CVD) risk. We aimed to prospectively examine the effect of antiretroviral therapy (ART) initiation on monocyte cholesterol metabolism *in vivo*.

**Methods:** In a multi-centre, prospective study, 28 genes involved in regulation of monocyte cholesterol metabolism (sensing, uptake, endogenous cholesterol synthesis and efflux), inflammation and mitochondrial function were measured using quantitative PCR arrays in RNA extracted from monocytes derived from ART-naïve adults at baseline and at 4 and 12 weeks post ART initiation. Data are presented as median (IQR), with genes expressed as a ratio to three housekeeping genes (*ACTB*, *TBP*, *RPL13*). Within- and between-group (PI/non-PI) differences were compared using Wilcoxon signed rank and Mann-Whitney U tests respectively.

**Results:** Of 85 HIV-positive subjects, (median (IQR) age 37 (31, 44) years, 70 (82%) male, 62 (73%) Caucasian, CD4+ T-cell count 369 (297, 547) cells/mm<sup>3</sup>, HIV RNA 42,181 (19,474, 107,593) copies/ml), 30 (35%) initiated protease inhibitor (PI) based regimens. ART initiation was accompanied by a coordinated downregulation of cholesterol sensing (*SREBF1*, *SREBF2*), uptake (*LDLR*) and endogenous cholesterol synthesis (*HMGCR*, *PMVK*, *ACAT2*) genes at weeks 4 and 12 consistent with appropriate intracellular responses to increased availability of intracellular cholesterol (table 1). However, cholesterol efflux pathways were dysregulated, with expression of *ABCA1* and its regulators (*NR1H3* and *PPARα*) downregulated while *SR-B1* gene expression, indicative of an alternative efflux pathway, was upregulated (table 1). ART initiation was accompanied by an expected downregulation of inflammatory pathway genes (*TLR4*, *NFKB1* and *NLRP3*, all  $p < 0.05$ ) and increased expression of mitochondrial RNA (*MT-CTB*,  $p < 0.01$ ). There was no significant difference in the pattern of gene expression changes with initiation of PI versus non-PI based ART.

**Conclusions:** This, the first study to prospectively examine M/M gene expression, demonstrates a molecular signature consistent with further increased availability of intracellular cholesterol and disrupted cholesterol efflux pathways with ART initiation despite expected appropriate downregulation of inflammatory pathways. How these changes impact on CVD risk remains to be determined.

Gene function	Gene	Week 4		Week 12	
		% change	P value	% change	P value
Cholesterol sensing	<i>SREBF2</i>	-18.1 (-43.3, +21.4)	0.0003	-14.7 (-40.0, +8.4)	<0.0001
	<i>SCAP</i>	-1.2 (-14.0, +16.8)	0.85	+2.8 (-7.5, 16.1)	0.17
Cholesterol regulation	<i>PPARα</i>	-20.0 (-1.4, -32.6)	<0.0001	-15.0 (-27.0, -0.6)	<0.0001
	<i>NR1H3</i>	-21.2 (-43.7, +21.8)	0.0002	-30.1 (-48.0, +8.4)	<0.0001
Cholesterol uptake	<i>LDLR</i>	-35.4 (-54.2, +10.5)	<0.0001	-37.4 (-61.9, -7.2)	<0.0001
	<i>CD36</i>	-2.6 (-20.5, +23.8)	0.90	+2.4 (-11.5, +36.2)	0.26
Cholesterol synthesis	<i>HMGCR</i>	-3.6 (-24.0, +16.5)	0.049	+5.2 (-17.0, +17.3)	0.68
	<i>PMVK</i>	-10.0 (-22.6, +4.6)	<0.0001	-13.2 (-30.0, +2.5)	<0.0001
	<i>ACAT2</i>	-19.5 (-28.2, -4.3)	<0.0001	-20.0 (-29.5, +5.7)	<0.0001
Cholesterol efflux	<i>ABCA1</i>	-14.1 (-37.2, +19.7)	0.007	-25.2 (-43.2, +10.8)	0.0002
	<i>SR-B1</i>	+23.2 (+6.9, +45.3)	<0.0001	+26.7 (-10.0, +45.6)	<0.0001

## 666 Metabolic Profiles After Switch From Failing First-Line ART in the Second-Line Study

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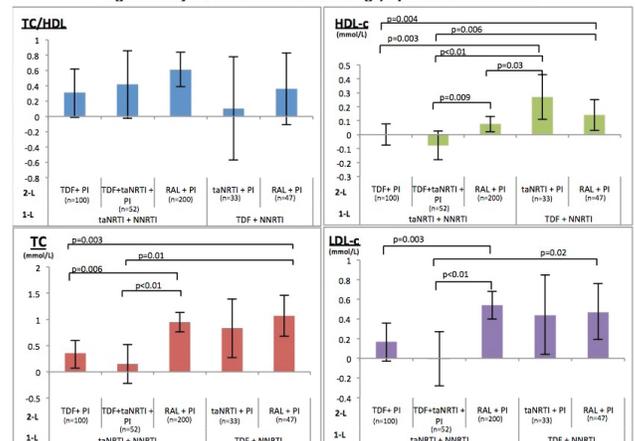
**Background:** There is little data on metabolic changes associated with boosted-lopinavir (r/LPV) containing second-line antiretroviral therapy (ART) after virological failure of first-line NNRTI+2N(t)RTI. We hypothesized that second-line ART containing thymidine analogue (ta)-NRTI+3[F]TC would confer a less favorable metabolic profile compared to TDF+3[F]TC.

**Methods:** SECOND-LINE was an open-label RCT conducted in 15 high- and middle-income countries. 541 participants received ritonavir-boosted lopinavir (LPV/r) with 2-3N(t) RTIs or raltegravir (RAL). N(t)RTIs were chosen by site investigators. 210 patients had DXA-scan soft tissue measurement at weeks 0 and 96. Participants were categorized into groups according to 2<sup>nd</sup>-line ART (ta-NRTI+3[F]TC+r/LPV; TDF+3[F]TC+r/LPV; TDF+ta-NRTI+/-3[F]TC+r/LPV; RAL+r/LPV), and pre-switch ART (TDF+3[F]TC+NNRTI or ta-NRTI+3[F]TC+NNRTI). For the TDF+ta-NRTI+/-3[F]TC+r/LPV group we combined participants who did (n=40/63) and did not (n=23/63) receive 3[F]TC. We analyzed the associations between (i) on-study second-line ART group and (ii) first-to-second-line ART switch and changes in fasted metabolic parameters [total cholesterol (TC), LDL-cholesterol (LDL-c), HDL-c, TC/HDL-c ratio, triglycerides (TG) and blood sugar (BSL), all reported in mmol/L] from baseline to week 96 after adjusting for confounders. We explored the association between metabolic and soft tissue changes in the DXA-scan subset. Linear regression methods were used.

**Results:** 454 participants were analyzed. (i) on-study analysis: those on RAL+r/LPV had greater increase in TC (adjusted mean change (aMC)=0.65, 95%CI 0.33, 0.96), LDL-c (aMC=0.38, 95%CI 0.15, 0.61) and HDL-c (aMC=-0.076, 95%CI -0.0013, 0.16) compared to TDF+3[F]TC+r/LPV. Participants who received ta-NRTI+3[F]TC+r/LPV experienced greater HDL-c increase compared to those on TDF+3[F]TC+r/LPV (aMC=0.22, 95%CI 0.05, 0.39). Those on RAL+r/LPV had a BSL increase when compared to both taNRTI+3[F]TC+r/LPV (aMC=0.89, 95%CI 0.09, 1.70) and TDF+3[F]TC+r/LPV (aMC=0.47, 95%CI -0.01, 0.92). Analysis (ii) metabolic changes by switch arm are displayed in Figure 1. Our exploratory analysis showed that a 1kg increase in trunk fat mass was independently associated with an increase in TC ( $\beta=0.098$  mmol/L, 95%CI 0.025-0.17) and LDL-c ( $\beta=0.081$  mmol/L, 95%CI 0.032-0.14).

**Conclusions:** These results describe the expected metabolic changes in patients switching from 1st- to 2nd-line N(t)RTI-containing or -sparing ART according to new WHO recommendations.

Figure 1: Adjusted mean metabolic change, by 1L to 2L switch arm.



**667 Variation in EraP Influences Risk for HLA-B\*57:01 Positive Abacavir Hypersensitivity**Rebecca Pavlos<sup>1</sup>; Kaija Strautins<sup>1</sup>; Ian James<sup>1</sup>; Simon Mallal<sup>1</sup>; Alec Redwood<sup>1</sup>; Elizabeth J. Phillips<sup>2</sup><sup>1</sup>Inst for Immunology & Infectious Diseases, Murdoch Univ, Murdoch, Australia; <sup>2</sup>Vanderbilt Univ Sch of Med, Nashville, TN, USA

**Background:** Abacavir (ABC) binds non-covalently to the floor of the peptide-binding groove of HLA-B\*57:01, altering the chemistry and shape of the antigen binding cleft. This allows previously intolerized self-peptides to be presented by HLA-B\*57:01 which upon T-cell receptor binding initiates a CD8+ T-cell response and a clinical hypersensitivity reaction (HSR). Endoplasmic reticulum aminopeptidases (ERAPs) trim peptides for MHC Class I presentation, influencing the degree and specificity of the CD8+ T-cell response. Genetic variation within ERAP adds to the positive predictive value (PPV) of the HLA class I risk allele in autoimmune diseases such as HLA-B27 positive ankylosing spondylitis. Considering the altered peptide repertoire mechanism of ABC HSR we hypothesize that variation in ERAP may help explain why 45% carrying HLA-B\*57:01 can tolerate ABC.

**Methods:** SNPs within ERAP1 (rs27044, rs17482078, rs30187, rs27434, rs2287987) were examined in HLA-B\*57:01+ ABC HSR patch test positive (PT+)(n=53) and HLA-B\*57:01+ ABC tolerant(n=22) with sequence-based typing. Rs2248374, a tag SNP for functional ERAP2 haplotypes was also examined. Haplotype A is tagged by the (A) allele, while haplotype B is tagged by rs2248374(G). Fisher exact tests and multiple logistic regressions were used to compare genotypes between the ABC HSR PT+ and tolerant groups.

**Results:** HLA-B\*57:01+ ABC tolerance was associated with rs27434(GG) (18/22(82%) vs 24/53(45%) in ABC HSR PT+, p=0.005). This SNP maps to the active site within ERAP1 (AA356). For an HLA-B\*57:01 positive population the estimated PPV for rs27434 genotypes for ABC HSR PT+ is AA(100%), AG(77%) and GG(40%). A missense mutation within the domain junction (rs30187(C)) important in conformation change of ERAP1 (AA528), was overrepresented in HLA-B\*57:01+ ABC tolerant individuals (p=0.04). Analysis indicated linkage between rs27044 and rs30187, rs17482078 and rs2287987, and between rs30187 and rs27434 (all p < 0.0001). In a multivariable model with rs27434(GG), the ERAP2 SNP (rs2248372(G)) that tags haplotype B which is characterized by a truncated protein, was decreased in tolerant individuals (p = 0.005).

**Conclusions:** ERAP and particularly ERAP1 variants are important in the development of ABC HSR. ERAP activity may influence the repertoire of peptides presented by HLA-B\*57:01 or influence early changes in immunodominant epitope selection. This provides a potential pathogenic mechanism for the development of ABC HSR or ABC tolerance in HLA-B\*57:01 carriers.

**668 AZT/NNRTI Induce Greater Adipose Tissue Mitochondrial Toxicity Than AZT/PI**Robert T. Maughan<sup>1</sup>; Elena Alvarez<sup>1</sup>; Anthony Kelleher<sup>2</sup>; David Cooper<sup>3</sup>; Andrew Carr<sup>4</sup>; Patrick Mallon<sup>1</sup>; for the HAMA001 Study Investigators<sup>1</sup>Univ Coll Dublin, Dublin, Ireland; <sup>2</sup>Univ of New South Wales, Sydney, Australia; <sup>3</sup>Kirby Inst, Sydney, Australia; <sup>4</sup>St Vincent's Hosp, Sydney, Sydney, Australia

**Background:** Use of AZT in antiretroviral therapy (ART) is associated with subcutaneous adipose tissue (SAT) and mitochondrial toxicity (MtTox). However, detailed molecular analyses of the additional contribution of protease inhibitors (PI) and non-nucleoside reverse transcriptase inhibitors (NNRTI) to this toxicity are lacking.

**Methods:** In a prospective cohort study, ART-naïve HIV+ subjects initiating ART containing AZT/PI, AZT/NNRTI or non-AZT/non-PI underwent assessments of clinical, demographic parameters and limb fat (LF) by DXA at wks 0, 12, 24 and 48. Mitochondrial DNA (mtDNA) and expression of 55 key insulin signalling, lipid metabolism and mitochondrial function genes were measured by qPCR in fasting SAT biopsies from wks 0, 2 and 48. Associations between treatment groups and parameter changes were analysed by adjusted longitudinal marginal models, gene expression by LIMMA in R, with data presented as model parameter estimates (PE [95% CI]).

**Results:** 23 subjects were recruited, median [IQR] age 39 [34, 48] yrs, 91% Caucasian, 87% male, CD4+ count 132 [58, 228] cells/mm<sup>3</sup>, log HIVRNA 4.97 [4.51, 5.57] cps/ml. Over 48 weeks, LF increased as expected with non-AZT/non-PI ART (+30.5 [-11.9, +49.1] g/wk) with significantly lower LF gains in the AZT/PI and AZT/NNRTI groups (-15.7 [-45.7, +14.2], P=0.005 and +4 [-29.3, +21.3] g/wk, P=0.012 respectively). Initiation of ART led to decreased expression of inflammatory genes in groups overall, including *CCL2* (P=0.017) and *IL6* (P=0.017, P=0.033). The largest mtDNA decreases occurred with AZT/NNRTI ART (-10.2 [-20, -0.6] cps/cell/wk), being significantly greater than both AZT/PI and non-AZT/non-PI ART groups (P.E. +3.2 [-8, +14.3], P=0.001 and +2.2 [-5.2, +9.5], P=0.015 respectively, fig.1). Consistent with MtTox, this group also had upregulated expression of the mitochondrial biogenesis gene *TFAM* (P=0.012) and decreases in key metabolic genes *LEP* and *INSR* at week 48 (P=0.013 and P=0.049 respectively), none of which were observed in the other two groups. In fact expression of several key adipocyte genes increased at week 48; *PPARG* and *FABP4* with AZT/PI (P=0.021 and P=0.001) and *Adiponectin* with non-AZT/non-PI (P=0.006).

**Conclusions:** These data suggest greater SAT MtTox with AZT/NNRTI ART than either AZT/PI or non-AZT/non-PI. However, changes in LF did not differ significantly between AZT-treated groups. These findings support emerging data on NNRTIs contribution to thymidine-NRTI toxicity, suggesting additive MtTox as an underlying mechanism.

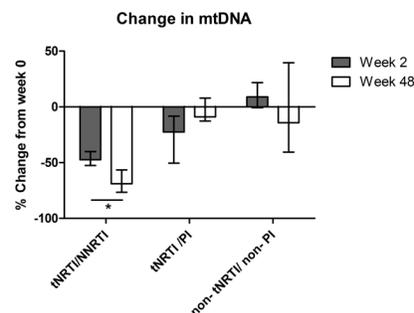


Fig. 1: Change in adipose tissue mitochondrial DNA (mtDNA). Data are median (interquartile range) % change from week 0. Group specific changes were compared using longitudinal marginal models. \*P<0.05

**669 Regulation of Telomerase Activity in PBMCs Exposed to Antiretroviral Drugs**Natalia C. Stella Ascariz<sup>1</sup>; Rocío Montejano<sup>1</sup>; Laura Pintado<sup>2</sup>; Susana Monge<sup>3</sup>; José I. Bernardino<sup>1</sup>; Ignacio Pérez-Valero<sup>1</sup>; Maria L. Montes<sup>1</sup>; Jesús Mingorance<sup>1</sup>; Rosario Perona<sup>2</sup>; Jose R. Arribas<sup>1</sup><sup>1</sup>Inst for Hlth Rsr of La Paz Univ Hosp, Madrid, Spain; <sup>2</sup>Inst de Investigaciones Biomédicas, Madrid, Spain; <sup>3</sup>Univ de Alcalá, Madrid, Spain

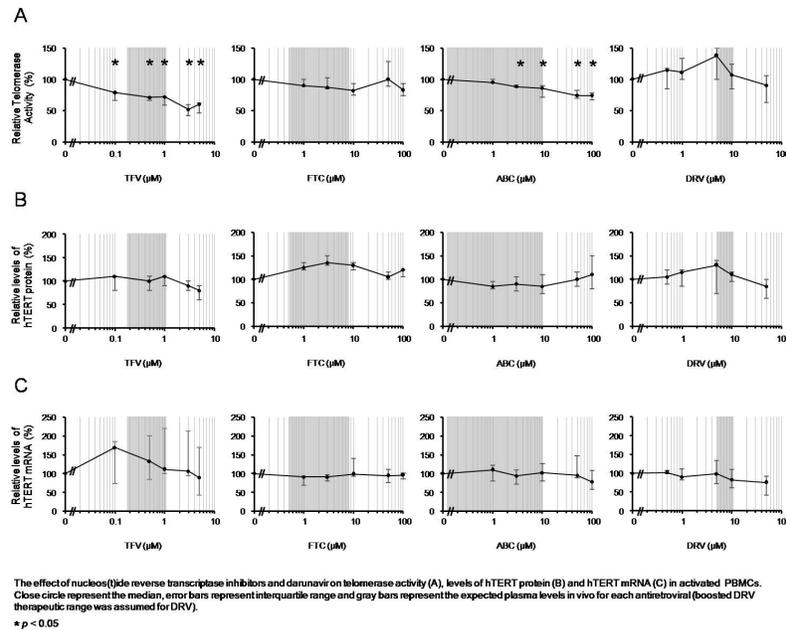
**Background:** Telomere attrition is one of the hallmarks of aging. It is unknown if this mechanism contributes to accelerated aging in HIV infected patients. One single prior study has shown that tenofovir (TFV) is a potent inhibitor of telomerase activity in vitro (J Inf Dis 2013; 207:1157). We sought to confirm this finding and to evaluate the possible impact of different antiretrovirals on expression of telomerase genes.

**Methods:** Activated peripheral blood mononuclear cells (PBMCs) from healthy donors were treated with increased concentration of TFV, abacavir (ABC), emtricitabine (FTC) or darunavir (DRV) for 72 hours. We determined telomerase activity, levels of human telomerase reverse transcriptase (hTERT) protein and expression of the genes coding for the different telomerase complex subunits (hTERT, TERC, DKC1, TIN2, TRF1 and TRF2). All results are expressed relative to untreated cells. Telomerase activity was measured using telomeric repeat amplification protocol (TRAP), levels of hTERT were determined by western blot and mRNA levels were measured by qPCR using TAQman probes in

duplicate. Experiments for each antiretroviral were repeated at least 5 times with PBMCs from different donors. Statistical significance was calculated using Wilcoxon signed rank test.

**Results:** TFV and ABC induced a significant dose-dependent decrease of telomerase activity within the therapeutic dose range in vivo (Fig A). Inhibition induced by TFV at 0.5  $\mu$ M and 1  $\mu$ M was median [(IQR), (min-max)] 29% [(29%-34%), (12%-39%)],  $p=0.042$  and 28 [(28%-41%), (25%-47%)],  $p=0.042$ . For ABC at 3  $\mu$ M and 10  $\mu$ M inhibition was 12 [(9%-13%), (8%-17%)],  $p=0.043$  and 14 [(10%-29%), (7%-40%)],  $p=0.043$ . We did not detect changes in levels of hTERT protein (Fig B) or in expression of hTERT (Fig C) and other telomerase genes (not shown). Exposure to FTC or DRV did not affect telomerase activity, levels of hTERT protein or mRNA levels of telomerase genes (Fig A, B, C).

**Conclusions:** Our results suggest that TFV and ABC but not FTC or DRV, inhibit telomerase activity within the therapeutic range. This inhibition does not involve changes in expression levels of telomerase genes or hTERT protein. The in vivo relevance of these findings remains to be elucidated.



## 670 Risk Factors of Short Telomere Length and Decreased Mitochondrial DNA in HIV Patients

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**Background:** Premature aging is one of the important issues in HIV/AIDS. Telomere length (TL) shortening and alterations of mitochondrial biogenesis are recognized as markers of cellular aging. In this study, we measured the leukocyte TL and mitochondrial DNA copy number to nuclear DNA (mtDNA) in combination antiretroviral therapy (cART) treated HIV patients and uninfected healthy controls. We investigated the association between TL and mtDNA, and examined which clinical parameters determined TL and mtDNA in young and middle-aged HIV patients.

**Methods:** Three hundred and fifty-five HIV patients on cART for > 6 months, and 141 HIV-uninfected controls were enrolled. Relative TL and mtDNA in leukocytes were estimated by quantitative real-time polymerase chain reaction. Linear regression analysis was used to determine the factors that associate with TL and mtDNA. We assessed several variables associated with HIV infection (CD4, HIV-RNA before cART), cART (duration, regimen), and other factors (age, smoking, BMI, hypertension, HOMA-IR, etc.). Variables found to be important in univariate analysis were multivariate model candidates.

**Results:** Of 355 HIV patients, 182 were aged less than 40 (young), and 173 were aged over 40 (middle-aged). Of 141 HIV uninfected controls, 64 were young and 77 were middle aged. In HIV patients, TL was significantly shorter and the rate of decline by age was greater than in controls. mtDNA also decreased significantly in HIV patients than in controls. TL was positively associated with mtDNA in young ( $r=0.441$ ,  $p<0.0001$ ) and middle-aged HIV patients ( $r=0.232$ ,  $p=0.0022$ ), but in controls, positive correlation was seen only in the middle aged ( $r=0.248$ ,  $p=0.03$ ). Multiple linear regression analysis showed that in young patients, nucleoside reverse transcriptase inhibitor (NRTI) use was independent factor of short TL ( $p=0.013$ ) and decreased mtDNA ( $p=0.02$ ). On the other hand, in middle-aged patients, protease inhibitor (PI) use ( $p=0.035$ ) as well as NRTI use ( $p<0.0001$ ) was independent factor of short TL. Other clinical parameters were not significantly related to TL and mtDNA.

**Conclusions:** In patients with cART, TL and mtDNA was positively correlated in young and middle-aged generation. NRTI use was a common risk factor of short TL and decreased mtDNA. In middle-aged patients, PI use was also risk factor of decreased mtDNA. To minimize the premature aging of HIV patients, we must optimize the cART. NRTI or PI sparing regimen is one of the candidates, especially in middle-aged patients.

## 671 CRP, D-dimer, and Oxidized LDL Predict Myocardial Infarction in Treated HIV Infection

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**Background:** The immunologic pathways that increase myocardial infarction (MI) risk in HIV-infected individuals on suppressive antiretroviral therapy (ART) and the specific role of oxidized low-density lipoprotein (oxLDL) in this process remain unclear.

**Methods:** HIV-infected cases with ART-mediated viral suppression (<400 copies/ml) who developed a confirmed Type 1 MI (plaque rupture/thrombosis) within the subsequent 3 years of an available plasma specimen were sampled from CNICS (an 8 site practice-based network) from 2001-2012. Each MI case was matched using density sampling to  $\leq 3$  controls by calendar time, age, gender, race, duration of viral suppression, and CD4 count. Associations between plasma biomarkers and subsequent MI were assessed by conditional logistic regression. Biomarkers associated with MI in unadjusted models ( $P<0.10$ ) were assessed in adjusted models to assess independence.

**Results:** A total of 51 ART-suppressed cases with subsequent Type 1 MI and 122 matched controls were selected. Among all participants, 78% were men, 56% were non-white race, and median values were: age, 50 years; duration of viral suppression, 36 months; and CD4 count, 574 cells/mm<sup>3</sup>. Among cases, the biological specimen preceded the MI by

a median of 3 (IQR: 1-9) months. Higher levels of the inflammatory marker hsCRP, the coagulation marker D-dimer, and oxLDL predicted MI in unadjusted models (Table). hsCRP remained significantly predictive of MI after adjustment for oxLDL (aOR 1.81, P=0.021), but not after D-dimer adjustment. D-dimer and oxLDL remained independently predictive of MI in fully adjusted models. Those currently taking abacavir (n=32) had higher median D-dimer levels than those on abacavir-sparing regimens (243 vs. 203 ng/ml, P=0.04), but D-dimer remained predictive of MI even among those on abacavir-sparing regimens (OR 2.06, P=0.032). We found no evidence for a relationship between Type 1 MI risk and other markers of inflammation (IL-6, sTNFR1), myeloid activation (sCD14, sCD163), gut epithelial barrier integrity (I-FABP), interferon response (IP-10), or CMV IgG titer.

**Conclusions:** While inflammation predicts Type 1 MI in treated HIV infection, hypercoagulability appears to be a more proximate predictor in the same pathway. OxLDL may also independently contribute to MI risk in this setting, representing a novel interventional target. Lack of an association between other markers and short-term Type 1 MI risk does not exclude a potential impact on atherosclerosis and longer-term cardiovascular outcomes.

Table: Relationship between Biomarkers and Subsequent MI during Suppressive ART

Biomarker	Odds Ratio per IQR increase* (95% CI)	P Value	Adjusted Odds Ratio per IQR increase* (95% CI)	P Value
hsCRP	1.81 (1.12 to 2.92)	0.016	1.32 (0.75 to 2.33)	0.33
D-dimer	1.94 (1.18 to 3.19)	0.009	2.05 (1.12 to 3.76)	0.02
oxLDL	1.76 (1.10 to 2.82)	0.019	1.89 (1.14 to 3.12)	0.013
IL-6	1.21 (0.84 to 1.75)	0.31	-	-
sTNFR1	1.09 (0.82 to 1.46)	0.56	-	-
sCD14	1.17 (0.74 to 1.82)	0.50	-	-
sCD163	0.73 (0.43 to 1.26)	0.27	-	-
I-FABP	0.99 (0.62 to 1.56)	0.95	-	-
IP-10	0.71 (0.42 to 1.21)	0.21	-	-
Anti-CMV IgG index†	1.61 (0.78 to 3.34)	0.20	-	-

\*Each biomarker was modeled continuously and log-transformed to satisfy model assumptions.  
 †Restricted to CMV-seropositive participants only (n=140)

672 Lack of Associations of Oxidized Lipoproteins With Atherosclerosis in HIV: ACTG A5260

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**Background:** The role of oxidized lipoproteins (ox-lipids) in the pathogenesis of cardiovascular disease (CVD) in chronic HIV infection remains poorly defined.

**Methods:** A5260s enrolled 328 HIV-infected antiretroviral treatment (ART) naïve participants without known CVD or diabetes mellitus. All participants were randomized to receive tenofovir disoproxil fumarate-emtricitabine plus atazanavir/ritonavir (ATV/r), darunavir/r (DRV/r), or raltegravir (RAL) as part of a large prospective clinical trial (A5257). The present analyses include 234 virologically suppressed subjects who achieved plasma HIV-1 RNA <50 copies/ml by week 24 and maintained through 3 years. Carotid intima media thickness (CIMT) was evaluated at study entry and then annually. Oxidized low-density lipoprotein (LDLox) was determined by ELISA and oxidized high-density lipoprotein (HDLox) by a novel biochemical assay. Ox-lipid fold changes from baseline were examined after 24 and 96 weeks (ratios of 1.0 indicate no change); pairwise treatment group comparisons used Wilcoxon rank-sum tests with p-values adjusted for false discovery rate control. Association analyses of ox-lipids with rate of CIMT progression over 3 years used mixed effects linear regression with ox-lipids categorized by quartiles.

Participant characteristics of this virologically suppressed cohort were similar to the full substudy population. The cohort was 90% men, 48% white, 29% black, 19% Hispanic; median age was 36 years. At baseline, 12% had a 10 year risk of hard coronary heart disease ≥6%; 13% had metabolic syndrome; prevalence of carotid lesions was low (9%). Median CD4+ cell count and HIV-1 RNA was 338 cells/mm<sup>3</sup> and 4.6 log<sub>10</sub> copies/ml, respectively. Over 96 weeks, HDLox declined with ATV/r and DRV/r; LDLox increased in all groups. Treatment group differences were apparent only with HDLox at week 24 between RAL and ATV/r, and RAL and DRV/r (p<0.025). Associations between the CIMT rate of progression and baseline and on-treatment ox-lipids were not apparent (p≥0.40, Table). These results remained consistent upon further adjustment for lipoprotein concentration.

**Conclusions:** Contrary to prior limited data that oxidized lipoproteins are associated with subclinical atherosclerosis, we did not find evidence of an association between early on-treatment levels of HDLox or LDLox and the rate of IMT progression estimated over 3 years on ART. Larger studies involving other CVD endpoints are needed to further study the role of ox-lipids in HIV-associated CVD.

	Mean Fold Change [95% Confidence interval]						Estimated Rate of CCA IMT Change (µm/year) [95% CI]	
	Week 24			Week 96			Baseline	Week 24
	ATV/r	RAL	DRV/r	ATV/r	RAL	DRV/r	Upper Quartile (vs Lower)	Upper Quartile (vs Lower)
<b>HDLox (no units)</b>								
Baseline median [Q1-Q3]	0.95	1.06	0.96	0.88	0.97	0.88	-1.16	-0.69
= 0.99 [0.84-1.22]	(0.89, 1.01)	(0.99, 1.13)	(0.89, 1.03)	(0.82, 0.95)	(0.90, 1.03)	(0.82, 0.95)	[-6.52, 4.20]	[-5.86, 4.49]
<b>LDLox (UL)</b>								
Baseline median [Q1-Q3]	1.12	1.04	1.17	1.12	1.11	1.10	2.28	0.31
= 49.0 [39.5-59.6]	(1.04, 1.19)	(0.96, 1.12)	(1.10, 1.24)	(1.05, 1.20)	(1.04, 1.18)	(1.01, 1.20)	[-3.10, 7.65]	[-4.88, 5.51]

673 Statin Effects on oxLDL in Relationship to Plaque and Arterial Inflammation in HIV

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**Background:** Circulating oxidized LDL (oxLDL) levels are elevated in HIV-infected patients and have been associated with subclinical atherosclerosis. Statins reduce plaque on coronary computed tomography angiography (cCTA) in HIV-infected individuals but whether this effect is related to changes in oxLDL is unknown. Thus, we investigated the impact of statins on serum oxLDL and the relationship between changes in oxLDL and coronary atherosclerosis on cCTA.

**Methods:** This randomized, double-blind, placebo-controlled trial included HIV-infected subjects on stable anti-retroviral therapy with subclinical coronary atherosclerosis and LDL-cholesterol < 130 mg/dL. Subjects were assigned to treatment with either atorvastatin or placebo for 12 months. Subjects underwent cCTA and measurements of serum oxLDL, sCD14, sCD163, lipoprotein phospholipase A<sub>2</sub> (Lp-PLA<sub>2</sub>), and fasting lipids including direct LDL at baseline and end of the study. Multivariate regression modeling was performed to examine the effects of change in oxLDL on change in non-calcified plaque volume, controlling for baseline CD4 count, log viral load, 10-year Framingham risk, and change in direct LDL.

**Results:** Forty HIV-infected subjects were enrolled and assigned to placebo (n=21) or atorvastatin (n=19). There was no difference in serum oxLDL at baseline between the two groups. After 12 months, serum oxLDL decreased -22.7% [95% CI -28.7 to -16.7] in the atorvastatin group and increased 7.5% [95% CI -3.3 to 18.4] in the placebo group (p < 0.0001 for comparison of change between groups). Change in oxLDL, but not direct LDL, significantly correlated with changes in non-calcified plaque volume, total plaque volume, and high risk coronary plaque features of positive remodeling and low attenuation (Table 1). The relationship between changes in oxLDL and non-calcified plaque volume was independent of the baseline 10-year Framingham risk, CD4 count, viral load, and change in direct LDL. Change in oxLDL was strongly related to change in Lp-PLA<sub>2</sub> (r=0.34, p=0.04) but not other immune markers in our study.

**Conclusions:** Statins significantly reduce oxLDL levels in HIV-infected patients, and reductions in oxLDL relate strongly to improvements in coronary atherosclerosis, independent of traditional cardiovascular risk factors. Reductions in oxLDL, via effects on arterial inflammation, may be one mechanism through which statins improve atherosclerosis in HIV-infected individuals.

**Table 1: Spearman Correlations Between Change in Plaque Characteristics and Change in Lipids and Inflammatory Markers**

	Change in Non-calcified Plaque Volume (mm <sup>3</sup> )	Change in Total Plaque Volume (mm <sup>3</sup> )	Change in Positively Remodeled Plaque (# segments)	Change in Low Attenuation Plaque (# segments)
Change in oxLDL (U/L)	<b>ρ = 0.50; p = 0.002</b>	<b>ρ = 0.34; p = 0.04</b>	<b>ρ = 0.34; p = 0.047</b>	<b>ρ = 0.41; p = 0.02</b>
Change in Lp-PLA <sub>2</sub> (ng/mL)	<b>ρ = 0.44; p = 0.007</b>	<b>ρ = 0.34; p = 0.04</b>	<b>ρ = 0.34; p = 0.04</b>	<b>ρ = 0.36; p = 0.03</b>
Change in Direct LDL (mg/dL)	ρ = 0.26; p = 0.12	ρ = 0.27; p = 0.11	ρ = 0.17; p = 0.31	ρ = 0.14; p = 0.41
Change in Total Cholesterol (mg/dL)	ρ = 0.16; p = 0.34	ρ = 0.14; p = 0.43	ρ = 0.09; p = 0.59	ρ = 0.29; p = 0.09
Change in HDL Cholesterol (mg/dL)	<b>ρ = -0.32; p = 0.05</b>	ρ = -0.20; p = 0.23	ρ = -0.20; p = 0.24	ρ = -0.30; p = 0.07
Change in Triglycerides (mg/dL)	ρ = 0.23; p = 0.16	ρ = 0.06; p = 0.71	ρ = -0.16; p = 0.35	ρ = 0.29; p = 0.09

Data are Spearman's rank correlation coefficients. Significant p-values are shown in bold. Abbreviations: oxLDL = oxidized LDL, CRP = C-reactive protein, Lp-PLA<sub>2</sub> = Lipoprotein Phospholipase-A<sub>2</sub>, LDL = Low-density lipoprotein, HDL = High-density lipoprotein, sCD14 = soluble CD14, sCD163 = soluble CD163. Data relating change in oxLDL to plaque parameters exclude two outliers in the placebo group. Sensitivity analyses including these subjects show similar results in terms of directionality and significance of relationship for non-calcified plaque volume.

**674 The Effect of Rosuvastatin on Vascular Disease Differs by Smoking Status**

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**Background:** Smoking is an important contributor to cardiovascular disease (CVD) risk and is highly prevalent in the HIV population. We have shown that rosuvastatin improves markers of inflammation (soluble tumor necrosis factor alpha receptor-II, interferon γ inducible protein 10 and lipoprotein associated phospholipase A2), immune activation [soluble CD14, proportion of tissue factor positive patrolling (CD14dimCD16+) monocytes and proportion of activated CD4+ and CD8+ T cells (CD38+HLA-DR+)] and arrests common carotid artery intima media thickness (IMT) progression in HIV+ adults. Whether smoking status modifies these effects of rosuvastatin is unknown.

**Methods:** The SATURN-HIV study is a randomized placebo-controlled trial to evaluate the effect of rosuvastatin (10 mg daily) on immune activation and subclinical vascular disease in HIV+ adults on stable antiretroviral therapy (ART) with LDL ≤ 130 mg/dL. Here we assessed whether smoking status modifies the effect of rosuvastatin on select outcome measures that differed between groups over the study. ANCOVA was used to model each outcome including a group by smoking status interaction. Stratum specific estimates are provided where the interaction was significant.

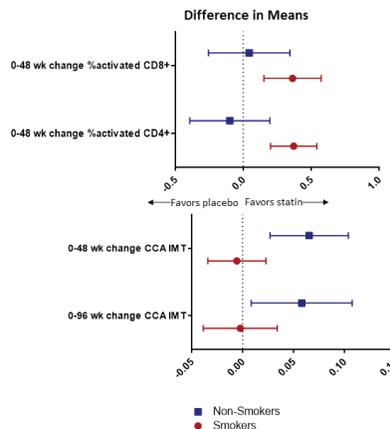
**Results:** 147 adults were included (72 rosuvastatin; 75 placebo). Groups were similar at baseline. Overall, 78% were men, 68% were black with median age 46 years and CD4 count of 613 cell/mm<sup>3</sup>. 78% had HIV-1 RNA < 50 cps/mL, 63% were current smokers (median packs per day 0.5) and another 16% past smokers. There was a significant group by smoking status interaction for 0-24 (p=0.01) and 0-48 (p<0.01) week changes in proportion of activated CD4+ T cells and a trend towards significance for 0-48 week change in activated CD8+ T cells (p=0.07). The interaction was also significant for 0-48 week change in CCA IMT (p<0.01) and trended towards significance for 0-96 week change in CCA IMT as well (p=0.06). The figure shows stratum specific adjusted means for these outcomes. No effect modification by smoking was detected for changes in markers of inflammation or monocyte activation.

**Conclusions:** Current smoking modifies the effect of rosuvastatin on CCA IMT and T-cell activation, such that the beneficial effect of rosuvastatin on CCA IMT was not apparent in smokers. In HIV+ smokers, interventions such as rosuvastatin are unlikely to be as important as smoking cessation in CVD risk reduction.

**Mean Change in T cell Activation and Carotid Intima Media Thickness for Rosuvastatin and Placebo Groups by Smoking Status**

	Rosuvastatin	Placebo	p-value
<b>Activated CD4+ T Cells</b>			
0-48 wk change			
Smokers	-0.511	-0.138	<0.01
Non-smokers	-0.365	-0.463	0.51
<b>Activated CD8+ T Cells</b>			
0-48 wk change			
Smokers	-0.613	-0.248	<0.01
Non-smokers	-0.506	-0.462	0.77
<b>CCA IMT</b>			
0-48 wk change			
Smokers	0.018	0.012	0.7
Non-smokers	-0.024	0.042	<0.01
0-96 wk change			
Smokers	0.018	0.016	0.9
Non-smokers	0.008	0.066	0.02

Values shown are mean absolute change from baseline in log-transformed outcome adjusted for the baseline value of that outcome. P-values are for between group t tests.



### 675 Coronary Artery Calcification on Chest Computed Tomography in HIV-Infected Smokers

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**Background:** Lung cancer screening in heavy smokers with chest Computed Tomography (CT) is also an opportunity to diagnose other asymptomatic smoking-related complications. The objective of our study was to evaluate the prevalence of coronary artery calcification (CAC) on chest CT in a population of HIV-infected heavy smokers, and to identify risk factors for CAC.

**Methods:** Post-hoc analysis of systematic chest CT scans performed during the ANRS EP48 HIV-CHEST multicentre study, which evaluated the feasibility of early lung cancer diagnosis in HIV-infected heavy smokers. Subjects were aged  $\geq 40$  years, had a history of smoking of at least 20 pack-years, a CD4 T-lymphocyte nadir cell count  $< 350$  cells/ $\mu$ L, and a current CD4-T cell count  $> 100$  cells/ $\mu$ L. We used a modified, published, semi-quantitative CAC score. Two radiologists reviewed the images, and discordant scores were discussed until consensus. Factors associated with presence of CAC were identified using a logistic regression model.

**Results:** The 396 subjects enrolled had a median age of 50 years, 83% were men, median pack-years of smoking was 30, 90% of subjects had a HIV viral load  $< 50$  copies/mL, and median last CD4 count was 574 cells/ $\mu$ L. CAC were observed in 266 (67%, 95% confidence interval (CI) [63; 72]) subjects, and 57 subjects (14.5%) had a CAC score  $\geq 4$ , which has been shown to be significantly associated with cardiovascular death. In multivariate analysis, older age (per 10 years increase, with an odd ratio (OR) of 2.29, 95% CI [1.72; 4.04]), male sex (OR 2.00, 95% CI [1.17; 3.42]) and duration of antiretroviral treatment (per 5 years increase, OR 1.27, 95% CI [1.05; 1.54]) were associated with CAC. Cannabis inhalation, smoking in pack-years, nadir CD4 levels, last CD4 count, hepatitis C co-infection and a last HIV viral load  $< 50$  copies/ml were not associated with CAC.

**Conclusions:** In a population of HIV-infected heavy smokers, CAC prevalence was high (67%) on chest CT scans, and was associated with age and sex as well as antiretroviral treatment duration, but neither immunological nor virological factors. Chest CT assessment in HIV-infected smokers should include CAC scoring, but whether subjects with a high CAC score should benefit from screening for silent myocardial ischemia remains to be determined.

### 676 Incidence and Predictors of Hypertension Among HIV Patients in Rural Tanzania

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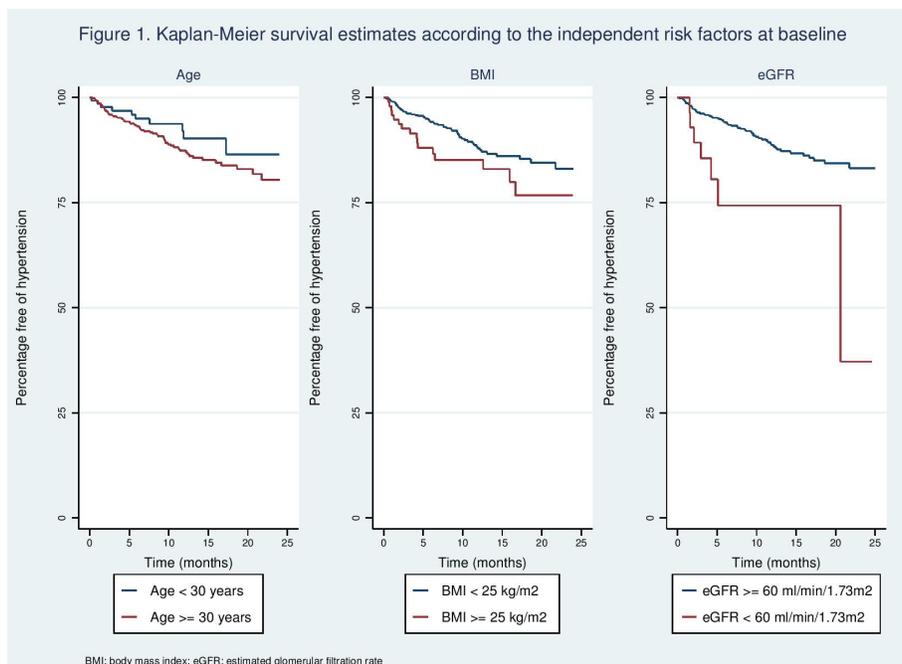
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**Background:** Management of non-communicable comorbidities is emerging as an essential part of HIV care in resource-limited settings. Yet, scarce data are available on the burden and epidemiology of cardiovascular risk factors among HIV patients in rural Sub-Saharan Africa. We explored the prevalence, incidence and predictors of hypertension development among ART (antiretroviral therapy)-naïve patients enrolled in a rural HIV clinic in southern Tanzania.

**Methods:** Prospective longitudinal study including patients enrolled in the Kilombero-Ulanga Antiretroviral Cohort (KIULARCO) between January 1, 2013 and March 2, 2015. Hypertensive patients at baseline, pregnant women and those exposed to ART before recruitment were excluded from the longitudinal analysis. Standardized blood pressure measurements were routinely performed at each visit. Incident hypertension was defined as systolic blood pressure (SBP)  $\geq 140$  mmHg and/or diastolic blood pressure (DBP)  $\geq 90$  mmHg on two consecutive visits. Cox proportional hazards models were used to assess the association of hypertension with demographic, clinical and treatment characteristics.

**Results:** Among 955 eligible subjects, 111 (11.6%) were hypertensive at baseline. Ten women who became pregnant during follow-up were excluded. The remaining 834 individuals contributed 7967 person-months (pm) to follow-up (median 231 days, IQR 119-421) and 80 (9.6%) of them developed hypertension during a median follow-up of 144 days (incidence rate 0.01 cases/pm). Median age at recruitment was 38 years (IQR 32-46), median CD4 count 188 cells/ $\mu$ L (IQR 66-367) and 62% were female. ART was started in 657/834 (79%) patients, with a median time on ART of 7 months (IQR 4-14). Cox regression models identified age (adjusted hazard ratio (aHR) per 10 years 1.34, 95% confidence interval (CI) 1.07-1.68,  $p=0.010$ ), body mass index (BMI) (aHR per 5 kg/m<sup>2</sup> 1.45, 95% CI 1.07-1.99,  $p=0.018$ ) and estimated glomerular filtration rate (eGFR) (aHR  $< 60$  versus  $\geq 60$  ml/min/1.73 m<sup>2</sup> 3.79, 95% CI 1.60-8.99,  $p=0.003$ ) as independent predictors of incident hypertension (Fig. 1).

**Conclusions:** Prevalence and development of hypertension are common among HIV patients in rural Tanzania. Traditional cardiovascular risk factors such as age, BMI or eGFR were predictive of incident hypertension, but no association was observed with immunological or ART status. These data support the implementation of routine hypertension screening and management strategies into integrated HIV programmes in rural Sub-Saharan Africa.



**677 Population-Level Decline in BMI and Blood Pressure Following Mass HIV Treatment**

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**Background:** Clinic-based cohort studies have found that antiretroviral therapy (ART) leads to an increase in BMI. The aim of this study is to determine whether the scale-up of ART is also associated with a population-level increase in BMI, blood pressure (BP), and prevalence of obesity and hypertension in a high HIV-prevalence (29% of adults HIV-infected) population in rural South Africa.

**Methods:** We conducted a cross-sectional anthropometric survey of height, weight, and BP in adults in rural KwaZulu-Natal, South Africa, before ART scale up (in 2004) and when ART coverage in the community was 25% (in 2010). 3,000 individuals were contacted for each survey. Anthropometric data was linked with data on HIV status collected as part of intensive HIV surveillance in the area.

**Results:** 2,252 and 2,088 individuals in 2004 and 2010, respectively, agreed to a height and weight measurement. BMI, and overweight and obesity prevalence declined between 2004 and 2010 in both females and males. Among females, BMI decreased from 29.9kg/m<sup>2</sup> to 29.1kg/m<sup>2</sup> (p=0.002), overweight prevalence from 30.1% to 28.8% (p=0.050), and obesity prevalence from 42.8% to 40.0% (p=0.020). Among males, the declines were even more marked with BMI decreasing from a mean of 24.2kg/m<sup>2</sup> to 23.0kg/m<sup>2</sup> (p<0.001), and overweight and obesity prevalence decreasing from 20.2% to 15.2% (p=0.005), and from 11.7% to 7.0% (p=0.001), respectively. Mean systolic BP also decreased significantly from 122.9mmHg to 118.2mmHg (p<0.0001) among females, and 128.4mmHg to 123.2mmHg (p<0.001) among males. Mean diastolic BP increased slightly from 79.6mmHg to 81.4mmHg (p<0.001) and 79.0mmHg to 80.7mmHg (p=0.005) among females and males, respectively. There were no significant changes in hypertension prevalence. Among HIV-infected individuals, BMI and systolic BP declined in females and did not change in males.

**Conclusions:** Population-level BMI and systolic BP declined significantly during the first seven years of ART scale-up in a high HIV prevalence community. This finding is likely due to ART substantially increasing survival of HIV-infected individuals in advanced disease stages and with substantial HIV-induced weight loss. For the large proportion of individuals who were overweight or obese prior to advanced HIV infection, the HIV-related decrease in BMI coupled with enrolment in chronic ART services may offer a unique opportunity to maintain healthy BMI levels through nutritional and lifestyle counselling at ART clinics.

**678 HIV Infection, ART Use, and Access to Care for NCDs in Agincourt, South Africa**

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**Background:** There is limited population-based data on the role of ART programs in the delivery of care for non-communicable diseases (NCDs) in Sub-Saharan Africa. The aims of this study were to assess differences in access to care for diabetes, hypertension and hyperlipidemia among those who are HIV-infected but have never used ART (HIV/Never ART) as compared to those who are HIV-infected but have ever accessed ART (HIV/Ever ART) and to assess the relationship between ART program participation and access to care for NCDs in the Agincourt sub-district of South Africa.

**Methods:** The Health and Aging in Africa: Longitudinal Studies of INDEPTH communities (HAALSI) Study is a cohort of adults aged 40 and over based in Agincourt, South Africa. The study began in November 2014 and will end in November 2015. The study consists of a survey of self-reported demographic, health and economic data as well as clinical biomarker testing including glycosylated hemoglobin, cholesterol, HIV antibody and blood pressure measurement. The survey also includes self-reported data on healthcare utilization. Multivariate logistic regression was used to assess the relationship between self-reported ART use and measurement of blood pressure, blood sugar and receipt of lifestyle modification advice from a healthcare provider.

**Results:** Among 4,767 participants, 979 (20.5%) were HIV-infected and 511 (10.7%) reported ever receiving ART. The HIV/Ever ART group reported higher rates of ever measured blood sugar (52.8% v. 38.8% in HIV/Never ART), ever measured blood pressure (74.4% v. 68.5%) and receipt of advice from a healthcare provider to change diet (13.3% v. 5.0%) and exercise (9.0% v. 2.6%). In multivariate logistic regression models, there was a significant association between ever receiving ART and having had a blood pressure (OR = 1.61, CI: 1.14 – 2.27) or blood sugar (OR = 1.94, CI: 1.43 – 2.63) measurement. Ever receiving ART was also associated with a greater odds of receiving recommendations from a healthcare provider to change diet (OR = 2.88, CI: 1.70 – 4.88) or exercise (OR = 2.39, CI: 1.18 – 4.81). All models were adjusted for age, sex, BMI, and educational attainment.

**Conclusions:** The HIV-infected population in Agincourt that had ever received ART reported greater access to preventive care for NCDs. The positive spillover effects from ART utilization to preventive care for other chronic conditions could provide a powerful vehicle for broader population health improvements via increased programmatic integration.

**Table 1.** Use of ART and Access to Care for NCDs in Agincourt, South Africa

	Ever Use of ART Odds Ratio + 95% CI
Ever Measured BP	1.61 (1.14 – 2.27)
Ever Measured Blood Sugar	1.94 (1.43 – 2.63)
Told to Change Diet	2.88 (1.70 – 4.88)
Told to Exercise	2.39 (1.18 – 4.81)

\*N = 728 for all models

\*All models adjusted for age, sex, BMI and educational attainment

**679 The Burden of NCDs Among the HIV-Infected Population in Agincourt, South Africa**

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**Background:** With increasing survival of HIV-infected populations due to ART, a greater burden of cardiometabolic disease may be unmasked. However, the population level impact of HIV and ART on cardiometabolic risk in communities that are severely affected by the HIV epidemic is poorly understood. The aim of this analysis was to assess differences in the burden of diabetes, hypertension, hyperlipidemia and chronic kidney disease (CKD) among those who were HIV-infected and had ever used ART (HIV/Ever ART) compared to the HIV-negative and HIV-infected population that had never used ART (HIV/Never ART) in the Agincourt sub-district of South Africa.

**Methods:** The Health and Aging in Africa: Longitudinal Studies of INDEPTH communities (HAALSI) Study is a population-based cohort study of about 5,000 adults aged 40 and older in Agincourt. Enrollment began in November 2014 and will close in November 2015. The study consists of a survey of demographic, health and economic data in addition to

total and LDL cholesterol measurement, dried blood spot HIV antibody, HIV viral load and blood pressure measurement. We calculated differences in means using one-way ANOVA and differences in the proportion of cardiometabolic risk factors across these groups using chi-squared tests.

**Results:** The study enrolled 4,767 participants, among which 979 (20.5%) were HIV-infected and 511 (10.7%) reported ever receiving ART. The mean age among the HIV/Ever ART group was 55.7 years compared to 63.8 years in the HIV-negative group and 56.0 years in the HIV/Never ART group. Mean BMI in the HIV-infected/Ever ART group was also significantly less at 24.9 v. 27.5 in the HIV-negative and 27.0 in HIV-infected/Never ART groups ( $p < 0.001$ ). In these groups, the prevalence of self-reported diabetes was lower (5.1% v. 7.4% and 4.1%) as was the prevalence of hypertension (35.2% v. 63.1% and 46.8%) and hypercholesterolemia (4.9% v. 7.6% and 4.3%). Self-reported CKD was greater among the HIV/Ever ART group at 7.5% v. 4.1% in the HIV-negative group and 2.6% in the HIV/Never ART group ( $p = .002$ ).

**Conclusions:** Both mean BMI and the burden of cardiometabolic risk factors were lower in the HIV/Ever ART group with the exception of CKD, which was highest in this group. This relationship may be due to selection into ART programs at advanced HIV disease stages that are in turn associated with weight loss. ART programs may be an appropriate setting for the delivery of preventive care for cardiometabolic disease among those with HIV in South Africa.

**680 Longer-Term Safety of Tenofovir Alafenamide in Renal Impairment**

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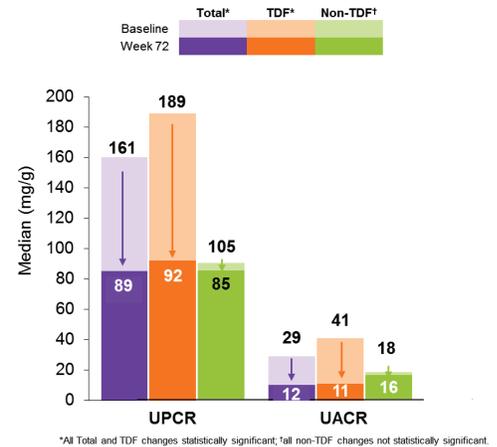
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**Background:** Tenofovir alafenamide (TAF) is a novel prodrug of tenofovir (TFV) that results in 91% lower plasma TFV levels compared to TDF. Switch to a once-daily single tablet regimen of elvitegravir, cobicistat, emtricitabine, and TAF (E/C/F/TAF) in HIV-1 infected patients with eGFR<sub>CG</sub> (Cockcroft-Gault) 30 to 69 mL/min was shown to be effective and safe through 48 weeks. Here, we report longer term results.

**Methods:** Virologically suppressed adults with stable eGFR<sub>CG</sub> of 30 to 69 mL/min had their treatment switched to open-label E/C/F/TAF. The primary endpoint was the change from baseline in glomerular filtration rate estimated using various formulae at 24 weeks. Longer term efficacy and safety data are described, including tests of renal function and bone mineral density (BMD).

**Results:** Of 242 subjects enrolled, mean age was 58 years (range: 24 – 82), 18% Black, 39% hypertension, 14% diabetes, and 65% were taking TDF-containing regimens prior to switch. Through Week 72, minimal change in eGFR<sub>CG</sub> was observed. Five patients (2.0%) with baseline eGFR <50 mL/min discontinued study drug for decreased creatinine clearance, none had evidence of proximal renal tubulopathy and all had risk factors for renal disease progression (diabetes and poorly controlled hypertension). Subjects who received TDF at baseline had significant improvements in proteinuria and albuminuria to levels seen with non-TDF regimens (Figure 1). The prevalence of significant proteinuria (UPCR > 200 mg/g) and albuminuria (UACR ≥ 30 mg/g) decreased from 42% to 18% and 49% to 28%, respectively. Hip and spine BMD increased significantly (mean % changes from baseline +1.50 and +1.91, respectively,  $p < 0.001$ ). 93% maintained HIV-1 RNA <50 copies/mL based on Missing = Failure analysis.

**Conclusions:** Through 72 weeks, switch to E/C/F/TAF was associated with minimal change in eGFR<sub>CG</sub>. Proteinuria, albuminuria and bone mineral density significantly improved. These data support the efficacy and safety of once daily E/C/F/TAF in HIV+ patients with eGFR 30-69 mL/min without dose adjustment.



**681 Renal Safety of Tenofovir Alafenamide in Patients at High Risk of Kidney Disease**

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**Background:** Compared with TDF, tenofovir alafenamide (TAF) results in significantly reduced plasma tenofovir (TFV) and has demonstrated less impact on surrogate markers of renal and bone health in multiple populations, but renal outcomes in treatment-naïve subjects at risk for chronic kidney disease (CKD) have not been characterized.

**Methods:** Treatment naïve HIV-1+ adults were randomized 1:1 to a single tablet regimen of elvitegravir, cobicistat, emtricitabine, with tenofovir alafenamide (E/C/F/TAF) or tenofovir disoproxil fumarate (E/C/F/TDF) once daily in two double blind studies. Assessments of renal function included serum creatinine and estimated GFR by Cockcroft-Gault (eGFR<sub>CG</sub>), and 4 measures of proteinuria: urine protein:creatinine (UPCR), urine albumin:creatinine (UACR), retinol binding protein:creatinine (uRBP:Cr), and beta-2-microglobulin:creatinine (uB2M:Cr). A post-hoc analysis of renal function by group with high risk vs low risk for development of CKD is described. High risk is defined as ≥2 renal risk factors: female gender, age ≥50 years, black race, use of NSAIDs, CD4 < 200 cells/uL, history of dyslipidemia, hypertension, diabetes, and clinical or subclinical renal events. Low CKD risk is defined as ≤1 risk factor.

**Results:** Combined, the two studies randomized and treated 1,733 participants. The proportion of participants with high CKD risk was similar by treatment arm (E/C/F/TAF 28%, E/C/F/TDF 32%). Among high CKD risk participants, significantly fewer subjects on E/C/F/TAF experienced a decline in eGFR to below 60 mL/min compared to E/C/F/TDF: 4.9% vs 9.6% ( $p = 0.044$ ). Participants with high CKD risk who initiated E/C/F/TAF also had significant declines in multiple measures of quantitative proteinuria (Table). Within the low CKD risk group, significantly fewer participants receiving E/C/F/TAF experienced a decline in eGFR by ≥25% (11.5% vs 24.9%,  $p < 0.001$ ). High rates of virologic suppression at week 48 were observed in both treatment groups in the high CKD risk category.

**Conclusions:** Among participants with both low and high CKD risk, participants receiving E/C/F/TAF had more favorable renal outcomes compared with those treated with E/C/F/TDF. These data provide further support for the improved renal safety profile of TAF.

Table

	E/C/F/TAF High CKD Risk N=245	E/C/F/TDF High CKD Risk N=273	P value
Median BL eGFR <sub>CG</sub> , mL/min	114.8	110.0	0.053
eGFR <sub>CG</sub> changes, mL/min	-6.6	-9.6	0.013
eGFR <sub>CG</sub> drop to <60 mL/min, % (n)	4.9% (12)	9.6% (26)	0.044
eGFR <sub>CG</sub> drop by ≥25%, % (n)*	14.8% (36)	27.3% (74)	<0.001
UPCR, % change from BL	-15.2	5.0	0.001
UACR, % change from BL	-10.3	-8.5	0.51
uRBP:Cr, % change from BL	-0.5	45.4	<0.001
uB2M:Cr, % change from BL	-39.7	-0.1	<0.001
Discontinuation due to Renal AEs, n	0	4**	
% with HIV RNA <50 copies/mL	90.6%	87.2%	

\*Acute kidney injury risk. \*\*Renal failure (2), GFR decreased (1), nephropathy (1).

## 682 Longer-Term Renal Safety of Tenofovir Alafenamide vs Tenofovir Disoproxil Fumarate

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**Background:** Compared with tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF) results in a 91% reduction in plasma tenofovir (TFV) exposure and has demonstrated less impact on surrogate markers of renal and bone health in multiple populations, but the clinical impact of these differences has not been fully characterized.

**Methods:** Treatment naïve HIV-1<sup>+</sup> adults were randomized 1:1 to a single tablet regimen coformulating elvitegravir, cobicistat, emtricitabine, with TAF (E/C/F/TAF) or TDF (E/C/F/TDF) once daily in two double blind studies. Assessments of renal function included serum creatinine and estimated GFR by Cockcroft-Gault (eGFR<sub>CG</sub>), and 4 measures of proteinuria: urine protein:creatinine (UPCR), urine albumin:creatinine (UACR), retinol binding protein:creatinine (RBP:Cr), and beta-2-microglobulin:creatinine (β-2-Mg:Cr). A post-hoc analysis of renal events in both studies through 96 weeks is described.

**Results:** Combined, the two studies randomized and treated 1,733 participants. Through 96 weeks, change from baseline eGFR<sub>CG</sub>, UPCR, UACR, RBP:Cr, and β-2-Mg:Cr, all favored E/C/F/TAF (p<0.001). There were no discontinuations for renal adverse events (AE) or cases of proximal tubulopathy in the E/C/F/TAF arm, while 6 participants discontinued due to a renal AE and 1 participant had subclinical tubulopathy in the E/C/F/TDF arm.

Fewer participants in the E/C/F/TAF arm had clinical events of new onset chronic kidney disease (CKD) and acute kidney injury (AKI, 50% decrease in eGFR<sub>CG</sub>), though differences were not statistically significant, while the differences in participants with significant proteinuria (UPCR >200 mg/g) and albuminuria (UACR >30 mg/g) were statistically significant (Table).

**Conclusions:** Through 96 weeks, biomarker analysis indicated that renal tubular function was less affected by E/C/F/TAF and that clinically significant renal events were less frequent in subjects receiving E/C/F/TAF compared with those treated with E/C/F/TDF. These data provide further support for the improved renal safety profile of TAF.

Table

Participants, n (%)	E/C/F/TAF (N=866)	E/C/F/TDF (N=867)	p-value
Renal Events leading to Discontinuation	0	6*	0.03
Subclinical tubulopathy	0	1**	NS
New onset CKD***	24 (2.8%)	32 (3.7%)	0.34
Acute Kidney Injury (AKI)****	3 (0.3%)	11 (1.3%)	0.06
UPCR >200 mg/g	27	42	0.030
UACR >30 mg/g	37	54	0.001
Medical History			
Diabetes mellitus	25 (3%)	40 (5%)	-
Hypertension	119 (14%)	147 (17%)	-
Cardiovascular disease	10 (1%)	14 (1%)	-
Hyperlipidemia	91 (11%)	104 (12%)	-

\*blood creatinine increased, glomerular filtration rate decreased, renal failure, nephropathy, renal failure, fanconi syndrome acquired/glycosuria; \*\*continued study drug at the Week 96 visit, but had confirmed abnormality in any 2 of the following at 2 consecutive post-baseline visits: serum creatinine increase ≥0.4 mg/dL, ≥1 grade decrease in phosphatemia, normoglycemic glycosuria, or ≥2 grade increase in proteinuria; \*\*\*Chronic kidney disease is defined as subjects who have maintained low eGFR measured by CG (< 60 mL/min) or high UACR (urine albumin to creatinine ratio > 30 mg/g) for at least 90 days; \*\*\*\*50% decline in eGFR<sub>CG-95</sub>

## 683 Clinical Risk Factors for Severe Tenofovir (TDF) Associated Renal Tubulopathy

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**Background:** Tenofovir (TDF) is associated with treatment-limiting renal tubulopathy (RT) which may manifest as proximal tubulopathy (PT) and/or acute tubular injury (ATI) on renal biopsy. The risk factors for RT remain poorly defined.

**Methods:** Cases of TDF treatment limiting PT (≥2 of normoglycaemic glycosuria, hypophosphataemia <2 mg/dL, protein-creatinine ratio >300 mg/g) and/or ATI were identified retrospectively in 7 major HIV treatment centres contributing to the UK CHIC cohort. Poisson regression was used to investigate factors associated with RT; age; ethnicity; calendar year of TDF start (fixed covariates); hepatitis B/C status; CD4 cell count; HIV RNA; time on TDF and protease inhibitor (PI) based regimes (time-updated covariates) were considered for inclusion in the model.

**Results:** 15983 subjects received TDF for >4 weeks, of whom 69 (0.4%) were diagnosed with treatment limiting RT between Oct 2002-July 2013 (PT: n=52; ATI: n=17) after a median (IQR) of 43 (26, 67) months of TDF exposure. At presentation, RT was characterised by normoglycaemic glycosuria (83%), hypophosphataemia (67%) and proteinuria (94%); eGFR decline >25% from baseline (start of TDF-containing regimen) was present in 54% of cases and exposure to ritonavir-boosted PI (44% lopinavir, 35% atazanavir, 16% darunavir, 5% other) in 83% of cases. Compared to subjects without RT, those with RT were older (mean age 45.7 vs. 40.7 years), more likely to be white (91% vs. 74%), male (90% vs. 80%), have a prior AIDS diagnosis (41% vs. 26%) and lower nadir CD4 cell count (median 129 vs. 190 cells/mm<sup>3</sup>) (p<0.01 for all). No differences in baseline HBV/HCV status, HIV RNA and eGFR (95 vs. 96 mL/min/1.73m<sup>2</sup>) were observed. In multivariable analysis, older age, ethnicity, time on TDF, CD4 cell count and PI based regimes remained independently associated with the development of RT (Table 1).

**Conclusions:** Severe TDF-associated RT was uncommon in this cohort and accompanied by significant eGFR decline in only half of all cases. Age and PI co-administration were risk factor for the development of RT, and black ethnicity was protective. Baseline eGFR did not identify subjects at increased risk of treatment-limiting RT.

**Table 1: Factors associated with developing TDF associated renal tubulopathy**

	Univariate			Multivariate <sup>§</sup>		
	RR	95% CI	P-value	RR	95% CI	P-value
<b>Age at baseline</b>	<b>1.30</b>	<b>(1.16, 1.46)</b>	<b>&lt;0.0001</b>	<b>1.33</b>	<b>(1.18, 1.50)</b>	<b>&lt;0.0001</b>
<b>Ethnicity (black vs. white/other)</b>	<b>0.28</b>	<b>(0.12, 0.64)</b>	<b>0.003</b>	<b>0.27</b>	<b>(0.12, 0.63)</b>	<b>0.003</b>
<b>Calendar year at TDF start</b>						
2000-2003	1			1		
2004-2007	0.46	(0.26, 0.81)	<b>0.007</b>	0.60	(0.33, 1.07)	0.083
2008-2010	0.31	(0.15, 0.63)	<b>0.001</b>	0.54	(0.25, 1.17)	0.116
2011-2014	0.39	(0.15, 0.97)	<b>0.043</b>	0.72	(0.26, 2.00)	0.532
<b>Time on TDF (per year increase)*</b>	<b>1.11</b>	<b>(1.01, 1.21)</b>	<b>0.025</b>	<b>1.12</b>	<b>(1.01, 1.23)</b>	<b>0.026</b>
<b>Years on ARVs at TDF start</b>	1.06	(1.01, 1.11)	<b>0.030</b>	1.00	(0.94, 1.05)	0.900
<b>ARV regime (PI based vs NNRTI based)*</b>	<b>4.05</b>	<b>(2.44, 6.72)</b>	<b>&lt;0.0001</b>	<b>3.99</b>	<b>(2.38, 6.68)</b>	<b>&lt;0.0001</b>
<b>CD4 cell count (per 50 cell increase)*</b>	<b>0.93</b>	<b>(0.88, 0.98)</b>	<b>0.006</b>	<b>0.92</b>	<b>(0.88, 0.97)</b>	<b>0.003</b>

<sup>§</sup>Time updated <sup>§</sup> adjusted for fixed covariates: age, ethnicity, calendar year of TDF start, years on ARVs prior to TDF start, time updated covariates: ARV regime, time on TDF and CD4 cell count  
 RR: risk ratio, CI: confidence interval, TDF: tenofovir disoproxil fumarate, ARV: antiretroviral, PI: protease inhibitor, NNRTI: non-nucleoside reverse transcriptase inhibitor

**684 Short-Term Renal Impact of Tenofovir Among HIV-Infected Patients in North America**

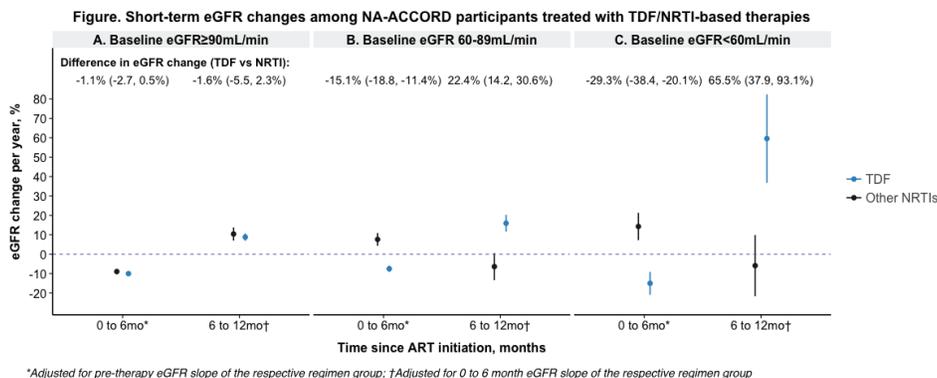
**Ruibin Wang<sup>1</sup>**; Gregory M. Lucas<sup>2</sup>; Michelle Estrella<sup>3</sup>; Michael Shlipak<sup>4</sup>; Marianne Harris<sup>5</sup>; Michael A. Horberg<sup>6</sup>; Mari M. Kitahata<sup>7</sup>; Angel M. Mayor<sup>8</sup>; Sonia Napravnik<sup>9</sup>; Alison Abraham<sup>1</sup>  
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**Background:** The short-term renal effect of tenofovir disoproxil fumarate (TDF) remains uncertain. Our objective was to assess estimated glomerular filtration rate (eGFR) changes within 1 year of TDF initiation among HIV-infected patients in care in North America.

**Methods:** NA-ACCORD participants who initiated TDF or other nucleoside reverse transcriptase inhibitor (NRTI)-based antiretroviral therapy (ART) in 2000-2009 were included. Baseline was defined as date of TDF/NRTI initiation. To assess eGFR (CKD-EPI) changes during the first year of ART, nonlinear mixed effects joint models were used with a knot at month 6. Persons who discontinued TDF or other NRTI within 1 year of initiation were censored at the time of ART cessation. We stratified analyses on baseline eGFR and accounted for informative dropout due to end-stage renal disease (ESRD) or death. Models adjusted for age, race, sex, baseline CD4 cell count and viral load, prior ART exposure, diabetes mellitus, hypertension, hepatitis C infection and pre-TDF/NRTI eGFR loss.

**Results:** 18,596 HIV+ adults were included with median (interquartile range) baseline age 43 years (37-49), eGFR 102 mL/min (88-114) and CD4 295/mm<sup>3</sup> (157-462). 71% were TDF-treated. 35% were blacks; 78% were men. Among persons with baseline eGFR ≥90mL/min, eGFR changes in the first year following ART initiation were similar in the two ART groups [Figure A]. In the first 6 months of ART, eGFR changes per year (95% CI) were -8% (-6, -9) and -15% (-9, -21) in the TDF group and 8% (4, 10) and 14% (7, 21) in the other NRTI group, for persons with baseline eGFR 60-89 and <60mL/min, respectively. Compared to NRTI users, TDF users with eGFR <90mL/min had faster loss of renal function during the initial 6 months of ART [Figure B, C]. From 6 to 12 months, TDF users with reduced kidney function at baseline experienced significant renal recovery (eGFR 60-89: 16%/yr [95% CI: 11, 20]; eGFR <60: 60%/yr [95% CI: 37, 82]) whereas NRTI users did not (eGFR 60-89: -6%/yr [95% CI: -13, 1]; eGFR <60: -6%/yr [95% CI: -22, 10]). Consistent patterns were observed among individuals who stayed on therapy for at least 1 year.

**Conclusions:** In this large study among HIV+ patients in care in North America, TDF was associated with short-term eGFR decline and subsequent recovery among those with reduced kidney function at baseline (eGFR <90mL/min). Investigation into whether this acute eGFR decline is associated with longer-term risk of ESRD is underway.



**685 Associations of Tenofovir Disoproxil Fumarate With Urine Biomarkers of Kidney Damage**

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**Background:** Tenofovir disoproxil fumarate (TDF) is widely prescribed for the treatment of HIV, but has been associated with the development of kidney disease. We hypothesized that TDF exposure would be associated with novel biomarkers of kidney tubular injury and fibrosis.

**Methods:** This cross-sectional study of 884 HIV-infected men from the Multicenter AIDS Cohort Study evaluated associations of cumulative exposure to TDF and other antiretroviral medications (ARVs) with four urine biomarkers: interleukin-18 (IL-18) and kidney injury molecule-1 (KIM-1), proximal tubular injury markers; pro-collagen type III

N-terminal pro-peptide (PIINP), a tubulointerstitial fibrosis marker; and albumin-creatinine ratio (ACR), a marker of glomerular injury. We used multivariable robust regression analyses and the least absolute shrinkage and selection operator (LASSO) method to determine which of multiple ARVs were associated with each biomarker.

**Results:** 573 (65%) participants were current TDF users, and 112 (13%) were former users. In adjusted analyses that controlled for LASSO-selected ARVs simultaneously, each year of TDF exposure was associated with 3.3% higher urine IL-18 (95%CI: 0.8%, 5.8%), 3.4% higher KIM-1 (1.1%, 5.7%), and 3.1% higher PIINP (0.8, 5.5). By contrast, the associations of TDF with ACR did not reach statistical significance (2.8% per year; -0.6%, 6.2%). Lopinavir/ritonavir exposure was associated with higher IL-18 (3.7% per year; 0.8%, 6.7%) and ACR (6.8%; 2.6%, 11.1%), and efavirenz exposure was associated with lower IL-18 (-3.0%; -4.8%, -1.3%) and KIM-1 (-3.9%; -5.7%, -2.2%).

**Conclusions:** Cumulative TDF exposure was associated with biomarkers of proximal tubular injury and fibrosis in HIV-infected men. These biomarkers may be useful for early detection and monitoring of subclinical toxicity from TDF.

## 686 Nature of Immunosuppression and Chronic Kidney Disease Risk in HIV-Positive Persons

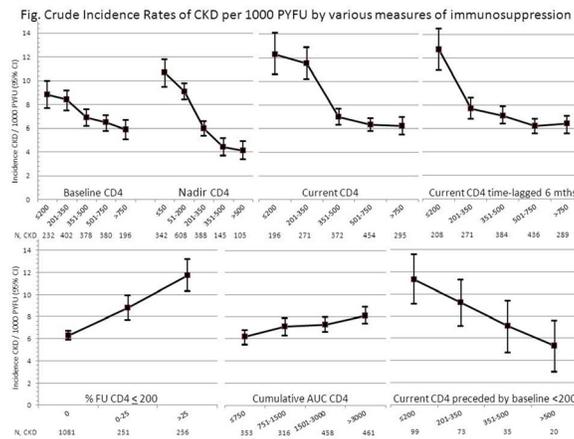
**Lene Ryom**<sup>1</sup>; Jens D. Lundgren<sup>1</sup>; Peter Reiss<sup>2</sup>; Michael Ross<sup>3</sup>; Christoph A. Fux<sup>4</sup>; Philippe Morlat<sup>5</sup>; Eric Fontas<sup>6</sup>; Colette Smith<sup>7</sup>; Amanda Mocroft<sup>7</sup>; for the D:A:D Study Group  
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**Background:** Immunosuppression is a known independent predictor of chronic kidney disease (CKD) in HIV-positive persons, but the nature of the association between different measures of immunosuppression and CKD is unknown.

**Methods:** D:A:D study participants without CKD and with  $\geq 2$  Cockcroft Gault (estimated glomerular filtration rate) eGFR measurements after 1/1/2004 (baseline) were followed until the earliest of CKD (eGFR  $\leq 60$ , confirmed  $\geq 3$  months apart), last eGFR plus 6 months or 1/2/2014. Measures of immunosuppression included baseline, current and nadir CD4, 6-months' time-lagged CD4, % of follow-up time (%FU) with CD4  $\leq 200$ , time-averaged AUC for CD4 and CD4 recovery (baseline CD4  $\leq 200$  followed by current CD4  $> 200$ ). Poisson regression models were used to determine the relationship between CKD and each measure of immunosuppression accounting for relevant confounders, and tested for interactions with the D:A:D CKD risk score, demographics, HCV and HIV-related factors. Akaike Information Criteria (AIC) was used to indicate which measures were better CKD predictors.

**Results:** Of the 33,144 persons included in analyses 1,588 developed CKD (incidence rate (IR) 7.2 [95%CI 6.8-7.5]/1000 PYFU) during a median 7.2 years FU (IQR 5.0-8.9). Those included were predominately white (47.6%), male (74.0%) with a baseline median age of 41 years (IQR 35-47) and median CD4 of 440 (292-626). The crude CKD IR varied for different measures of immunodeficiency (Fig). Univariately, all measures of immunosuppression were significantly associated with CKD, most strongly for nadir CD4 ( $> 500$  vs.  $\leq 50$ , IR 0.39 [0.31-0.48]) and %FU CD4  $\leq 200$  ( $> 25\%$  vs. 0%, IR 1.86 [1.62-2.13]). Multivariately, the strongest CKD predictor was %FU CD4  $\leq 200$  ( $> 25\%$  vs. 0%, 1.29 [1.11-1.30]). There was a significant ( $p < 0.0001$ ) interaction between %FU CD4  $\leq 200$  and the D:A:D CKD risk score; those at lowest estimated CKD risk had a significantly higher CKD IR ( $> 25\%$  vs. 0%, 3.57 [2.23-5.70]) compared to those at highest CKD risk ( $> 25\%$  vs. 0%, 1.24 [1.05-1.46]). There was no significant interaction between measures of immunosuppression and ethnicity, age, HIV-RNA, ART status or use of nephrotoxic antiretrovirals including tenofovir, indinavir, atazanavir/r and lopinavir/r.

**Conclusions:** The strongest association between CKD and immunosuppression was observed for the relative duration of severe immunosuppression, which was of greatest importance in persons at low estimated CKD risk. These new data support aggressive ART to maintain/restore immune function.



## 687 Traditional and Viral Factors Associated With Iohexol-Based GFR Slope Over 3 Years

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**Background:** Monitoring kidney function is important in HIV-positive persons, but creatinine-based estimates of glomerular filtration rate (GFR) have limitations. There are little to no data assessing longitudinal GFR trends in HIV-positive persons using a gold-standard measure of GFR.

**Methods:** We measured GFR based on iohexol plasma disappearance (iGFR) annually for 3 years in non-diabetic, HIV-positive and HIV-negative volunteers with normal estimated kidney function. Additionally, we measured carotid intima-media thickness and pulse wave velocity at baseline. We used mixed linear models to evaluate factors associated with baseline iGFR and iGFR slope.

**Results:** 191 HIV-positive and 100 HIV-negative, predominantly black individuals (median age 49 years) participated in the study and completed a median (IQR) of 4 (3, 4) annual iGFR assessments. The average baseline iGFR values were lower in HIV-positive compared with HIV-negative participants (103.2 vs. 110.8, mL/min/1.73m<sup>2</sup>, P=0.004), despite similar estimated GFR by the CKD-EPI equation (average eGFR, 101 mL/min/1.73m<sup>2</sup> in both groups). Subsequent iGFR decline was not significantly different in HIV-positive and HIV-negative subjects (iGFR slope, -1.94 vs. -3.28 mL/min/1.73m<sup>2</sup> per year, respectively, P=0.092). In the HIV-positive group, lower baseline iGFR values were significantly associated with the presence of carotid plaque (98.3 vs. 109.4 mL/min/1.73m<sup>2</sup>, P<0.001) and hepatitis C virus coinfection (100.0 vs. 107.1 mL/min/1.73m<sup>2</sup>, P=0.024). A non-suppressed HIV RNA level at baseline was associated with a significantly more rapid iGFR decline compared with individuals with HIV RNA < 400 copies/mL (-4.69 vs. -1.31 mL/min/1.73m<sup>2</sup> per year, P=0.005). Other factors significantly associated with iGFR slope in HIV-positive participants included albuminuria and glycosylated hemoglobin. Increased pulse wave velocity, a measure of vascular stiffness, had a near statistically significant association with more rapid iGFR decline.

**Conclusions:** Despite similar estimated GFR in the two groups, HIV-positive participants had significantly lower baseline iGFR than HIV-negative participants. Subsequent 3-year iGFR slope was similar in the two groups. Among HIV-positive subjects, hepatitis C coinfection was associated with a lower baseline iGFR and a non-suppressed HIV RNA at baseline was strongly associated with a more rapid iGFR decline.

### 688 The Racial Survival Paradox in HIV+ End-Stage Renal Disease (ESRD) Patients

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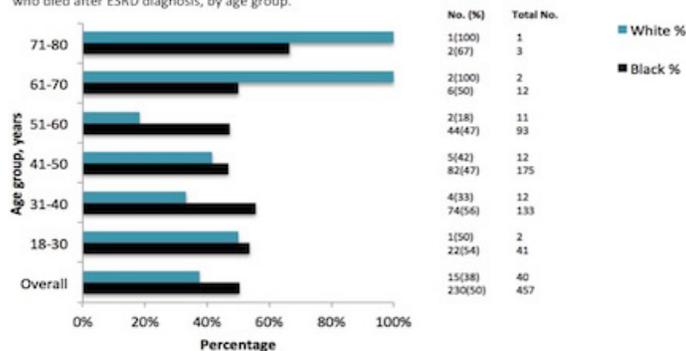
**Background:** The survival paradox among ESRD patients in the general population suggests older black patients have better survival compared to whites, but that this association is reversed at younger ages. As survival after ESRD in HIV-infected adults has not been well described, we sought to investigate the existence of the racial survival paradox in HIV-infected adults.

**Methods:** Adults ( $\geq 18$  years old) participating in one of 12 contributing cohorts to the NA-ACCORD with validated ESRD diagnosis from 1 Jan 2000 to 31 Dec 2009 were included and followed from ESRD diagnosis to death or censoring. Demographic data, smoking, diabetes, treated hypertension, hypercholesterolemia, statin use, past clinical AIDS diagnosis, CD4 count, ART and HIV viral load status, prior tenofovir exposure, and hepatitis B and C co-infection at ESRD diagnosis were examined for their relationship with death using adjusted hazard ratios (aHR) and 95% confidence intervals (CI) from Cox proportional hazard models.

**Results:** 540 adults with ESRD contributed 1,958 person-years and 255 deaths. Median follow-up time was 2.5 years. Death more likely to be among those who are black, those with heterosexual or injection drug use HIV transmission risks, smokers, those with past AIDS defining illness, low ( $< 200$  cells/mm<sup>3</sup>) CD4 count, and detectable ( $> 400$  copies/mL) HIV RNA. At ESRD diagnosis, 52% of participants were not prescribed ART, 29% were prescribed ART but not virologically suppressed, and 19% were prescribed ART and virologically suppressed. The racial survival paradox was apparent among HIV-infected persons, though the number of white ESRD patients was very small (Figure 1). In multivariate models, older age ( $\geq 60$  years vs.  $< 40$  years, aHR=1.77 [1.01, 3.10]), hypercholesterolemia (aHR=1.59 [1.13, 2.24]), an AIDS defining illness (aHR=3.08 [2.21, 4.48]), no ART prescription (aHR=18.16 [7.31, 45.16]), and tenofovir use prior to ESRD (aHR=2.16 [1.38, 13.38]) increased the risk of death, and a higher CD4 count was protective (CD4  $\geq 500$  vs.  $< 200$  cells/mm<sup>3</sup>, aHR=0.41 [0.27, 0.61]).

**Conclusions:** The suggestion of the racial survival paradox in HIV-infected adults motivates the need for understanding the factors contributing to higher mortality among younger ( $< 60$ yo), black, HIV-infected adults with ESRD.

Figure 1: A visualization of the racial survival paradox: Age-stratified proportion of adults with HIV and ESRD who died after ESRD diagnosis, by age group.



### 689 Activation and Senescence Markers in HIV Patients With Chronic Kidney Disease

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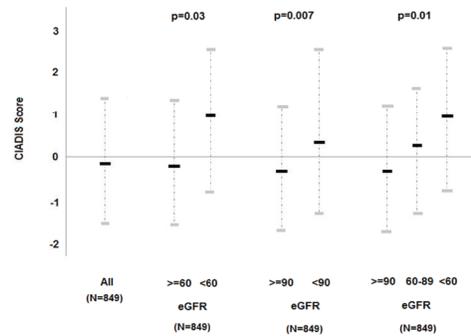
**Background:** ART-treated HIV-infected individuals remain at higher risk for chronic kidney disease (CKD) than the general population and multiple causes are hypothesized. We evaluated the association between T cell immune activation (IA) and -senescence (IS), and CKD in these patients.

**Methods:** Patients with undetectable viral load and an estimated Glomerular Filtration Rate (eGFR) measurement were part of the cross-sectional CIADIS substudy (2011-2013) of the ANRS CO3 Aquitaine cohort. The CIADIS score was determined with CD4 and CD8 activation (DR+), maturation (T naive, memory) and senescence (CD57+CD28-) markers (Duffau *et al.* AIDS in press); positive values represent a phenotype with high IA and IS, negative values a profile with low IA/IS and with high proportion of naive T cells. The association between the CIADIS score and CKD was evaluated for two separate outcomes: i) eGFR  $< 60$  mL/min/1.73m<sup>2</sup> and ii) eGFR  $< 90$  mL/min/1.73m<sup>2</sup>. Logistic regression models adjusted for age, sex, tenofovir (TDF) use and non-HIV related comorbidities (diabetes, cardiovascular events, dyslipidemias, hypertension and cancer) were constructed. In a subgroup analysis, urine protein-creatinine ratio (uPCR)  $> 30$  mg/mmol was combined with eGFR  $\geq 90$  to define early kidney dysfunction.

**Results:** We included 849 patients with a median age of 51 years; 74% were men, 23% at CDC stage C; they were on ART for a median of 13 years, 85% of patients had an ongoing or previous TDF-containing regimen. eGFR was  $\geq 90$ , 60-89 and  $< 60$  in 68%, 28% and 4% of patients, respectively. The median CIADIS score was -0.1 (IQR -1.5;1.5) and increased significantly with decreasing kidney function (Figure). In univariable analysis: an increase of the CIADIS score was significantly associated with an eGFR  $< 60$ , (OR=1.2; 95% CI 1.0-1.5;  $P=0.04$ ) and eGFR  $< 90$  (OR=1.1; 95% CI 1.0-1.2;  $P<0.01$ ). After adjustment, the CIADIS score remained significantly associated with an eGFR  $< 60$  (OR=1.3; 95% CI 1.0-1.6;  $P=0.03$ ) only. Among 221 patients with an eGFR  $\geq 90$  and available uPCR, 9% had an early kidney dysfunction. No difference in the score was found whether uPCR was  $\leq 30$  or  $> 30$  ( $P=0.46$ ).

**Conclusions:** Higher IA and IS levels were independently associated with advanced CKD. Although other factors may contribute to kidney damage, persisting T-cells activation and senescence could induce inflammation and thus play a direct role even in successfully treated patients. Follow-up continues; longitudinal analysis will allow studying the development of CKD according to IA/IS profiles.

Figure: Description of CIADIS score between eGFR groups in the ANRS CO3 Aquitaine Cohort – CIADIS substudy 2011-2013



Legend: eGFR, estimated Glomerular Filtration Rate. P/C, Ratio proteinuria on creatininuria. Black lines show the median values of CIADIS score, dotted lines between grey lines represent the interquartile range. The Mann-Whitney test was used to compare the score between groups defined by eGFR. A median value of CIADIS score above 0 represents an immune phenotype with higher T cell activation, expression of terminally differentiation and senescence markers whereas negative values of CIADIS score represent a less activated and less senescent profile.

## 690 Soluble CD163 Predicts Incident Chronic Lung and Kidney Disease in HIV-1 Infection

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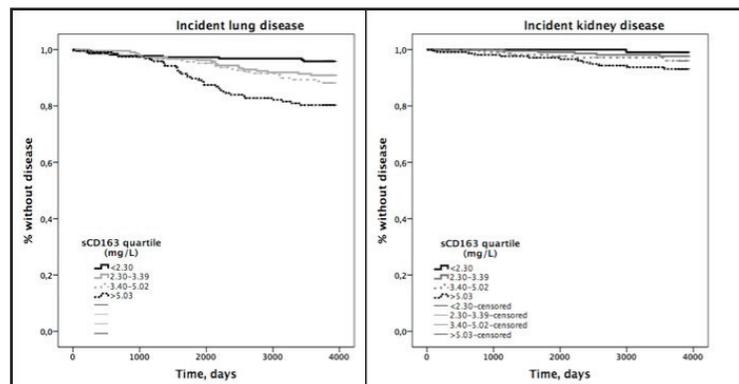
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**Background:** Despite the success of antiretroviral therapy (ART), HIV infection is still associated with an increased risk of comorbidity compared to that of the background population. We recently showed that plasma soluble CD163 (sCD163), a marker of monocyte/macrophage activation, was associated with all-cause mortality. Here we hypothesized that elevated sCD163 may predict non-AIDS comorbidity in HIV-infected individuals.

**Methods:** Prospective single-center cohort study (n = 933). Plasma sCD163 was quantified at study entry in 2004/05. Comorbidity (cancer, cardiovascular disease (CVD), diabetes, and kidney, liver and lung diseases) was identified by ICD-10 diagnosis codes and registry linkage. Associations between sCD163 and incident comorbidity was examined using multivariable Cox proportional hazards models adjusted for age, sex, race, transmission category, prior comorbidity, CD4 T lymphocyte count, HIV RNA, and ART.

**Results:** Median age of study participants was 43 years, 72% were men, 79% were white, and 86% were on ART. At baseline, 19% had comorbidity. During 10.5 years of follow up, there were 360 cases of incident non-AIDS comorbidity in 330 individuals. In multivariate analysis, highest quartile sCD163 concentration was associated with incident chronic lung disease (adjusted hazard ratio (aHR), 3.20; 95% confidence interval (CI): 1.41 to 7.24) and incident chronic kidney disease (aHR, 8.43; 95% CI: 1.73-41.01), when compared with lowest quartiles. sCD163 level was not associated with incident cancer, CVD, diabetes, or liver disease.

**Conclusions:** These results suggest that monocyte/macrophage activation may be involved in the pathogenesis of HIV-associated lung and kidney disease. Measuring sCD163 may identify HIV infected patients at risk for chronic lung and kidney disease.



## 691 Use of Urine Biomarker-Derived Clusters to Predict CKD Risk and All-Cause Mortality

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**Background:** Individual urine biomarkers are associated with CKD incidence and all-cause mortality in the setting of HIV infection, but their combined utility for prediction remains unknown.

**Methods:** We measured 8 urine biomarkers in 902 HIV+ women: NAG, KIM-1, alpha 1 microglobulin (α1m), IL-18, NGAL, ACR, L-FABP, and AAG. A group-based cluster method classified each participant into 3 distinct clusters using the three most distinguishing markers (NAG, KIM-1, and α1m). We evaluated associations of each cluster with incident CKD (defined as eGFR<sub>cs</sub><60) and all-cause mortality, adjusting for traditional and HIV-related risk factors.

**Results:** Over 8 years of follow-up, 177 CKD events and 128 deaths occurred. As shown in the Table, incidence of CKD and mortality increased incrementally across the 3 clusters. After multivariable adjustment, cluster 3 remained associated with a nearly 3-fold risk of both outcomes compared with cluster 1. Addition of the clusters to the multivariable

model improved discrimination for CKD ( $c$ -statistic=0.72 to 0.76,  $p=0.0029$ ), but only modestly for mortality ( $c$ =0.79 to 0.80,  $p=0.099$ ). Clusters derived with all 8 markers were no better for discrimination than the 3-biomarker clusters.

**Conclusions:** For predicting incident CKD in HIV-infected women, clusters developed from three urine-based kidney disease biomarkers were as effective as a panel of eight biomarkers in improving CKD risk discrimination.

Association of biomarker-derived cluster* with incident CKD and all-cause mortality in HIV+ women			
	Cluster 1 n=289	Cluster 2 n=435	Cluster 3 n=94
<b>Incident CKD</b>			
Event rate	13%	21%	50%
Unadjusted Risk Ratio (95%CI)	Ref	1.7 (1.2, 2.4)	3.9 (2.7, 5.6)
Adjusted Risk Ratio (95%CI)	Ref	1.6 (1.1, 2.3)	2.9 (2.0, 4.3)
<b>All-cause mortality</b>	Cluster 1 n=301	Cluster 2 n=470	Cluster 3 n=131
Event rate	7%	13%	34%
Unadjusted Hazard Ratio (95%CI)	Ref	1.9 (1.1, 3.0)	5.4 (3.2, 9.0)
Adjusted Hazard Ratio (95%CI)	Ref	1.5 (0.9, 2.5)	2.8 (1.6, 4.8)

\* Clusters were derived using NAG,  $\alpha$ 1m, and KIM-1. Adjusted models control for traditional kidney risk factors and HIV-related risk factors.

## 692 Differences in Urine Metabolomes With HIV Infection and Antiretroviral Drug Exposure

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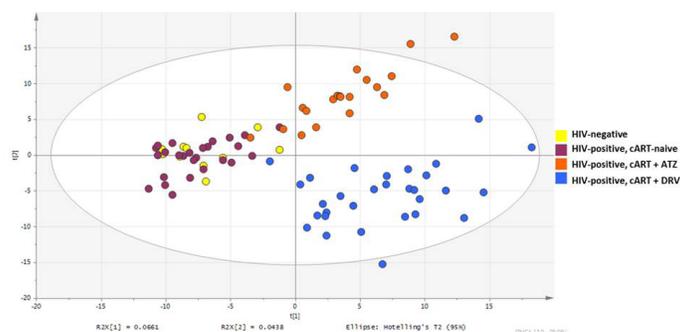
**Background:** Metabolomics is the analysis of small molecular weight compounds (<1000 Daltons) in biological samples. Few metabolomic studies have analysed the effect of combination antiretroviral therapy (cART) using urine in HIV-infected patients. We investigated the effect of cART on the urinary metabolome of HIV-infected patients.

**Methods:** Fasted urine samples from randomly chosen HIV-infected patients and negative controls were analysed by non-targeted metabolomics using liquid chromatography mass spectrometry (LC-MS). Participants were grouped into 3 groups: HIV-infected on cART, cART-naïve and HIV-negative. Principal components analysis (PCA) was used to plot metabolomic differences associated with HIV and cART status. A supervised modelling approach identified metabolites associated with cART. Analysis of variance was used to compare differences in metabolomes between groups.

**Results:** 89 participants (cART-naïve: 26; on cART: 50; negative: 13) were included (mean age 42.1 [SD 9.9] years, 100% male, 97.8% white). All those on cART (median CD4 525 [IQR 403,690] cells/ $\mu$ L, 100% VL <40 copies/mL) were on tenofovir/emtricitabine, ritonavir and atazanavir (ATZ, n=20) or darunavir (DRV, n=30). In cART-naïve participants, median CD4 was 490 (IQR 395,695) cells/ $\mu$ L. Serum renal and liver profiles were similar between all groups, except for higher bilirubin in those on ATZ. The PCA plot showed no difference in metabolomes between HIV-negative and HIV-infected groups, but discriminated between those on cART (ATZ versus DRV) and naïve groups (Figure). Bile acids (cholic acid glucuronide, cholic acid, glycocholic acid and norcholestanhexol glucuronide), which can be markers of liver toxicity, were significantly reduced in those on cART compared to cART-naïve ( $p<0.05$ ) indicating potential disruption of bile acid transporter mechanisms in the kidney/liver. The nucleoside 5-deoxy-5-(methylthio)adenosine, involved in cell apoptosis, was significantly reduced in both cART groups ( $p<0.05$ ), suggesting that cART may be effective in reducing HIV-induced CD4 cell apoptosis.

**Conclusions:** This is to our knowledge is the first urinary trace metabolomic study in HIV infection. There were significant differences between the urine metabolome of those on protease inhibitor-based cART compared with cART-naïve and negative groups. Changes in urine metabolomes associated with cART may be useful in monitoring toxicity or adherence. Further work is needed to assess the clinical utility of metabolomics in HIV-infected patients.

Principal components analysis (PCA) scores plot showing differences in metabolomes in urine samples from patients with differing HIV and cART status (positive electrospray dataset from liquid chromatography mass spectrometry [LC-MS] analyses)



## 693 Early Markers of Renal Dysfunction Among Cocaine Users With HIV and HCV Infection

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**Background:** Cocaine use is associated with increased kidney and cardiovascular disease in the general population, but its effects on these comorbidities in people with HIV and HCV infection are not well characterized.

**Methods:** Case-control study to examine effects of cocaine use on plasma metabolite profiles in relation to comorbidities in 104 subjects in 3 cohorts; cocaine users (mainly crack cocaine) matched to controls for demographics, HIV serostatus, ART use, CD4 counts, plasma HIV viral load (VL) (n=38 HIV-negative from ALIVE and Bioreclamation, n=36 HIV+ IDU from ALIVE [77% on ART], n=30 HIV+ from NNCT and CHARTER [100% on ART]; 52% cocaine users, 60% HCV+ (ALIVE subjects were HCV aviremic), 76% with VL <400 cps/ml.

Metabolomics was performed by LC/MS/MS and GC/MS. Integrative analysis of metabolite, laboratory, and clinical data used Metaboanalyst and R. Multivariate logistic regression was done in SAS.

**Results:** Participants were predominantly male, black, with high prevalence of HCV (60%, 75%, and 60%, respectively) and renal dysfunction (40% had estimated GFR (eGFR)  $\leq 90$ ). Of >300 metabolites detected, 15 distinguished cocaine users from controls in HIV- and HIV+ cohorts, mapping to oxidative stress, altered tryptophan catabolism, phenylalanine/tyrosine/dopamine, and linoleic acid metabolism ( $p < 0.05$ , FDR  $< 0.10$ ). Metabolites altered in cocaine users included uremic solutes indicative of early renal dysfunction, which correlated inversely with eGFR (c-glycosyl-tryptophan [c-glyTrp], pseudouridine, N6-carbamoyl-threonine, kynurenine, N-acetylated amino acids;  $p < 0.05$ ). Heme, a pro-oxidant with inflammatory and nephrotoxic activity, and kynurenine:tryptophan ratio (marker of tryptophan catabolism associated with immune activation) were also elevated. AUROC identified c-glyTrp and pseudouridine as uremic solutes with good classification power for eGFR  $\leq 90$  vs  $> 90$  (AUROC c-glyTrp single marker 83%; c-glyTrp combined with serum creatinine 95% vs 92% for creatinine alone;  $n = 95$ ). Cocaine use was associated with eGFR  $\leq 90$  in logistic models adjusted for older age, race, HIV, and HCV (OR 3.4, CI 1.3-8.7;  $p = 0.01$ ) and HCV status modified this association (OR 6.1, CI 1.6-23.6;  $p < 0.01$ ).

**Conclusions:** Cocaine use is associated with early markers of renal dysfunction in HIV and HCV infection. Tryptophan catabolism, oxidative stress, and pro-oxidant and nephrotoxic effects of circulating heme may contribute to mechanisms involved in cocaine-associated comorbidities that are augmented by these viral infections.

#### 694 Bone Loss With Antiretroviral Therapy Is Associated With Phosphaturia

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**Background:** Initial antiretroviral therapy (ART) is associated with bone mineral density (BMD) loss. ACTG A5303, a randomized trial of initial tenofovir disoproxil fumarate (TDF) vs maraviroc (MVC)-containing ART over 48 weeks showed greater bone loss with TDF, consistent with known bone effects of TDF. The mechanisms of TDF-associated bone loss are not known, but urinary phosphate wasting from TDF-induced renal tubulopathy may contribute to this loss.

**Objective:** To examine associations between phosphaturia and changes in BMD in ACTG A5303

**Methods:** The contributions of phosphaturia to changes in hip or spine BMD (based on dual energy x-ray absorptiometry) from baseline to week 48 were assessed using multivariable linear regression in subjects with complete data through week 48. Phosphaturia was estimated by calculating the area under the curve of the fractional excretion of phosphorus at weeks 0, 4, 24 & 48 (FEP-AUC). Adjusting variables included age, sex, race/ethnicity, BMI, week 48 viral load & changes in CD4 from baseline to week 48.

**Results:** Among the 163 participants included in this analysis, 15 (9%) were women. Women were significantly older than men (med age 41 vs 33 yrs;  $P = 0.01$ ). The mean FEP-AUC over 48 weeks was similar between women and men (9.8 vs 10.5%, respectively;  $P = 0.7$ ) but tended to be higher in women and men who were randomized to TDF vs MVC (10.8 vs 10.0%,  $P = 0.07$ ). Higher FEP-AUC was associated with significantly more bone loss in spine but not hip, which was evident mainly in women ( $P = 0.04$  for interactions with sex). Each 10% average FEP increase over 48 weeks was associated with BMD<sub>spine</sub> loss of -14.9% [95%CI: -15.4, -14.3%] in women, but only -0.9% [-4.7, 2.8%] in men. The significant differences between treatment arms persisted in these models, however, so the effect of TDF on BMD was only slightly attenuated by inclusion of FEP-AUC in the models, from -1.7% [-2.9, -0.5%] to -1.6% [-2.9, -0.5%] in BMD<sub>spine</sub> loss with TDF vs MVC.

**Conclusions:** Phosphaturia was associated with bone loss with the initiation of either TDF or MVC over 48 weeks, that was evident primarily in women. The detrimental bone effects of TDF were not explained by phosphaturia, however.

#### 695LB Immunologic Effects of Maraviroc vs Tenofovir and Associations With Bone Loss

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**Background:** The anti-inflammatory and immune modulating effects of maraviroc (MVC) and their hypothetical clinical associations are unclear. We compared immune biomarker changes with MVC vs. tenofovir disoproxil fumarate (TDF) in ACTG A5303 where MVC led to less bone loss than TDF in initial ART.

**Methods:** A5303 was a 48 week double-blind, placebo-controlled trial conducted in the US. Participants were HIV-1-infected, ART-naïve with viral load (VL)  $> 1000$  c/mL, R5 tropism on Trofile, and were randomized 1:1 to MVC 150mg or TDF 300mg QD, stratified by VL  $<$  and  $\geq 100,000$  c/mL and age  $<$  and  $\geq 30$  yrs. All participants received darunavir 800mg, ritonavir 100mg, and emtricitabine 200mg QD. A total of 32 biomarkers were assayed at weeks 0 and 48, including soluble biomarkers measured by ELISA, CD4/CD8 counts, activated (CD38/HLA-DR) and senescent (CD28/CD57) T-cells, monocytes (CD14/CD16), B cells and NK cells. Hip BMD was determined using DXA scans at weeks 0 and 48. Analyses were as-treated. Wilcoxon signed-rank tests evaluated within group differences. Stratified Wilcoxon rank sum tests evaluated differences between treatment groups stratified by age stratum. Spearman correlations evaluated associations between %change in BMD and biomarkers. All tests were 2-sided and conservatively interpreted at 0.5% significance level.

**Results:** There were 230 participants in the as-treated population (MVC=119; TDF=111): 9% female; median age 33 yrs, 44% White, 31% Black, 22% Hispanic; median VL 4.5 log<sub>10</sub> c/mL. The MVC group had greater increases in CD4 count, smaller decreases in CD8 count, and smaller increases in CD4:CD8 ratio observed over 48 weeks compared to the TDF group (Table 1). There were significant declines in all soluble biomarkers except for IL-6 and sCD14 where the declines were apparent only in MVC group. No evidence of differences between treatment groups was found in changes in soluble or cellular biomarkers ( $p > 0.10$ ). Significant associations between hip BMD %change and soluble/cellular biomarkers were not found in either group, except for a marginal negative association with %change in IL-6 ( $r = -0.23$ ,  $p = 0.016$ ) found in TDF but not MVC group.

**Conclusions:** Initiating ART with MVC compared to TDF in A5303 resulted in greater increase in CD4 count and smaller decline in CD8 count, but less rise in CD4:CD8 ratio. Changes in soluble or cellular biomarkers of inflammation and immune activation were not different between MVC and TDF groups. A consistent immunologic driver to differential bone loss was not seen.

Marker	Treatment Group	Baseline Median (Q1, Q3)	Absolute Change Median (Q1, Q3)	p-value (within group)	p-value (between group)
CD4 [cells/mm <sup>3</sup> ]	MVC	385 (295, 493)	234 (131, 327)	<0.001	0.036
	TDF	391 (279, 518)	188 (94, 304)	<0.001	
CD8 [cells/mm <sup>3</sup> ]	MVC	867 (686, 1141)	-6 (-252, 175)	0.51	0.008
	TDF	863 (575, 1227)	-109 (-340, 59)	<0.001	
CD4:CD8 Ratio	MVC	0.43 (0.32, 0.60)	0.26 (0.13, 0.43)	<0.001	0.003
	TDF	0.48 (0.30, 0.68)	0.39 (0.21, 0.54)	<0.001	
IL-6 [pg/mL]	MVC	1.48 (0.98, 2.42)	-0.21 (-0.91, 0.25)	0.007	0.50
	TDF	1.77 (1.14, 2.49)	-0.12 (-0.83, 0.42)	0.12	
Ip-10 [pg/mL]	MVC	363 (235, 564)	-198 (-366, -91)	<0.001	0.75
	TDF	354 (244, 551)	-170 (-310, -97)	<0.001	
sTNF- $\alpha$ [pg/mL]	MVC	3619 (2826, 4409)	-1108 (-1658, -597)	<0.001	0.54
	TDF	3751 (3146, 4301)	-1198 (-1875, -641)	<0.001	
sCD14 [pg/mL]	MVC	17779 (15464, 23281)	-1178 (-3478, 1032)	0.001	0.17
	TDF	18526 (15511, 29902)	-103 (-2991, 2627)	0.41	
D-dimer [ng/mL]	MVC	266 (162, 429)	-82 (-210, -1)	<0.001	0.65
	TDF	231 (146, 434)	-61 (-211, -7)	<0.001	
sCD163 [ng/mL]	MVC	810 (579, 1145)	-250 (-469, -129)	<0.001	0.87
	TDF	850 (652, 1064)	-258 (-458, -136)	<0.001	

Table 1: Summary of CD4 and CD8 counts change and soluble biomarkers change from baseline to week 48

696 TDF and Quantitative Ultrasound Bone Density in African Patients on Second-Line ART

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**Background:** Several reports indicated that HIV-1 infected people have lower bone mineral density (BMD) than general population, independently of traditional osteoporosis risk factors. TDF-including regimens have been associated with greater loss of BMD than other regimens.

**Objective:** To identify low bone mineral density associated factors in African patients on a protease inhibitor (PI)-based second line antiretroviral treatment (ART).

**Methods:** It is a sub-study (ANRS 12250) of a multicenter randomized phase 3 trial that compared efficacy and safety of three second line combinations in Africa (ANRS 12169). Patients from Bobo-Dioulasso (Burkina Faso), Yaounde (Cameroun) and Dakar (Senegal) were randomized to receive either TDF/FTC/LPVr, ABC/ddI/LPVr or TDF/FTC/DRVr. The BMD was assessed by calcaneum quantitative ultrasound (Achilles, GE Healthcare) at baseline and every 6 months. Stiffness index was used to evaluate BMD and associated factors were determined by multiple linear regressions. Mixed models with random effects were used to determine associated factors during the follow-up.

**Results:** Out of 228 patients, 158 (69%) were included in Yaounde and 168 (74%) were women. At baseline, mean age was 40 ± 10 years, mean T-CD4 counts was 218 ± 139 cells/μl and mean viral load, 4.5 ± 0.7 log/ml. The mean duration of the first line ART was 55 ± 24 months and the mean baseline stiffness index was 103 ± 22. Independent factors associated with baseline BMD were sex (β=-10.57 [-17.90,-3.23] for women), age (β=-0.87 [-1.24,-0.50] per year), body mass index (BMI) (β=+0.80 [-0.07,1.52] per unit of BMI) and study site (β=+12.81 [6.48,19.14] for Yaounde). After 24 months of second line therapy, a reduction of 7.3% of mean stiffness index was observed, compared to baseline. The factors associated with BMD during the follow-up were similar to those found at baseline (table). Exposure to TDF or to LPV was not associated with greater loss of BMD over time.

**Conclusions:** BMD decreases after second line ART initiation in African patients independently of TDF or LPV exposition. Factors associated with BMD were age, sex, baseline BMI, study site and time of follow up.

Table: Bone mineral density associated factors in African patients during the first two years of follow-up on a second line antiretroviral treatment (ANRS12250).

	Multivariable		
	β	95% CI	P
<b>Follow-up (semester)</b>	<b>-0.89</b>	<b>[-1.43,-0.36]</b>	<b>0.001</b>
<b>Age (per ten years)</b>	<b>-6.75</b>	<b>[-9.38,-4.12]</b>	<b>0.000</b>
<b>Female</b>	<b>-9.64</b>	<b>[-15.45,-8.83]</b>	<b>0.001</b>
<b>Baseline BMI</b>	<b>0.82</b>	<b>[0.26,1.38]</b>	<b>0.004</b>
Physical activity	-0.96	[-2.52,0.60]	0.228
Duration < 36	Ref.		---
of first line [36-72]	6.42	[0.62,12.22]	0.073
ART ≥ 72 (months)	2.45	[-4.42,9.31]	
Year of inclusion	2.31	[-2.56,7.18]	0.353
<b>Yaounde</b>	<b>10.90</b>	<b>[5.70,16.10]</b>	<b>0.000</b>
TDF regimens	1.67	[-4.08,7.42]	0.570
LPV regimens	1.61	[-3.98,7.20]	0.572

697 Bone-Mineral Density After Switching to ATV/r+3TC: A Substudy of the AtLaS-M Trial

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**Background:** AtLaS-M is a randomized multi-center trial showing superior efficacy and safety of treatment simplification to a dual regimen with ATV/r+3TC (dual therapy, DT) as compared to continuing ATV/r+2NRTI (triple therapy, TT) in virologically suppressed HIV+ patients. Here, we report data of Bone Mineral Density (BMD) and Body Fat Distribution at 48 Weeks.

**Methods:** BMD at femoral neck and lumbar spine and body fat distribution as measured by DEXA and bio-markers of bone turnover were measured at baseline and 48w. Student t-test was used to compare means and multivariate regression to assess predictors of mean changes at 48 weeks

**Results:** 106 randomized patients were included in this sub-study, 60 from the DT and 46 from the TT arm. 82.1% were male, the most frequent risk factor was MSM contacts (50%), median age was 47 y (IQR 38-51), median BMI 24.1 kg/cm<sup>2</sup> (22.2-26), 49.1% were smokers, 88.9% were on a tenofovir-containing NRTI backbone with a median time of HAART exposure of 1.99 years (1.32-4.93). At BL, 62.3% of pts had a low BMD in any district and 68.8% a pathological Vitamin D level. No baseline difference was found between arms. At 48w, in the DT arm, BMD of the femoral neck showed a significant increase (mean change +1.93% p=0.002), while in the TT arm no significant modification was observed (mean change -1.13%; mean difference in change 3.06% p=0.01). DT arm also improved lumbar spine BMD at 48w (+0.53% p=0.21) while TT arm worsened (-1.3% p=0.14). Pts randomized to DT, showed a significant reduction of PTH (-9.3 vs +1.9 ng/L in TT, mean difference in change -5.9 ng/L p=0.002) and osteocalcin levels (-7.2 vs -1.3 ng/ml in TT; mean difference in change -5.92 ng/ml p<0.001) at 48w. Body fat distribution did not show changes within and between groups. At multivariate logistic regression, pts losing BMI had a lower probability to gain lumbar spine BMD at 48w (OR 0.72 95%CI 0.54-0.97, p=0.03), while pts with osteopenia at BL as compared to those with osteoporosis were more likely to improve (OR 2.96 95%CI 1.18-7.44 p=0.02). Remarkably, BMD gain at femoral neck observed in pts receiving DT was independent from their baseline NRTI backbone type.

**Conclusions:** In virologically controlled pts on ATV/r+2NRTI, a switch to DT with ATV/r+3TC was associated with significant improvements in BMD and markers of bone turnover after one year. Early diagnosis of BMD loss seems crucial since pts with osteoporosis benefit less from switch to DT as compared to those with a less advanced condition.

**698 Osteoporosis and Fractures in HIV-Infected Adults: Who Is Afraid of the Lumbar Spine?**

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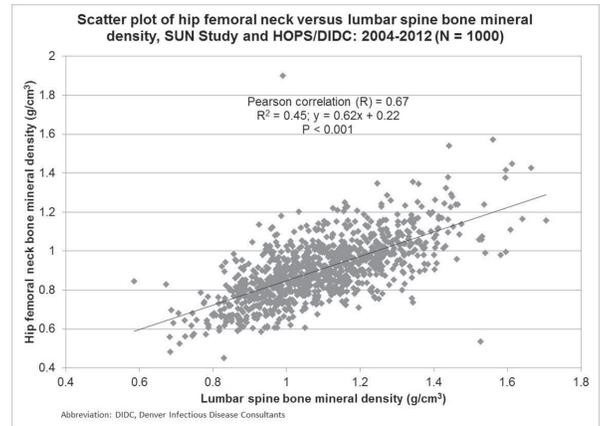
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**Background:** Although the World Health Organization recommends determining osteoporosis based on the bone mineral density (BMD) of the femoral neck, there are limited data comparing rates of osteoporosis based on assessment of the femoral neck versus lumbar spine among HIV-infected adults.

**Methods:** We analyzed available dual energy X-ray absorptiometry (DXA) values of the hip (left femoral neck) and lumbar spine (L1-L4) and clinical data collected prospectively during 2004-2012 from two CDC-sponsored HIV cohort studies, the HOPS and the SUN Study. We identified patients with low BMD (osteopenia or osteoporosis, defined by T-scores of -1.0 to >-2.5, and ≤-2.5, respectively), at the femoral neck, lumbar spine, or both anatomical sites. Cox proportional hazards models were used to determine factors associated with incident fracture using femoral neck, lumbar BMD, or both sites to determine osteoporosis.

**Results:** Characteristics of 1000 patients with both femoral neck and lumbar spine BMD values were: median age 43 years (interquartile range [IQR] 36-49), 83% male, 67% non-Hispanic white, median CD4+ cell count [CD4] 461 cells/mm<sup>3</sup> [IQR 312-659]. During 4066 person-years (py) of observation after DXA during study, there were 85 incident fractures (20.9 per 1000py) including 22 fragility fractures (5.4 per 1000py). Prevalences of osteopenia and osteoporosis at the femoral neck only, lumbar spine only, and using the lower of the two BMD values were 35.5%, 30.6% and 44.0% and 3.7%, 7.1% and 8.4%, respectively. Among 71 patients with lumbar spine osteoporosis, 24 also had femoral neck osteoporosis. There was a direct linear association between femoral neck and lumbar spine BMD (Figure). Analyses of risk for fracture that defined osteoporosis based on using femoral neck BMD only, lumbar spine BMD only, and the lower of the two BMD values, respectively, identified the same risk factors: being a current or former tobacco smoker (aHR 1.64, 1.61 and 1.61, all P < 0.05) and the presence of osteoporosis (aHR 4.10, 3.17 and 3.01, all P < 0.001).

**Conclusions:** In a large convenience sample of U.S. HIV-infected adults, adding lumbar spine data to osteoporosis assessment more than doubled diagnoses made with femoral neck data alone. Utilizing both anatomical sites expands the opportunity to identify persons at risk for fragility fractures and to intervene with therapies and recommend lifestyle changes to prevent fractures.



**699 Fractures Occur at a Younger Age in HIV+ Men in the Multicenter AIDS Cohort Study**

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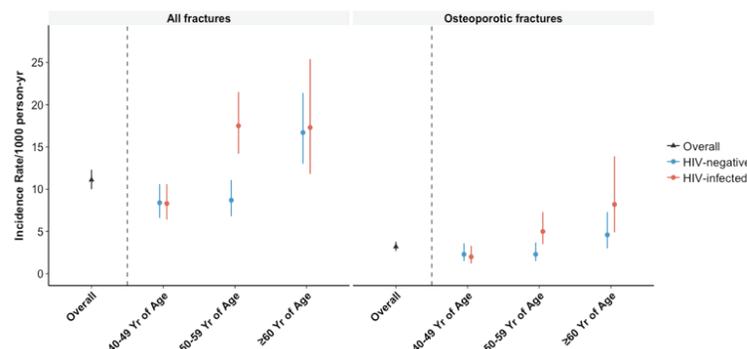
**Background:** Current guidelines recommend osteoporosis screening for HIV-infected men > 50 years old, but fracture data supporting this recommendation are limited.

**Methods:** Between 1984 and 2014, self-reported bone fractures were ascertained at semi-annual visits in 1302 HIV-infected and 1302 HIV-uninfected men over age 40. The study outcomes were: 1) all fractures (excluding fractures of skull, face, digits) 2) fragility fractures (fractures of vertebral column, femur, wrist, humerus). Incidence rates per 1000 person-years (py), adjusted incident rate ratios (aIRR) and 95% confidence intervals [, ] were estimated using Poisson regression with an interaction term for age (40-49, 50-59, ≥60 years) and HIV serostatus. Additional covariates included were race, body mass index (BMI), hypertension, diabetes, hepatitis C-coinfection, estimated glomerular filtration rate (eGFR), current smoking and alcohol use.

**Results:** At index visit, HIV-infected and uninfected men were similar by age (median 40 years), BMI (median 25 kg/m<sup>2</sup>), eGFR (median 103 vs 99 mL/min/1.73 m<sup>2</sup>), alcohol use, presence of diabetes or hypertension. HIV-infected men were more likely to be hepatitis C virus infected (10% vs 6%, p<0.001), smokers (38% vs 31%, p<0.001) and non-white (41% vs 27%, p<0.001). Among HIV-infected men, fracture incidence rate was higher in 50-59 year-olds (yo) (IR=17.5 [14.2, 21.5]) compared to 40-49 yo (IR=8.3 [6.4, 10.6]), but was similar among uninfected men aged 50-59 yo (IR=8.7 [6.8, 11.1]) and 40-49 yo (IR=8.4 [6.6, 10.6]) (Figure). Compared to younger (age 40-49) uninfected men, 50-59 yo HIV-uninfected men had a similar rate (aIRR=1.02 [0.72, 1.44]) whereas ≥60 yo HIV-uninfected men had higher rate (aIRR=1.84 [1.29, 2.63]). Among HIV-infected men, the rate of incident fracture among 40-49 yo was similar to that observed among similarly aged HIV-uninfected men (aIRR=0.99 [0.7, 1.4]), but increased in 50-59 yo men (aIRR=1.99 [1.44, 2.74]) and ≥60 yo men (aIRR=1.88 [1.17, 3.01]). The incidence rate for osteoporotic fractures showed similar trends. Of all covariates, hypertension was consistently associated with an increased rate of all fractures (aIRR=1.33 [1.04, 1.71]).

**Conclusions:** Bone fracture incidence increased with age among HIV-infected and uninfected men but was higher among HIV-infected men. A significant fracture increase was found among 50-59 yo HIV-infected men, highlighting the importance of osteoporosis screening in HIV infected men above the age of 50.

Figure. Incidence rates of all fractures and osteoporotic fractures per 1000 person-year, stratified by age and HIV status.



**700 Racial Differences in Bioavailable Vitamin D With Supplementation: ACTG A5280**

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**Background:** Vitamin D and calcium (VitD/Ca) supplementation prevents bone loss after ART initiation for both blacks and non-black HIV-infected individuals despite racial differences in vitamin D levels. Low levels of total 25-hydroxyvitamin D (25OHD) are common among black, but bioavailable 25OHD may be preserved since vitamin D binding protein (VDBP) levels are also lower in blacks.

**Methods:** ACTG A5280 is a 48-week, randomized, double-blind, placebo-controlled study evaluating the effect of vitamin D3 (4000 IU/day) and calcium carbonate (1000 mg/day) supplementation in HIV-infected participants initiating ART with efavirenz/emtricitabine/tenofovir DF. In this secondary analysis, total 25OHD (D2 and D3), VDBP, estimated bioavailable 25OHD, and parathyroid hormone (PTH) levels were assessed at baseline and 48 weeks. Wilcoxon signed-rank tests and Wilcoxon rank sum tests were used to test change within and differences between race groups (black vs non-black), respectively.

**Results:** Of 165 eligible participants enrolled, 129 participants (40 blacks and 89 non-blacks) had calciotropic hormone data at baseline and 48 weeks. At baseline, blacks had lower total 25OHD [median (Q1,Q3) 22.6 (15.8, 26.9) vs. 31.1 (23.1, 38.8) ng/ml, p<0.001] and VDBP [125.6 (79.0, 264.0) vs. 289.6 (207.7, 393.2) ug/ml, p<0.001] but higher bioavailable 25OHD [2.9 (1.5, 5.2) vs. 2.0 (1.5, 3.0) ng/ml, p=0.022], and similar PTH levels than non-blacks. In the placebo arm, VDBP and PTH levels increased similarly over 48 weeks in blacks and non-blacks, but total and bioavailable 25OHD levels remained unchanged (Table). In the VitD/Ca arm, bioavailable 25OHD increased more in blacks than non-blacks (median 3.9 vs. 1.1ng/mL, p<0.001), but change in VDBP did not differ between race groups. VitD/Ca prevented the expected PTH increase after ART; the effect appeared to be greater in blacks than non-blacks but did not reach statistical significance (p=0.11).

**Conclusions:** Despite a total 25OHD in the insufficient range (median <30ng/ml), black HIV-infected individuals had higher bioavailable 25OHD at baseline, and greater increases in bioavailable 25OHD with VitD/Ca supplementation than non-blacks. In addition, VitD/Ca may prevent rise in PTH more in blacks than non-blacks. Measurement of bioavailable 25OHD may help to elucidate racial differences in calciotropic hormone physiology.

**Table: Absolute and Percent Change from 0 to 48wks by race [(Median (Q1, Q3))]**

Variables	Change 0 - 48 week	VitD/Calcium				Placebo			
		n	black	non-black	p	n	black	non-black	p
Total 25OHD	%change (%)	64	141.7* (43.3, 260.6)	60.9* (44.7, 106.2)	0.11	65	-10.2 (-20.3, 32.7)	3.8 (-15.1, 14.8)	0.31
	Abs. change (ng/mL)	64	32.2* (14.3, 42.6)	22.5* (14.2, 34.1)	0.27	65	-2.5 (-6.3, 4.3)	1.3 (-5.5, 4.7)	0.25
VDBP	%change (%)	60	8.2 (-1.4, 17.5)	10.4* (0.0, 23.4)	0.79	63	11.2* (-3.3, 28.5)	4.3* (-6.6, 15.1)	0.24
	Abs. change (ug/mL)	60	9.4 (-0.2, 28.3)	27.0* (0.0, 60.0)	0.098	63	25.9* (-4.8, 40.6)	11.2 (-6.9, 38.7)	0.43
Bioavailable 25OHD	%change (%)	59	127.0* (40.5, 264.0)	58.3* (17.3, 95.7)	0.021*	61	-8.8 (-36.8, 14.1)	2.5 (-18.3, 16.8)	0.18
	Abs. change (ng/mL)	59	3.9* (1.4, 7.3)	1.1* (0.4, 2.2)	<0.001*	61	-0.1 (-1.4, 0.1)	0.1 (-0.4, 0.5)	0.063
PTH	%change (%)	65	-4.2 (-19.5, 16.7)	6.7* (-5.8, 26.5)	0.16	69	19.6* (-1.2, 54.5)	16.4* (-6.2, 46.6)	0.68
	Abs. change (pg/mL)	65	-0.9 (-8.2, 4.2)	1.7 (-1.9, 7.2)	0.11	69	5.9* (-0.4, 12.2)	4.3* (-1.4, 12.3)	0.49

\* p<0.05 within group change from baseline.  
# p<0.05 difference between race groups (black vs. non-black) in change from baseline.

**701 Blood Microbiome in Treatment-Naive HIV-1-Infected Persons Developing Hyperglycemia Under ART**

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**Background:** Increased bacterial translocation from the gut lumen into the bloodstream has been reported in HIV-1-infected persons. To investigate the role of bacterial translocation on the onset of metabolic diseases, we studied the relationship between the blood microbiome and the occurrence of hyperglycemia in HIV-1-infected persons before antiretroviral (ART) initiation and after 2 years on ART.

**Methods:** We carried out a nested case control study in naive HIV-1-infected persons with normal glucose levels at initiation of ART in the ANRS COPANA cohort. We compared 37 patients (43 years; range 38-51) who developed type 2 diabetes or impaired fasting plasma glucose (>5.6mmol/L) and 102 controls (35 years; range 29-44) with normal glucose values during the follow-up. Patients and controls were matched according to age, sex, type of ART and duration of follow-up. The median duration of follow-up was 24 months (range 13-35). At baseline, the blood microbiome was assessed using 16S targeted metagenomics sequencing (VAIOMER SA, Labège). Plasma adipokines (adiponectin, leptin) and inflammatory markers (C-reactive protein, I-FABP, lipoprotein binding protein, sCD163, sCD14, IL-1RA) concentrations were assessed.

**Results:** We observed a higher proportion of taxa belonging to the class Clostridia, the order Clostridiales the family Clostridiaceae and the genus Clostridium sensu stricto in cases as compared with controls (table). However no significant differences were observed in inflammatory markers and adipokines between the two groups. p-value are from rank sum Wilcoxon test or Mc Nemar test

**Conclusions:** Interestingly, it has been shown (PMID:23023125/Nature 2012) that the proportion of the class Clostridia in the gut is associated with type 2 diabetes. We propose that bacterial translocation of the class Clostridia may play a role in the onset of hyperglycemia in HIV-1-infected patients.

	cases				control			P-value	
	Median	Mean	Q1	Q3	Median	Mean	Q3		
relative abundance %									
class/clostridia	3.512	5.587	1.452	6.498	0.029	1.897	0.015	2.2606	0.001
order/clostridiales	3.512	5.587	1.452	6.498	0.029	1.896	0.015	2.2605	0.001
family/clostridiaceae	10.782	10.782	6.490	15.075	0.020	0.501	0.0126	1.1555	0.030
genus/clostridium sensu stricto	10.601	10.601	6.375	14.827	0.02	0.499	0.0126	1.1555	0.030

## 702 WITHDRAWN

703 **Incidence and Risk Factors for Overweight and Obesity After Initiation of ART**

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**Background:** The scaling up of HIV treatment services in sub-Saharan Africa has resulted in a large increase in the number of people currently receiving antiretroviral therapy (ART) in this region. Increased ART coverage in-turn has enabled more patients to survive to ages that have been associated with a higher prevalence of NCDs, including overweight and obesity. To our Knowledge, no study has previously assessed risk factors for these two outcomes in a sub-Saharan population.

The objective of this study therefore was to describe the incidence of overweight and obesity after ART initiation and to explore risk factors for these two outcomes in patients receiving care in Dar-es-Salam, Tanzania.

**Methods:** We used cox proportional hazards models to investigate risk factors of incident overweight and obesity after ART initiation.

**Results:** 13,172 (26%) patients became overweight [median follow-up of 0.8 years, interquartile range (IQR): 0.2-2.4 years], and 6996 (11%) patients became obese [median follow-up 1.3 years, IQR: 0.3-3.3-y years]. The incidence rate of obesity was 6.0 per 100 person-years (95% C.I.: 5.9-6.1). In multivariate analyses, female sex, being married, later year of ART initiation, higher baseline BMI and high adherence were all associated with increased risk for overweight and obesity. Being on an Efavirenz based regimen was associated with a 16% increased risk for obesity as compared to being on a Nevirapine based regimen (RR: 1.16, 95% C.I.: 1.07-1.25). Patients on Stavudine containing regimen had higher risk for obesity as compared to those on Zidovudine containing regimen (RR: 1.09, 95% C.I.: 1.01-1.18).

**Conclusions:** Overweight and obesity are increasingly becoming common outcomes after ART initiation in this sub-Saharan population. Long-term follow up of HIV patients will require regular screening and targeted interventions for patients with specific risk factors for these two outcomes.

704 **Measurement of Abdominal Fat Changes in HIV-Infected Individuals Initiating Therapy**

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**Background:** Visceral adipose tissue (VAT) accumulation remains common with contemporary HIV treatment and may be an important cardiovascular disease (CVD) risk factor. We examined whether changes in waist circumference and self-reported fat gain are valid methods of estimating change in abdominal fat for those initiating antiretroviral therapy (ART).

**Methods:** Prospectively collected data from A5257, a treatment initiation study, and its metabolic substudy, A5260s, were used for this analysis. ART-naïve HIV-infected participants were randomized to one of three ART regimens. Objective changes in abdominal CT-measured VAT and total adipose tissue (TAT) and DXA-measured trunk fat were tested for association with measured waist circumference changes (by Spearman correlation) and categories of self-reported abdominal fat changes (by regression modeling) between entry and week 96. General linear models compared waist circumference and self-reported fat changes.

**Results:** The study population (N=328) was predominantly male (90%) and white non-Hispanic (44%) with a median age of 36 years and BMI of 25 kg/m<sup>2</sup>. Waist circumference changes between entry and week 96 ranged from -19.2 cm to 38.4 cm (N=287). At week 96, 53% indicated "Lost/No Change", 39% "Gained Some/Somewhat Larger", and 8% "Gained A Lot/Much Larger" as their self-reported changes in abdominal fat (N=295). Trunk fat, VAT, and TAT changes differed between the self-reported groups (ANOVA p<0.0001), and the ordering of the groups was as expected. A strong correlation was found between waist circumference changes and CT and DXA changes (trunk fat:  $\rho=0.70$ , p<0.0001; VAT:  $\rho=0.52$ , p<0.0001; TAT:  $\rho=0.66$ , p<0.0001). While waist circumference changes explained a greater proportion of the variation of VAT, TAT, and trunk fat, self-reported fat change still explained a significant proportion of these fat measures after controlling for waist circumference (p<0.05).

**Conclusions:** After initiation of ART, changes in self-reported abdominal fat and measurements of waist circumference each correlated directly with imaging-derived measures of abdominal fat. These simple measures can be used as reliable, affordable tools for central body fat assessment.

705 **Metabolic Alterations and Physical Function in Older HIV-Infected Adults**

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**Background:** HIV-infected older persons have accelerated rates of physical function decline compared to HIV-uninfected persons of similar age and gender. The biological mechanisms for this are not well-understood but may include disordered metabolism. To identify possible metabolic perturbations, we conducted metabolic profiling in a cohort of older HIV-infected patients on antiretroviral therapy (ART) who had completed physical performance measures.

**Methods:** Physical performance testing (8-foot walk, grip strength, 30-second chair stand, and 6-minute walk) was performed in 117 HIV-infected older persons ( $\geq 50$  years old) on ART with undetectable HIV-1 viral loads in the Duke HIV Clinic, in Durham, NC. Targeted metabolic profiling of acylcarnitines and amino acids was conducted using mass spectrometry on plasma collected at time of physical performance testing. Metabolites were then correlated with physical performance and a comorbidity index (1 low – 5 high) using Spearman's Rank Correlation Coefficient.

**Results:** 117 participants were enrolled (mean age 60.3 years; 70% male, 59% black, 38% white, non-Hispanic; mean BMI 28.6 kg/m<sup>2</sup>; mean length of time since diagnosis 15.3 years, mean current CD4 count 691 cells/mm<sup>3</sup>). Results from all 4 physical performance tests showed significantly impaired performance when compared to normal reference ranges for HIV-uninfected adults matched by age and gender.

Short-chain acylcarnitines, including odd chain length dicarboxyls representing incomplete oxidation products of amino acid metabolism, were negatively associated with 8-foot walk speed and positively associated with the co-morbidity index. C18 acylcarnitines were positively associated with several functional measures, including the 30-second chair stand, 6-minute walk and 8-foot walk speed. Long-chain dicarboxyl acylcarnitines were positively associated with the co-morbidity index. The amino acids serine and methionine were negatively associated with the co-morbidity index.

**Conclusions:** Older HIV-infected persons have diminished physical performance compared to HIV-uninfected controls. Individual metabolites appear to be associated with impaired physical performance and a higher comorbidity index, including the dicarboxyl short and long chain acylcarnitines which have been associated with excess mortality in other populations. Further study will be required to assess the significance of these associations.

#### 706 Superior Glucose Tolerance and Metabolite Profiles in Women vs Men on Long-term ART

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**Background:** HIV-infected men on antiretroviral therapy (ART) are at higher risk of incident diabetes mellitus compared to women in some epidemiologic studies. We used a metabolomics approach to determine whether a sex difference in plasma amino acids, acylcarnitines, and organic acids predictive of diabetes and impaired energy metabolism is present in healthy, HIV-infected males versus females on the same ART regimen with long-term virologic suppression.

**Methods:** We enrolled 70 HIV patients (43% female) on efavirenz, tenofovir, and emtricitabine (Atripla) with HIV-1 RNA <50 copies/mL for over 2 years, a CD4+ count >350 cells/μL, and no history of diabetes, cardiovascular disease, statin use, or heavy alcohol use. Hemoglobin A1c (HbA1c) and homeostatic model assessment 2 (HOMA2) fasting insulin sensitivity were measured. Liquid chromatography/mass spectrometry was used to quantitate fasting plasma branched chain and aromatic amino acids (isoleucine, leucine, valine, phenylalanine, and tyrosine) predictive of incident diabetes in the Framingham cohort, and C3 and C5 acylcarnitines and organic acids indicative of impaired energy metabolism. We assessed the relationship of sex and metabolic parameters using regression models adjusted for age, race, CD4+ count, smoking, duration of ART, and fat mass index (FMI; defined as DEXA total fat [kg] / height [m]<sup>2</sup>).

**Results:** The median age was 45 years (IQR 39, 50) and median CD4+ count 701 cells/μL (IQR 540, 954). Males and females did not differ by race distribution, smoking prevalence, or median age, body mass index (BMI), CD4+ count, or duration of ART. HbA1c was <6.5% for all participants, indicating adequate insulin release, and did not differ by sex. However, females had higher HOMA2 insulin sensitivity compared to males (p<0.01), and lower plasma levels of isoleucine, leucine, valine, and phenylalanine (p<0.01 for all), and tyrosine (p=0.06). Females also had lower C3 and C5 acylcarnitines (p<0.01 for all), but no difference in alpha-hydroxybutyrate, compared to men. Results were similar when FMI was replaced with BMI in the model.

**Conclusions:** Women on Atripla with long-term virologic suppression had superior glucose tolerance and lower plasma metabolomic biomarkers associated with diabetes risk compared to men. Further studies are needed to determine how these findings compare to HIV-infected individuals on other ART regimens and HIV-uninfected persons, and whether they could reflect sex-differences in regional, hepatic or myocellular adiposity.

#### 707 Reduced Dicer and Brown Adipose Tissue (BAT) Gene Expression in HIV Lipodystrophy

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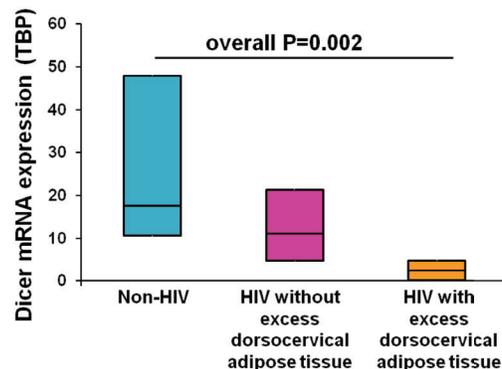
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**Background:** HIV patients are at increased risk for cardiometabolic disease secondary to depot-specific alterations in adipose function, but mechanisms remain poorly understood. BAT plays a key role to enhance energy expenditure (EE). The endoribonuclease Dicer has been linked recently to modulation of brown and white adipocyte differentiation. We previously demonstrated that Dicer knockout mice develop lipodystrophy and transformation of BAT to white adipose tissue (WAT). Thus, we hypothesized that reduced Dicer and BAT gene expression from abdominal subcutaneous (SC) WAT would relate to severity of the HIV lipodystrophic phenotype.

**Methods:** 18 HIV (9 with and without clinically apparent lipodystrophy, marked by excess dorsocervical adipose tissue [DCAT]) and 9 non-HIV subjects underwent punch biopsy of the abdominal SC adipose tissue in a cross-sectional study. We assessed abdominal SC WAT specific expression of Dicer and other adipose related genes. The Wilcoxon test was used to compare HIV and non-HIV groups and HIV with and without excess DCAT as confirmed by MR imaging. Correlations were assessed by Spearman's.

**Results:** HIV subjects with long duration of HIV and ART use demonstrated excess DCAT vs. non-HIV subjects (9.8±1.0 vs. 6.6±0.8 cm<sup>2</sup>, P=0.02) with similar BMI. Dicer expression was decreased in HIV vs. non-HIV (median[IQR] 4.9 [1.9,11.9] vs. 17.7 [10.7,47.9], P=0.01), as were PGC1a, ZIC1, PRDM16, DIO2, and HSP60 (all P≤0.03). Dicer expression was decreased in HIV with vs. without excess DCAT (2.5 [0.0,4.9] vs. 11.2 [4.8,21.5], P=0.006) in addition to brown [PGC1a (P=0.002), ZIC1(P=0.004), LHX6 (P=0.03), PRDM16 (P=0.001), PAT2 (P=0.008), P2RX5 (P=0.02)], beige [TMEM26 (P=0.004), CD137 (P=0.008)] and other genes [DIO2 (P=0.002), leptin (P=0.003), HSP60(P=0.0004)]. Among all subjects, downregulation of Dicer correlated with downregulation of UCP1, PGC1a, ZIC1, LHX8, PRDM16, PAT2, P2RX5, TMEM26, CD137, DIO2, leptin, and HSP60 (all P≤0.01). Excess DCAT negatively correlated with PGC1a, ZIC1, LHX8, PRDM16, PAT2, P2RX5, TMEM26, DIO2, leptin and HSP60 (all P<0.04). Resting EE normalized to fat free mass correlated with UCP1, LHX8, PAT2, TMEM26, and HSP60 (all P≤0.04).

**Conclusions:** Our results demonstrate reduced Dicer in relationship to downregulation of multiple BAT related genes in SC WAT in HIV lipodystrophic patients and may provide a novel mechanism for metabolic dysregulation. A strategy to increase "browning" of WAT may yield a novel target to improve cardiometabolic health in HIV.



DICER mRNA expression in A.U. normalized to TBP (TATA-binding protein) in abdominal white adipose tissue among non-HIV and HIV subjects with and without excess dorsocervical adipose tissue. Box plot represents 25th and 75th percentile and line within the box represents median. P value represents overall P by Kruskal-Wallis Test.

**708 Hospitalizations With AIDS and Chronic End-Organ Conditions in HIV Outpatient Study**

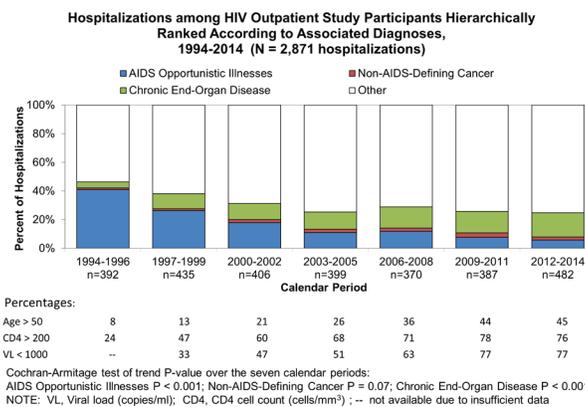
**Kate Buchacz**<sup>1</sup>; Linda A. Battalora<sup>2</sup>; Carl Armon<sup>3</sup>; Rachel Hart<sup>3</sup>; John T. Brooks<sup>1</sup>; for the HIV Outpatient Study  
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**Background:** Combination antiretroviral therapy (ART) has extended survival of HIV-infected patients in the United States (U.S.) resulting in more patients aging to experience chronic conditions. We assessed the frequency of select conditions at hospitalization among HIV-infected patients before and during the era of ART.

**Methods:** We analyzed data from HIV Outpatient Study (HOPS) participants seen at 9 U.S. HIV-specialty clinics during 1994-2014. We evaluated the percentage of hospitalizations of at least 1 night stay among HOPS participants with the following associated diagnoses (classified hierarchically): AIDS-defining opportunistic illnesses (OIs), non-AIDS defining cancers, chronic-end organ conditions (including cardiovascular, hepatic, renal, and pulmonary events), and other conditions, in seven three-year calendar periods during 1994-2014. We used Cochran-Armitage test of trend to analyze changes in frequency of associated conditions and characteristics of hospitalized patients over time, and Wilcoxon rank sum test to analyze changes in length of stay in hospital (LOS).

**Results:** Among 8,358 HOPS participants, 2,100 had been hospitalized with a total of 5,001 hospitalizations during 1994-2014 (29% for women, 47% for blacks, and 14% for Latino/Hispanic or other race/ethnicity patients). In analyses restricted to first hospitalization per patient per calendar period (n=2,871 hospitalizations), and comparing hospitalizations from 1994-1996 through 2012-2014, those with AIDS OIs fell from 45% to 6% (p < 0.001), those with chronic end-organ disease rose from 4% to 17% (p < 0.001), and those with non-AIDS defining cancers did not change significantly (Figure). The characteristics of patients at hospitalization also shifted: age > 50 years increased from 8% to 45%, CD4 cell count > 200 cells/mm<sup>3</sup> rose from 24% to 76% and HIV viral load < 1000 copies/mL rose over time to 77% (all p < 0.001). Median LOS was 7 days in the first period and 3 days in the last period (p < 0.001).

**Conclusions:** During this 21-year prospective HIV cohort study, coincident with more widespread use of increasingly effective ART, the percentage of hospitalizations associated with AIDS OIs decreased as the percentage associated with chronic end-organ conditions increased. Hospitalizations occurred increasingly among patients who were older, had higher CD4 cell counts and lower HIV viral loads. These findings highlight the growing need for preventative primary care.



**709 Ageing With HIV: Emerging Importance of Chronic Comorbidities in Patients Over 75**

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**Background:** As HIV-infected adults on successful ART are expected to have close to normal lifespans, they will increasingly develop age-related comorbidities. Only scarce epidemiological and clinical data are available in a geriatric HIV population.

**Methods:** From the prospective multicenter (15 sites) DatAIDS cohort, we selected patients with at least one visit since 2004 and aged over 75 at the latest visit (geriatric group). Demographic, immuno-virological characteristics, as well as current ART regimen and comorbidities were compared with the elderly population, aged 50 to 75 (elderly group). P values < .05 were considered significant. Statistical tests used Wilcoxon test or  $\chi^2$ , as appropriate.

**Results:** Of the 43,522 patients, characteristics of the 654 patients over 75 years (1.5%) were as follow: median age 78 years [IQR 76-82], 72% male, 31% HSH, with a median age at HIV diagnosis of 64 years [58-70] (age >75 at diagnosis in 10%) and a median duration of HIV infection of 15 years [10-20]. Death occurred in 146 patients (22%). Among the 430 patients alive at the censoring date (01/09/2014), 420 (98%) were receiving a triple therapy, a mono/dual therapy or at least 4 ARVs in 78%, 14% and 6% of cases, respectively. Main significant differences (p<0.05) between geriatric and elderly groups were: median age 78 vs. 56 years, hepatitis co-infection 9% vs. 24%, CMV serology positive 95% vs. 87%, low BMI 22% vs. 4%, overweight 6% vs. 29%, CDC stage C 34% vs. 29%, current smokers 5% vs. 37%, median CD4 cell count 494 vs. 560/mm<sup>3</sup>, age at ART initiation 64.5 vs. 44.5 years, number of previous ART regimen 6 vs. 5, and current NRTI-sparing regimen use 30% vs. 26%. Virologic success rate (HIV RNA < 50 c/mL) was 89.2% in both groups. The geriatric group had more frequent age-associated non communicable comorbidities (AANC) than the elderly group: 45.8% vs. 71.1% had no more than 1 AANC, 40.2% vs. 24.7% had 2 or 3 AANC and 14% vs. 4.3% had more than 4 AANC (all p<0.05). ART, immunological characteristics and details of AANC are presented in table.

**Conclusions:** In the HIV ageing population commonly defined as over 50, age-related comorbidities and undernourishment dramatically increased in the geriatric population, after 75, whose prevalence starts to be relevant. Despite an insufficient immunological reconstitution and more frequent unusual ARV regimens, rate of virological success is high.

	Elderly Group n = 12748 n (%)	Geriatric Group n = 430 n (%)	p
2 NRTIs + NNRTI	4182 (32.8)	126 (29.3)	0.111
2 NRTIs + PI	3591 (28.2)	110 (25.6)	0.216
2 NRTIs + INSTI	1234 (9.7)	43 (10)	0.850
CD4 > 500/mm <sup>3</sup> and CD4:CD8 > 1	2873 (24.5)	82 (20.7)	<b>0.001</b>
Diabetes	1195 (9.4)	96 (22.3)	<b>&lt;0.001</b>
Dyslipidemia	2700 (21.2)	120 (27.9)	<b>0.001</b>
Hypertension	2685 (21.1)	182 (42.3)	<b>&lt;0.001</b>
Chronic renal disease	1386 (10.6)	179 (35.2)	<b>&lt;0.001</b>
Cardiovascular event	1400 (10)	116 (27)	<b>&lt;0.001</b>
Neoplasia	1526 (12)	97 (22.6)	<b>&lt;0.001</b>
Osteoporosis	626 (4.9)	36 (8.4)	<b>0.002</b>

### 710 Incremental Association Between CD4:CD8 Ratio and Incidence of Non-AIDS Events

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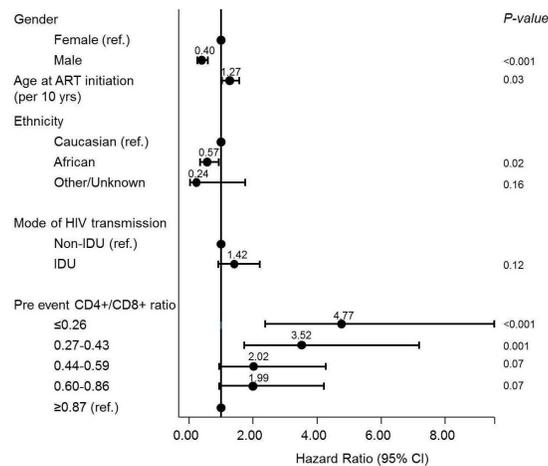
<sup>1</sup>HIV Molecular Rsr Group, UCD Sch of Med, Univ Coll Dublin, Dublin 7, Ireland; <sup>2</sup>Univ Coll Dublin, Dublin, Ireland; <sup>3</sup>Mater Misericordiae Univ Hosp, Dublin, Ireland

**Background:** Despite effective antiretroviral therapy (ART), people living with HIV (PLWH) still experience excess morbidity and mortality, with a growing awareness of the impact of non-AIDS defining events (NADE) and the factors potentially associated with the occurrence of these events including failure to normalise the CD4:CD8 ratio.

**Methods:** Adult PLWH enrolled in the Mater ID Cohort Study who commenced ART after January 1st 2001 were included in an analysis determining prevalence of and associations with AIDS events and NADE. Demographic, laboratory (including HIV RNA, CD4+ and CD8+ T-cell counts, CD4:CD8 ratio) and clinical events (AIDS and NADE) were collated. Multivariable Cox proportional hazards regression models explored factors independently associated with the progression to NADE. Data are reported as median (IQR).

**Results:** Of 550 PLWH, 317 (58%) were male, 299 (54%) Caucasian, 220 (40%) African, 114 (21%) Men who have sex with Men and 131 (24%) Injecting drug users (IDU). 128 (23%) were co-infected with Hepatitis C. At ART initiation median age was 34 (29, 40) yrs, and nadir CD4+ count 187 (80, 284) cells/mm<sup>3</sup>. Of 135 NADE in 2557 person years of follow (crude incidence 5.3 per 100 PYFU), the commonest were pneumonia (n=39), liver disease (n=17), cardiovascular disease (CVD) (n=14) and non AIDS malignancies (n=12). Of 23 deaths, 5 were AIDS related and 11 were NADE (malignancy (n=7), liver disease (n=2), CVD (stroke) (n=1), abdominal sepsis (n=1)). In multivariable Cox models, older age at ART initiation, IDU risk and lower quintiles of pre-event CD4:CD8 ratio were independently associated with an increased risk of non-AIDS defining events (see figure 1), with male gender and non-Caucasian ethnicity independently associated with reduced risk of NADE (see figure 1). A sensitivity analysis in those virally suppressed revealed similar associations with age at ART initiation (HR=1.59, 95% CI 1.23-2.05), gender (HR=0.33, 95% CI 0.20-0.56) and lower pre-event CD4:CD8 ratio (CD4:CD8 ratio ≤0.26; HR=3.11 (95% CI 1.44-6.71), CD4:CD8 ratio 0.27-0.43; HR=1.64 (95% CI 0.74—3.66), CD4:CD8 ratio 0.44-0.59; HR= 1.67 (95% CI 0.77-3.66), CD4:CD8 ratio 0.60-0.86; HR=1.26 (95% CI 0.54-2.93)).

**Conclusions:** This is the first study to show an incremental association between pre-event CD4:CD8 ratio and NADE. It is yet to be determined what impact, if any, strategies to improve CD4:CD8 ratio will have on prevalence of NADE.



### 711 Impact of Monocyte Metabolism on Serious Non-AIDS Events in HIV+ Individuals

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**Background:** Excessive glucose uptake and metabolism, mediated by cell surface expression of Glucose-transporter1 (Glut1) by monocytes, is associated with inflammation. Having shown that glucose metabolism is increased in inflammatory monocyte subsets and only partially normalized by ART, we analyzed metabolic activation of monocytes and its association with markers of cardiovascular disease risk.

**Methods:** We determined the relationship between monocyte subset Glut1 expression, markers of inflammation and coagulation in naïve and ART-treated HIV+ (n = 28) by linear regression analysis; markers significant in univariate analyses were used in a multivariate linear regression model; the final model derived by backward elimination. For functional analysis, monocyte were treated with a PI3K inhibitor (IC87114), and activated with LPS/IFN $\gamma$ . Glucose uptake and lactate levels in monocytes were measured

**Results:** In multivariate models, the levels of Glut1 (MFI) on the intermediate pro-inflammatory (CD14+CD16+) monocyte subpopulation were independently associated with levels of D-dimer (p=0.003) and ART treatment status (p=0.015), and inversely associated with HDL-cholesterol (p=0.018). In a simple correlation analysis, there was a significant relationship between the % of intermediate monocytes expressing Glut1 and plasma TNF levels (p=0.04). Metabolic parameters (plasma glucose, insulin, and triglyceride levels) were not associated with Glut1 expression on monocytes, possibly because Glut1 is predominantly regulated by inflammatory signals. CD4+ and CD8+ T activation (CD38, HLA-DR), were not associated with Glut1 expression on intermediate monocytes. Glucose metabolic inhibition by IC87114 prior to stimulation significantly inhibited glucose uptake (p=0.02, n=5) and L-lactate (p=0.03, n=5).

**Conclusions:** Elevated expression of Glut1 on intermediate monocytes in HIV+ patients with suppression on ART is associated with plasma biomarkers of cardiovascular risk. The PI3K signaling pathway in monocytes offers a potential drug target to counteract inflammatory-mediated Serious Non-AIDS Events in HIV+ individuals

### 712 IGF-1 Levels Predict Advanced Aging in HIV/HCV Coinfected Persons

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**Background:** Some diseases including liver cirrhosis occur at younger ages in persons with HIV than in persons without HIV. Insulin growth factor-1 (IGF-1) declines in healthy adults as they age. We explored whether IGF-1 explained the association between HIV, liver fibrosis, and age.

**Methods:** Subjects were from the AIDS Linked to the Intravenous Experience (ALIVE) cohort of former and current people who inject drugs in whom we previously showed that liver cirrhosis occurred 10 years earlier in persons with HIV compared to those without HIV after controlling for HCV, HBV, BMI, and alcohol (Kirk Ann Intern Med 2013). From 2006-2014, liver fibrosis was staged by transient elastography (TE) every six months. IGF-1 was quantified by ELISA (R&D Systems, Inc, Minneapolis, MN) in serum collected a) upon enrollment in the cohort b) contemporaneous with the earliest TE result and c) at the most recent visit. Correlates of initial IGF-1 levels were identified by multivariable linear regression. IGF-1 decline was determined by Wilcoxon rank sum tests. Intraperson IGF-1 variability was modeled using generalized estimating equations (GEE).

**Results:** Of 553 subjects, 158 (28.6%) were female, 536 (96.9%) were black, 490 (88.6%) had HCV, and 129 (23.3%) had HIV. The median total study interval was 16 yrs (IQR 15, 17), which included baseline to first TE (11 yrs [11, 12]) and first TE to most recent (5 yrs [4, 5]). At first TE, 337 (65.8%) participants had minimal liver stiffness (<8kPa), 103 (20.1%) had intermediate levels of liver stiffness (8-12.2 kPa), and 72 (14.1%) had evidence of cirrhosis ( $\geq 12.3$  kPa).

As previously published, liver stiffness was associated ( $p < 0.001$ ) with age, HIV, HCV RNA, BMI, and HBV. Interestingly, the initial IGF-1 level taken a median of 11 yrs earlier was inversely associated with liver stiffness (OR -11.6; 95% CI -22.0, -1.1;  $p = 0.03$ ), after adjusting for age, even though IGF-1 levels were also strongly and independently associated with age ( $p < 0.0001$ ). Declines in IGF-1 were also noted: at a rate of -1.84 pg/mL-yr in HCV mono-infected persons and at a rate of -1.47 pg/mL-yr in HIV/HCV co-infected persons ( $p < 0.0001$  for both compared to baseline). However, there was little evidence that IGF-1 levels (at baseline, the time of the TE, or the rate of change) altered associations of age and HIV with liver stiffness by GEE.

**Conclusions:** Although IGF-1 levels were strongly associated with age and liver stiffness, we found no support that IGF-1 contributes to how HIV-1 accelerates liver aging.

### 713 HLA-B57.01 Promotes Significantly Better Periodontal Health in HIV-Positive Patients

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**Background:** In HIV-negative subjects with chronic periodontitis a relationship between the individual HLA system (human leukocyte antigen) and the incidence of chronic periodontitis was observed. The presented study is the first that investigates the incidence of chronic periodontitis in HIV-positive patients in connection with the HLA system and in particular with the HLA-B57.01 status. This study hereby evaluates associations among periodontitis, the HLA system and additional risk factors in HIV-positive patients in multivariate analyses.

**Methods:** A group of 100 HIV-positive patients was enrolled in the study program. 45 patients were treatment naïve, whereas 55 patients were treated with combined antiretroviral therapy (cART). 19 patients presented a positive HLA-B57.01 status. The patients which received cART were treated with cART for at least 12 months. The periodontal testing included PSI-Score (Periodontal Screening Index), GI (Gingivalindex), Bleeding on Probing Index (BOP) and the DMF-T Score (decayed, missing, filled teeth). The testing was performed with a standardized periodontal probe by an experienced dentist.

**Results:** HIV-positive patients who were carriers of HLA-B57.01 had significantly lower PSI- ( $p < 0.001$ ), GI- ( $p < 0.001$ ) and BOP-Scores ( $p < 0.001$ ) in comparison to naïve HIV-positive patients as well as HIV-positive patients receiving cART, who were both not carriers of HLA-B57.01. A lower value of PSI-, GI- and BOP-Score equals a better periodontal health.

The adjusted odds ratio (OR) of periodontitis was decreased in patients who were carriers of HLA-B57.01 by measurement of PSI-Score (OR = 0.006, 95% confidence interval (CI) = 0.001 to 0.026), GI-Score (OR = 0.018, 95% confidence interval (CI) = 0.003 to 0.104) and BOP-Score (OR = 0.003, 95% confidence interval (CI) = < 0.001 to 0.011).

The DMF-T score showed no significant difference ( $p = 0.516$ ) between the HIV-positive patients with and without cART, regardless of their HLA-B57.01 status.

**Conclusions:** HLA-B57.01 was identified as an independent resistance indicator for generalized periodontitis in HIV-positive patients with respect to established cofactors for periodontitis.

The underlying biological mechanisms for this finding have to be investigated in the future, especially the questions if there are periodontitis-specific bacterial peptides that are expressed by HLA-B57.01 and if HLA-B57.01 molecules are able to influence the immune response against periodontopathogens.

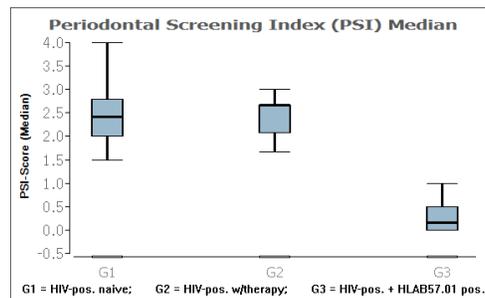


Fig.1: Periodontal Screening Index (PSI) Median

### 714 Association of Depressive Symptoms With Biomarkers in Veterans With and Without HIV

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**Background:** HIV infection is associated with increased cardiovascular disease risk. Recently, we found that HIV+ veterans with a diagnosis of clinical depression have higher risk of heart failure compared to HIV+ veterans without depression. To explain the physiological mechanism underlying this finding, we compared biomarkers of inflammation (IL-6), monocyte activation (sCD14) and altered coagulation (D-dimer) between HIV+ and HIV- veterans with and without depressive symptoms. Prior studies have separately linked higher IL-6 and D-dimer with HIV and depression. However, no study has examined these biomarkers in a cohort of HIV+ and HIV- people with and without depression. We hypothesize that HIV+ people with depression will have higher IL-6, sCD14 and D-dimer than people with one or neither of these conditions.

**Methods:** Participants in this study were from the Veterans Aging Cohort Study-Biomarker Cohort, a prospective cohort study of HIV+ and HIV- veterans who provided blood specimens for research. Depression was assessed using the Patient Health Questionnaire-9 (PHQ-9), with scores  $\geq 10$  indicative of clinically significant symptoms of major depressive disorder in the last two weeks. We used linear regression to assess the associations between depression and biomarker levels, adjusting for age, race/ethnicity, hypertension, obesity, smoking, hepatitis C infection, antidepressant medication, and HIV infection.

**Results:** In this sample of 2224 participants, 573 (24%) participants reported clinically significant depressive symptoms. PHQ-9 scores  $\geq 10$  were not associated with significant differences in IL-6, sCD14 or D-dimer in adjusted models (Table). Given a significant interaction between HIV and PHQ-9 in IL-6 models ( $P = 0.07$ ), we stratified this analysis by HIV status. PHQ-9  $\geq 10$  was associated with higher IL-6 among HIV- veterans [ $\beta$  (95% CI): 0.11 (-0.02, -0.25)] but not among HIV infected participants with HIV-1 RNA < or  $\geq 500$  copies/mL [0.00 (-0.12, 0.12) and -0.07 (-0.23, 0.09) respectively;  $p > 0.05$  for all].

**Conclusions:** We did not observe associations between depressive symptoms and IL-6, sCD14 or D-dimer. These findings suggest that the physiological pathways mediating depression and heart failure risk may not be captured by IL-6, sCD14, or D-dimer in HIV infected people. Future research is needed to elucidate the mechanisms driving depression-associated heart failure risk in HIV infected people.

**Table.** Association between symptoms of major depression (PHQ-9  $\geq$  10) and biomarker concentrations (continuous scale)

Sample	Predictor	log(IL-6)		log(D-dimer)		log(sCD14)	
		$\beta$ (95% CI)	P	$\beta$ (95% CI)	P	$\beta$ (95% CI)	P
Total cohort	PHQ-9 $\geq$ 10	0.043 (-0.035, 0.12)	0.28	0.07 (-0.024, 0.16)	0.15	0.017 (-0.011, 0.045)	0.23
HIV-	PHQ-9 $\geq$ 10	0.11 (-0.021, 0.25)	0.098	0.038 (-0.094, 0.17)	0.58	-0.013 (-0.055, 0.028)	0.53
HIV+ ( $<$ 500 HIV-RNA copies/mL)	PHQ-9 $\geq$ 10	-0.0032 (-0.12, 0.12)	0.96	-0.017 (-0.18, 0.15)	0.84	0.045 (-0.0044, 0.094)	0.074
HIV+ ( $\geq$ 500 HIV-RNA copies/mL)	PHQ-9 $\geq$ 10	-0.067 (-0.23, 0.091)	0.41	0.11 (-0.92, 0.31)	0.29	0.013 (-0.042, 0.069)	0.63

All models were adjusted for age, race/ethnicity, hypertension, obesity, smoking status, hepatitis C infection, and antidepressant medication use; Total cohort model was additionally adjusted for HIV infection

### 715 Higher Plasma Cell-Free DNA Levels Are Associated With Younger Age and HIV Infection

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**Background:** HIV infection and antiretroviral treatment are associated with mitochondrial dysfunction, cytotoxicity, and chronic inflammation; all processes that can contribute to the accelerated aging seen in persons living with HIV. Increased cell-free nuclear (cf-nDNA) and mitochondrial (cf-mtDNA) DNA levels are generally associated with poorer health outcomes, and cf-mtDNA is particularly pro-inflammatory. We investigated cf-DNA levels in participants of the CARMA cohort.

**Methods:** Our observational cross-sectional study included 92 HIV-infected (HIV+; aged 4-63y), 45 HIV-exposed uninfected (HEU; 2-18y), and 24 HIV-unexposed uninfected (HUU; 15-78y) participants. The latter two groups were combined into one HIV-uninfected group (HIV-). Fresh whole blood (WB) was spun at 14,000xg and the plasma filtered (0.45  $\mu$ m) to fully remove cells and platelets. mtDNA and nDNA levels were measured in both filtered plasma and WB via multiplex qPCR. Associations with age and HIV infection were investigated among children or adults by Student's *t*-test and by correlations. Significant univariate associations were further investigated through multivariable linear regression analyses.

**Results:** Within-participant cf-mtDNA were higher (median 6x) than cf-nDNA levels but the two were highly correlated across the HIV+ (n=92,  $r=0.80$ ,  $p<0.0001$ ) and HIV- (n=69,  $r=0.70$ ,  $p<0.0001$ ) groups. No correlation was seen between cf- and WB DNA levels. Age was negatively correlated with cf-mtDNA and nDNA in both the HIV+ (n=92,  $\rho\leq -0.26$ ,  $p\leq 0.013$ ) and HIV- (n=69,  $\rho\leq -0.35$ ,  $p\leq 0.004$ ) groups. HIV infection itself was associated with higher cf-mtDNA ( $p=0.004$ ) and cf-nDNA ( $p=0.003$ ) in pediatric participants (2-19y, n=72) but not in adults. In a multivariable model (n=161), younger age ( $p<0.0001$ ) and HIV infection (HIV+ vs. HIV-;  $p\leq 0.026$ ) were independently associated with higher cf-mtDNA and nDNA. Among HIV+ participants, CD4+ T cell count was positively correlated with both cf-mtDNA and nDNA (n=52,  $r\geq 0.36$ ,  $p\leq 0.009$ ). Too few participants had a detectable HIV pVL to investigate it as a predictor of cf-DNA. In a multivariable model of the HIV+ group (n=52), both younger age ( $p\leq 0.009$ ) and higher CD4+ count ( $p\leq 0.019$ ) were independently associated with higher cf-mtDNA and nDNA.

**Conclusions:** Children have more circulating cf-mtDNA and nDNA than older persons, and that those living with HIV have further increased levels, possibly impacting aging processes. A larger sample size will be needed to verify whether this association exists in adults.

### 716 Peak HIV Viral Load Is Associated With Increased Blood Mitochondrial DNA Mutations

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**Background:** People living with HIV experience accelerated aging. The oxidative stress theory of aging states that the accumulation of mtDNA mutations over time leads to tissue aging and dysfunction. Somatic mtDNA point mutations are implicated in age-associated diseases such as those seen in HIV+ individuals. Both the virus and the antiretroviral drugs can negatively impact mtDNA as well as increase oxidative stress and may therefore contribute to accelerated aging. We hypothesized that mtDNA somatic substitutions would increase with age, and HIV infection.

**Methods:** Participants in this cross-sectional study were HIV+ (n=92, 12 < 19y) and HIV- (n=72, 13 < 19y) female participants enrolled in the CARMA cohort, not infected with hepatitis C or B virus, and either current or never (but not past) smokers. Whole blood DNA was extracted and somatic mtDNA substitution mutation rates in the D loop region were quantified blindly via a next generation sequencing assay that distinguishes "true" mtDNA substitutions from background ones introduced via PCR or sequencing errors. After sorting and aligning, mtDNA somatic substitution rates per 100,000bp were calculated. Factors associated with mtDNA mutations were investigated through Spearman's correlations, Mann-Whitney tests, and ANCOVA of  $\log_{10}$  values as appropriate.

**Results:** Measured blood mtDNA mutation rates met quality control for 78 HIV+ and 64 HIV- individuals (median [IQR] (range) of 3.6 [2.3-6.1] (0.0-25.0)) aged 1-75 years. Ages were similar between the two groups ( $p=0.15$ ) and all HIV+ children had undetectable pVL while this was true for 59% of adults. Approximately 40% of adults were current smoker in both groups. A significant correlation was seen between mtDNA mutation rates and age ( $\rho=0.29$ ,  $p<0.001$ ) but there was no association with HIV+ status ( $p=0.96$ ) or smoking (current vs. never,  $p=0.84$ ). Among HIV+ adults, higher mtDNA mutation rates were associated with peak HIV pVL recorded ( $>$  vs.  $\leq 100,000$  copies/mL,  $p=0.015$ ) but not current HIV pVL ( $\rho=0.003$ ,  $p=0.67$ ), CD4+ count ( $\rho=0.015$ ,  $p=0.34$ ), or CD4 nadir ( $\rho=0.021$ ,  $p=0.25$ ). In a multivariable model of HIV+ adult participants that included age and peak pVL, only the latter ( $p<0.01$ ) remained independently associated with mtDNA mutations rates.

**Conclusions:** Somatic mtDNA mutations can be measured in blood and their rate increases with age, consistent with current theories of aging. Our results further suggest that high HIV viremia may also induce aging-like mtDNA damage.

### 717 Equivalent Decline in Inflammation Markers With Tenofovir Alafenamide and Tenofovir DF

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**Background:** Initiation of antiretroviral therapy (ART) reduces both immune activation and systemic inflammation. We examined changes in biomarkers of monocyte activation (sCD14, sCD163), and systemic (IL-6, hsCRP, sTNFR-I and D-dimer) and vascular (Lp-PLA2) inflammation in a randomized, controlled trial comparing elvitegravir/cobicistat/ emtricitabine/tenofovir alafenamide (E/C/F/TAF) to E/C/F/tenofovir disoproxil fumarate (TDF) in treatment-naïve adults.

**Methods:** We randomly selected 100 participants from each arm, stratifying by baseline CD4 and HIV-1 RNA, age, sex, geography, and excluding patients with cardiovascular disease and those who received statins. Plasma levels were assayed at baseline and weeks 12, 24, and 48 in batch. We summarized baseline levels and percent change from baseline, comparing between arms using Wilcoxon Rank Sum, and within arms and overall using Wilcoxon Signed Rank tests. We tested equivalence between arms using the two-one sided test (TOST). We compared arms with multivariable multiple regression (MMR) to account for correlations among biomarkers. We assessed the biomarkers' ability to differentiate between arms with the machine learning algorithm Random Forest using a ROC curve and variable importance.

**Results:** A total of 194 patients had evaluable samples (TAF, 98; TDF, 96); 19% women, 44% non-white, 25% VL>100,000 c/mL, and 28% smokers. Median baseline characteristics: age 33, CD4 405 cells/ $\mu$ L, and VL 4.7 log<sub>10</sub>c/mL were similar to the parent study. Baseline levels of biomarkers did not differ by arm. Overall (both arms pooled), biomarkers of systemic inflammation declined after ART initiation, with statistically significant declines from baseline for IL-6, d-dimer, sCD163 and sTNFR-1 by week 24. Lp-PLA2 levels increased slightly from baseline to 48 weeks (p=0.045). There were no differences in percent change from baseline between groups at weeks 12, 24, or 48 (p>0.05), except IL-6 at week 12 (p=0.012). All biomarkers were equivalent between arms by TOST (90% CIs within 80%-125%) except hsCRP (105.3%, 90%CI [80.7%, 137.4]). By MMR (p=0.9970) and Random Forest (ROC AUC=0.49; 95%CI 0.41-0.57; variable importance scores <0.5%), there was no differentiation between arms by any biomarkers.

**Conclusions:** After initiating ART with either E/C/F/TAF or E/C/F/TDF, there were equivalent declines in biomarkers of monocyte activation and systemic inflammation, supporting that TAF provides similar salutary modulation of inflammation as TDF.

**Table: Baseline and Percent Change in Biomarkers**

Median Biomarker Log10 ng/mL unless otherwise indicated	Baseline E/C/F/TAF (N=98)	Baseline E/C/F/TDF (N=96)	Baseline comparison p-value*	Overall Percent Change W12** (p-value)	Overall Percent Change W24** (p-value)	Overall Percent Change W48** (p-value)
hsCRP	3.32	3.12	0.19	-9.45 (0.55)	-5.55 (0.26)	-7.10 (0.12)
IL-6 (*Log <sub>10</sub> pg/mL)	0.18	0.13	0.44	-13.67(0.092)	-24.84 (<.001)	-22.63 (0.049)
d-dimer	2.45	2.37	0.17	-18.81 (<.001)	-15.54 (0.001)	-15.86 (0.005)
sCD14	3.21	3.20	0.95	-1.89 (0.88)	-1.45 (0.40)	-5.74 (0.061)
sCD163	2.85	2.87	0.54	-16.06 (<.001)	-23.34 (<.001)	-30.67 (<.001)
Lp-PLA <sub>2</sub>	2.29	2.30	0.47	1.17 (0.39)	-0.92 (0.84)	3.51 (0.045)
sTNFR-1 (*Log <sub>10</sub> pg/mL)	2.93	2.94	0.90	-5.11 (0.001)	-6.05(0.003)	-6.21 (0.008)

\*p-value for difference between treatment groups at baseline-Wilcoxon rank sum test

\*\*p-value for overall declines within the combined treatment groups-Wilcoxon signed rank test

**718 Similar Inflammatory Marker Levels With LPV/r+3TC Versus LPV/r+2NRTIs at 48 Weeks**

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**Background:** GARDEL was a randomized trial demonstrating the virologic non-inferiority of dual-therapy using lopinavir/ritonavir plus lamivudine (LPV/r+3TC) compared to triple therapy using lopinavir/ritonavir plus two investigator-selected nucleoside reverse transcriptase inhibitors (LPV/r+2NRTIs) in treatment-naïve HIV-infected adults. As residual inflammation has been associated with increased morbidity and mortality in HIV-infected patients, we compared levels of key inflammatory biomarkers between study arms over 48 weeks among GARDEL participants from Argentina.

**Methods:** Plasma collected at baseline, week 24 and week 48 visits was stored at -80C for batched analysis of sCD14, MCP-1, TNF, IL-6, D-dimer and hsCRP. Generalized Estimating Equations with an identity/logit link as appropriate were used to model the impact of dual versus triple ART on each biomarker at 48 weeks, controlling for baseline levels. Additional models estimated the change in biomarker levels over 48 weeks after adjustment for age, sex, baseline CD4 count and baseline log<sub>10</sub> HIV RNA.

**Results:** Of 192 GARDEL participants enrolled in Argentina, 171 had samples available from both the baseline and at least one follow-up visit and were eligible for inclusion in the analysis. Median (interquartile range) age was 35 (28,44) years, baseline CD4 count 308 (218,413) cells/mm<sup>3</sup>, baseline HIV RNA was 5.14 (4.67,5.59) log<sub>10</sub> copies/mL. Most participants were Hispanic/Latino (76%), men (79%) with clade B (56%) or BF (27%) infection; only 2% were hepatitis C co-infected. Of the 80 (47%) participants receiving triple therapy, 70 (41% of total) used zidovudine/lamivudine, 7 (4%) used tenofovir plus 3TC or FTC and 3 (2%) switched between these backbones. Overall, over 48 weeks, significant changes were seen in sCD14 (+154 ng/mL, 95%CI=32.8,275), MCP-1 (-0.045 log<sub>10</sub> pg/mL, 95%CI=-0.0679,-0.022), TNF (-0.235 log<sub>10</sub> pg/mL, 95%CI=-0.262,-0.208), and D-dimer (-0.157 log<sub>10</sub> ng/mL, 95%CI=-0.208,-0.106), while no change was seen in hsCRP (-0.0242, 95%CI=-0.133,0.0852) and the odds of having undetectable IL-6 was similar (OR=1.46, 95%CI=0.88,2.43). Dual therapy was not associated with significantly different biomarker levels at 48 weeks relative to triple therapy (Table).

**Conclusions:** In addition to having virologic non-inferiority, LPV/r+3TC dual therapy is associated with similar levels of inflammatory markers over 48 weeks compared to LPV/r+2NRTIs triple therapy in treatment-naïve adults. The increase in sCD14 over time requires further study.

**Differences in inflammatory biomarkers at 48 weeks for dual versus triple therapy**

Biomarker	Estimate (95% CI)	p
CD14s ng/ml	99.1 (-61.7,260)	0.23
Log <sub>10</sub> (MCP-1 pg/ml)	-0.03 (-0.06,0.00)	0.07
Log <sub>10</sub> (TNF- $\alpha$ pg/ml)	0.02 (-0.01,0.05)	0.16
Undetectable IL-6 <sup>a</sup>	1.18 (0.65,2.15)	0.59
Log <sub>10</sub> (D-Dimer ng/ml)	0.00 (-0.07,0.08)	0.92
Log <sub>10</sub> (CRP ng/ml)	0.08 (-0.06,0.23)	0.26

<sup>a</sup> odds ratio of having a detectable value is presented

**719 Frailty Is Associated With NNRTI-Based Initial ART and Modifiable Risks in ACTG 5322**

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**Background:** Frailty is described in HIV infection, particularly among persons with greater historical immune suppression. The impact of initial antiretroviral therapy (ART) selection on subsequent frailty has not been well described in the current ART era.

**Methods:** HIV+ participants aged  $\geq 40$  years who received an initial ART regimen as participants in an ACTG randomized clinical trial completed a frailty assessment (4 meter walk, grip strength, and self-reported weight loss, exhaustion, and low physical activity) at A5322 enrollment, a longitudinal study. Participants meeting previously-defined thresholds in 3-5 categories were considered frail, 1-2 pre-frail, and 0 non-frail. Characteristics were compared between frailty groups. Ordinal logistic regression models examined associations with an increase in frailty (from non-frail to pre-frail/frail or non-frail/pre-frail to frail). Covariates with category-specific p-value  $< 0.10$  in univariate models were retained in the final multivariable model.

**Results:** There were 1016 HIV+ participants with frailty exams; median age was 51 (IQR 46, 56) years; 19% were female, 30% Black and 20% Hispanic; 51% with CD4 nadir  $< 200$  cells/ $\mu$ L. At A5322 entry, median CD4 was 616 (449, 821) cells/ $\mu$ L, 95% had HIV-1 RNA  $< 200$  copies/mL, with a median of 7.8 (IQR 4.4, 12.0) years since ART initiation. Frailty criteria were met in 6%; 38% were pre-frail; 56% non-frail. Among frail participants, 90% had exhaustion, 89% low physical activity, 66% weak grip strength, 60% slow walk time, and 27% weight loss. In univariate models, no significant associations were seen between greater frailty and nadir CD4; prior exposure to zidovudine, didanosine or stavudine; time since ART initiation; substance use; or liver disease. Female sex, CD4  $< 350$  cells/ $\mu$ L, CD4/8, pre-ART HIV RNA, Hepatitis C, cardiovascular and renal disease, hypertension, diabetes, and cancer were significantly associated with advancing frailty in univariate but not multivariable analyses; significant covariates in the multivariable model are shown in the table.

**Conclusions:** Among older HIV+ adults, modifiable factors (smoking, low physical activity, obesity) were strongly associated with increased frailty, and provide ideal targets for future interventions. The apparent association between frailty and NNRTI-based initial ART requires further investigation. The lack of association between frailty and current/historical immunosuppression may reflect a highly compliant study population or a survivor effect.

**Association between an increase in frailty\* and demographics, HIV characteristics, ART and comorbidities**

Covariates	Univariate		Multivariable	
	Odds Ratio (95% CI)	P-value <sup>^</sup>	Odds Ratio (95% CI)	P-value
Neurocognitive impairment**	2.52 (1.80, 3.52)	$< 0.001$	2.14 (1.43, 3.19)	$< 0.001$
Age 50-59 (vs 40-49) years	1.87 (1.43, 2.45)	$< 0.001$	1.83 (1.34, 2.52)	$< 0.001$
$\geq 60$ (vs 40-49) years	1.89 (1.32, 2.71)	$< 0.001$	1.47 (0.93, 2.30)	0.06
$\leq$ High school education	2.35 (1.82, 3.02)	$< 0.001$	1.56 (1.13, 2.17)	0.007
Insurance: private/other (vs Medicare/caid)	0.31 (0.24, 0.42)	$< 0.001$	0.57 (0.41, 0.80)	$< 0.001$
None (vs Medicare/caid)	0.58 (0.41, 0.82)	0.002	0.73 (0.48, 1.12)	0.16
Initial ARV: NNRTI-based (vs 2 NRTI/PI)	1.35 (1.1, 1.83)	0.05	1.58 (1.11, 2.26)	0.01
Integrase-based (vs 2 NRTI/PI)	1.26 (0.83, 1.9)	0.28	1.0 (0.60, 1.66)	$> 0.9$
Current/prior smoker	1.44 (1.12, 1.84)	0.004	1.40 (1.03, 1.90)	0.03
Current alcohol use	0.48 (0.37, 0.62)	$< 0.001$	0.6 (0.45, 0.81)	$< 0.001$
Low physical activity <sup>†</sup>	2.1 (1.63, 2.7)	$< 0.001$	1.93 (1.45, 2.59)	$< 0.001$
Obesity	1.54 (1.18, 2.01)	0.001	1.5 (1.08, 2.09)	0.01

\* Increase in frailty from non-frail to pre-frail/frail or non-frail/pre-frail to frail using ordinal logistic regression; \*\* using the ALLRT Neuroscreen (Trailmaking A+B and Digit Symbol) with impairment defined as  $\geq 1$  SD below 0 on  $\geq 2$  normalized scores, or  $\geq 2$  SD below 0 on  $\geq 1$  score; <sup>^</sup> covariates with category-specific p-value  $< 0.10$  from univariate analysis were retained in the final multivariable model; <sup>†</sup> low activity defined as  $< 3$  days/week of moderate to vigorous physical activity; CI, confidence interval; ART, antiretroviral therapy; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; PI, protease inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; obesity, body mass index  $\geq 30$  kg/m<sup>2</sup>

**720 Prevalence of Falls Among Older Women in the Women's Interagency HIV Study**

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**Background:** HIV+ women may be at greater risk for reduced bone density and falls, leading to fractures as they age.

**Methods:** We quantified self-report of any fall,  $\geq 2$  falls, and fear of falling (FOF) in the prior 6 months in 1,412 HIV+ and 660 HIV- women enrolled in the Women's Interagency HIV Study (WIHS). Associations of demographics, behavioral factors, comorbid conditions, and CNS active agents (i.e. antidepressants, antipsychotics, anticonvulsants, sedatives, or muscle relaxants) with odds of any fall and  $\geq 2$  falls (vs. none) were evaluated using logistic regression.

**Results:** HIV+ women were older than HIV- women (median 49 vs 47yr,  $p < 0.0001$ ), and more likely to report neuropathy (21% vs 14%,  $p = 0.0003$ ), but less likely to be obese (47% vs 59%) or use marijuana currently (17% vs 25%). HIV+ women had greater exposure to CNS active agents than did HIV- women: 41% vs 33% used at least 1 agent. At least one fall was reported in 243 HIV+ (18%) and 119 HIV- (18%) women ( $p = 0.87$ ), with  $\geq 2$  falls reported in 113 HIV+ (9%) and 65 HIV- (10%) women ( $p = 0.73$ ). FOF did not differ by HIV status: 21% of women rated their FOF as "a little", 7% "quite a bit" and 10% "very much". Among women with no FOF, only 9% reported any fall; however, among women with "a little", "quite a bit" and "very much" FOF, 28%, 47% and 37% ( $p < 0.0001$ ) reported falls, respectively. Factors associated with any fall in multivariate analysis included age  $\geq 60$  (aOR=1.93, 95% CI: 1.09-3.44 vs age  $< 39$ ), current marijuana use (aOR=1.98, 95% CI: 1.32-2.98) depressive symptoms (aOR=1.55, 95% CI: 1.18-2.03 for CES-D  $\geq 16$  vs  $< 16$ ), impaired memory (aOR=2.18, 95% CI: 1.54-3.07), neuropathy (aOR=1.55, 95% CI: 1.86-4.69), obesity (aOR=1.39, 95% CI: 1.06=1.82), and number of CNS active agents (aOR=2.95, 95% CI: 1.86-4.68 for  $\geq 3$  agents vs 0). Risk factors for multiple falls were similar, including current marijuana use, depressive symptoms, impaired memory, neuropathy, and number of CNS active agents (all  $p < 0.02$ ). HIV infection was associated with neither any fall nor multiple falls in multivariate analyses.

**Conclusions:** In this cross-sectional study of older women, falls were associated with factors affecting cognition and obesity, and were not associated with HIV status.

Longitudinal studies are needed to determine the rate of progression of fall risk by HIV status and whether the clinical consequences of falls differ by HIV status as these women age.

**721 Factors Associated With Limitations in Daily Activity Among Older HIV+ Adults**

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**Background:** Aging HIV+ adults may have a greater burden of geriatric syndromes, including diminished ability with independent activities of daily living (IADLs). Improved understanding of limitations can assist providers in incorporating interventions to prevent or delay future decline.

**Methods:** A5322 follows HIV+ adults  $\geq 40$  years who previously initiated ART within an ACTG clinical trial. IADL function was assessed at entry in 8 categories (housekeeping, money management, cooking, transportation, telephone use, shopping, laundry, medication management); those who needed assistance in  $\geq 1$  category were considered IADL impaired. Associations were examined in logistic regression models; covariates with category-specific p-value  $< 0.10$  were retained in the final multivariable model. Additionally, frequency of IADL impairment by frailty status was examined. Frailty included a 4-m walk, grip strength, and self-reported weight loss, exhaustion and low physical activity; participants meeting criteria in 3-5 categories were considered frail; in 1-2, pre-frail; and in 0, non-frail.

**Results:** Of 1015 HIV+ participants with IADL assessed (19% female, 30% Black, 20% Hispanic), median age was 51 (IQR 46, 56) years; 51% had CD4 nadir  $< 200$  cells/ $\mu$ L. At A5322 entry, median CD4 was 616 (449, 822) cells/ $\mu$ L, 95% had HIV-1 RNA  $< 200$  copies/mL, with a median 7.8 (IQR 4.4, 12.0) years since ART initiation. There were 11% with 1 IADL

impairment; 6% had  $\geq 2$  IADL impairments. The most common impairments involved housekeeping (48%), transportation (36%), shopping (28%), laundry (20%), and cooking (15%). Of the 999 who also had frailty assessed, frail participants were more likely to report IADL impairment (52% had  $\geq 1$  impairment vs. 21% of pre- and 11% of non-frail). In univariate models, no significant associations were seen between IADL impairment and nadir/current CD4, HIV-1 RNA, initial ART regimen, prior exposure to ZDV, D4T, or DDI; years since ART initiation; substance use; obesity or weight change since ART initiation; renal disease; hypertension; or cancer. Neuroimpairment, public insurance (vs private/other), education, and low physical activity were associated with impairment in the final model (Table).

**Conclusions:** In HIV+ older adults, IADL impairment occurs more frequently among those with neuroimpairment or frailty. Modifiable risk factors (smoking, low physical activity) provide targets for interventions to help maintain independent living.

Associations between demographics, HIV characteristics and co-morbidities with IADL impairment ( $\geq 1$  category)

Variables	Univariate		Multivariable	
	Odds Ratio (95% CI)	p-value*	Odds Ratio (95% CI)	P-value
Neuroimpairment*	3.48 (2.37, 5.12)	<0.001	2.18 (1.34, 3.54)	<b>0.001</b>
Female sex	1.7 (1.17, 2.48)	0.005	1.27 (0.78, 2.07)	0.34
Black (vs White, Non-Hispanic)	1.84 (1.24, 2.73)	0.003	0.97 (0.59, 1.6)	0.90
Hispanic (vs White, non-Hispanic)	2.68 (1.79, 4.0)	<0.001	1.23 (0.69, 2.22)	0.48
Age at entry 50-59 yrs (vs 40-49yrs)	1.43 (1.0, 2.03)	0.05	1.30 (0.84, 2.01)	0.23
$\geq 60$ years (vs 40-49 yrs)	1.30 (0.80, 2.11)	0.29	0.82 (0.43, 1.57)	0.55
$\leq$ High school education	3.28 (2.35, 4.57)	<0.001	2.16 (1.38, 3.35)	<b>&lt;0.001</b>
Insurance: None vs Medicare/caid	0.88 (0.59, 1.32)	0.54	0.99 (0.58, 1.68)	0.90
Private/Other vs Medicare/caid	0.22 (0.15, 0.34)	<0.001	0.43 (0.26, 0.70)	<b>&lt;0.001</b>
Entry CD4/CD8 $\leq 0.4$	1.65 (1.04, 2.62)	0.03	1.43 (0.66, 3.09)	0.36
Hepatitis C antibody +	2.14 (1.39, 3.29)	<0.001	1.60 (0.93, 2.76)	0.09
Current/prior smoker	1.67 (1.19, 2.36)	0.003	1.52 (0.98, 2.35)	0.06
Current alcohol use	0.61 (0.44, 0.86)	0.004	0.89 (0.59, 1.35)	0.57
Low physical activity*	2.15 (1.52, 3.05)	<0.001	1.96 (1.30, 2.95)	<b>0.001</b>
Cardiovascular disease	1.95 (1.08, 3.51)	0.03	1.89 (0.93, 3.81)	0.08
Liver disease	4.79 (1.19, 19.33)	0.03	3.58 (0.44, 29.11)	0.23
Diabetes	2.18 (1.45, 3.27)	<0.001	1.55 (0.92, 2.63)	0.10

\*Covariates with a category specific p-value <0.10 from univariate analysis were retained in the final multivariable model. \*using the ACTG Neuroscreen (Trailmaking A+B and Digit Symbol) with impairment defined as  $\geq 1$  SD below 0 on  $\geq 2$  normalized scores, or  $\geq 2$  SD below 0 on  $\geq 1$  score, \*\* <3 days/week of moderate/vigorous physical activity.

722 Fitness Characteristics of United States Air Force Members With HIV Infection

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**Background:** HIV infection has been associated with early cardiovascular disease, reduced muscle mass, and adverse metabolic effects. To better understand the impact of HIV on physical fitness, we evaluated longitudinal fitness characteristics for active duty United States Air Force (USAF) members with and without HIV infection.

**Methods:** Since 2004, USAF members perform a standardized fitness test every 6-12 months with a composite score (CS) comprised of abdominal circumference (AC), push-ups (PU), sit-ups (SU), and 1.5-mile run. Scores in each area are based on decade of age, gender, and amount of repetitions or elapsed time. A CS  $\geq 75$  is required to pass and  $\geq 90$  is defined as excellent. Male USAF members with HIV infection (n=172) and fitness tests between 2004-2014 were compared with male HIV-negative controls (~10 per case; n=1,636) matched by age at military service entry and rank category. Body mass index (BMI) was also analyzed. Fitness tests for cases were divided into two groups for comparison by paired t-tests: before HIV diagnosis (pre-HIV) and after HIV diagnosis (post-HIV). Fitness tests for cases in each category were also compared to tests for controls of the same age. Random effects regression analyses were also performed to compare characteristics for cases and controls.

**Results:** The majority of cases were enlisted members (91.3%) with mean age of 21.3 ( $\pm 3.5$ ) and 28.1 ( $\pm 6.4$ ) years at military service entry and HIV diagnosis, respectively. Cases had lower AC and BMI both Pre- and Post-HIV compared to controls (Table). Pre-HIV tests showed similar CS but lower scores for muscle strength components (SU and PU) compared to controls. Similar results were observed Post-HIV versus controls, however Post-HIV CS was significantly greater. Among cases, Post-HIV values of CS, SU, and PU were significantly greater compared to Pre-HIV values. Panel regression analyses controlling for background characteristics showed that cases had lower BMI, AC and PU scores regardless of Pre- or Post-HIV status, however the odds of an excellent CS were 75.1% higher for Post-HIV cases than for controls.

**Conclusions:** USAF members with HIV exhibit a high level of physical fitness with greater overall CS and excellent scores than HIV-uninfected controls. Although muscle strength scores were lower compared to controls, these components improved Post-HIV. These results suggest that USAF members can effectively maintain muscle mass and cardiovascular performance after HIV diagnosis.

Characteristic	Pre-HIV Cases	Controls	Pre-HIV Cases vs. Controls*	Post-HIV Cases	Controls	Post-HIV Cases vs. Controls*	Pre-HIV vs. Post-HIV Cases*
Fitness tests (n)	574	14,001		1247	16,442		
Composite Score	84.9 (8.4)	85.4 (9.2)	0.468	87.1 (9.1)	85.7 (9.6)	0.050	0.004
Abdominal Circumference (inches)	32.5 (3.3)	33.3 (3.0)	0.004	32.4 (3.2)	33.3 (3.0)	<0.001	0.608
Sit-ups (n)	50.6 (9.2)	52.3 (8.0)	0.037	51.7 (7.8)	52.6 (7.8)	0.116	<0.001
Push-ups (n)	50.4 (11.2)	53.0 (10.5)	<0.001	51.5 (9.7)	53.3 (7.8)	<0.002	0.018
Run time (min)	11:85 (2:09)	11:98 (1:93)	0.323	11:88 (1:69)	12:02 (2:18)	0.336	0.056
Body Mass Index	25.0 (3.4)	26.2 (3.4)	<0.001	25.2 (3.3)	26.3 (3.4)	<0.001	<0.001

\*P-values; Values are number (n) or mean (SD)

723 Frailty in HIV-Infected Patients Is Associated With Increased insulin Resistance

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**Background:** Physical frailty develops earlier in HIV-infected than in uninfected individuals. In the general population, frailty is associated with insulin resistance.

**Methods:** We examine the association between frailty and indices of glucose metabolism in HIV-infected patients on stable antiretroviral therapy (ART). This subgroup analysis utilized baseline data from the Hawaii Aging with HIV-Cardiovascular Study. Frailty was defined as a syndrome meeting 3 or more of 5 phenotypic criteria: weakness, assessed by grip strength; slowness, assessed by the 15 feet walking time; low physical activity; low energy or self-reported exhaustion; and unintentional weight loss. Patients with 1 or 2 criteria were considered Pre-Frail and those with 0 phenotypic traits were considered Non-Frail. Insulin resistance was assessed by Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and the Oral Disposition Index (DIo), a dynamic measure of insulin resistance using a 2 hour oral glucose tolerance test (OGTT). Biomarkers (sE-selectin,

sVCAM-1, sICAM-1, MMP-9, tPA-1, hsCRP, IL-6, IL-8, IL-10, TNF- $\alpha$ , MCP-1, IFN- $\alpha$ ) were assessed by multiplex Luminex assay. Analysis of covariance was used to determine group differences.

**Results:** 79 HIV-infected subjects (45 Non-Frail, 28 Pre-Frail, 6 Frail): 90% men, median age of 51 years, median CD4 count of 479.0 cells/mm<sup>3</sup>, 84% with plasma HIV RNA < 50 copies/mL. The three groups had similar clinical and laboratory assessments except for body mass index (BMI). Median BMI (Q1,Q3) for the Non-Frail, Pre-Frail, and Frail groups was 26.1 (24.5,27.5), 25.8 (24.1,27.2), and 29.8 (26.0,37.5) kg/m<sup>2</sup>, respectively, with a difference noted between Pre-Frail and Frail groups. There were no differences in biomarkers except for IL-6. Median IL-6 for the Non-Frail, Pre-Frail, and Frail groups was 1.26 (1.02,1.68), 2.20 (0.90,3.61), 2.54 (2.46,5.75) pg/mL, respectively, with differences between Non-Frail and Frail groups. Glucose measures at 90 and 120 min of the OGTT were significantly higher in the Frail than in the Non-Frail group. HOMA-IR and Dlo were higher in the Frail group compared to the Non-Frail and Pre-Frail groups even after adjustment for age and BMI. IL-6 correlated with HOMA-IR ( $\beta=0.26$ ,  $p=0.03$ ) but not Dlo.

**Conclusions:** Frailty in HIV-infected subjects was associated with increased insulin resistance. IL-6 was elevated in frailty and correlated with HOMA-IR. The roles of inflammatory processes and insulin resistance in frailty require further study.

	Non-Frail	Pre-Frail	Frail
Fasting glucose, mg/dL	89 (81, 93)	89.5 (81, 97)	90.5 (88.3, 96.5)
30 min glucose, mg/dL	141 (124, 154)	156 (130, 185)	154 (150, 160)
60 min glucose, mg/dL	130 (102, 155)	158 (138, 183) *	171 (140, 193)
90 min glucose, mg/dL	166 (87.8, 129)	124 (81.5, 147)	138 (123, 203) *
120 min glucose, mg/dL	92 (75, 112)	107 (84, 124)	134 (118, 144) *
Fasting insulin, mg/dL	7.1 (4.3, 10.3)	7.3 (4.78, 9.35)	18.9 (10.7, 26.0) *
Insulin sensitivity	0.0203 (0.0140, 0.0335)	0.0197 (0.0154, 0.0302)	0.0077 (0.00567, 0.0150) **
HOMA-IR	1.46 (0.88, 2.26)	1.56 (1.08, 2.04)	4.21 (2.36, 5.65) **
Dlo	2.38 (1.71, 3.90)	1.91 (1.41, 3.48)	1.25 (1.08, 1.59) *

\*  $p < 0.05$  with Non-Frail as the referent variable  
 \*\*  $p < 0.05$  with Pre-Frail as the referent variable  
 HOMA-IR was calculated using the formula: [fasting glucose (mg/dL.) X fasting insulin (microIU)] /405  
 Oral Disposition Index (Dlo) is a dynamic measure of insulin resistance, is calculated as the ratio of the change in insulin to the change in glucose from 0 to 30 minutes divided by the fasting insulin level ( $(\Delta I_{0-30} / \Delta G_{0-30}) \times (1/\text{fasting insulin})$ ). A low Dlo is associated with an increased risk of diabetes mellitus.

## 724 HIV gp120 in the Lungs of HAART-Treated Individuals Impairs Pulmonary Immunity

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**Background:** Antiretroviral therapy (ART) has improved overall survival but HIV+ individuals remain at increased susceptibility to chronic lung disease and pneumonia. HIV associated immune activation and oxidative stress persist despite ART and HIV proteins have been implicated in this process. We hypothesised that gp120 plays a role in dysregulating lung immunity in HIV+ individuals despite ART.

**Methods:** We recruited 14 healthy, viral hepatitis-uninfected, ART treated HIV+ non-smokers (9 male, 9 white, mean age 42, mean 372 weeks ART) and 10 matched HIV- controls for bronchoalveolar lavage (BAL) to obtain lung mononuclear cells and fluid (BALF). Cells were analysed by flow cytometry and gp120 measured by ELISA. Alveolar macrophages (AM) and 14-day human monocyte derived macrophages (hMDM) exposed to 10-100ng/mL of gp120 were challenged with pneumococci, and responses characterised by microscopy, flow cytometry and viable bacterial counts. Data were compared with t test and if non-parametric Mann-Whitney or Wilcoxon test and considered significant if  $p < 0.05$ .

**Results:** gp120 was detected by ELISA in the BALF of 45% of HIV+ at concentrations 13 to 78 ng/mL. Of the HIV+ individuals, those with detectable BALF gp120 had significantly lower plasma CD4 counts (mean  $470 \pm SEM43$  vs  $756 \pm 80$  cells/mL,  $p=0.0087$ ). HIV+ individuals had a significant BAL lymphocytosis compared with controls ( $12.4 \pm 1.9$  vs  $7.6 \pm 1.1$  %,  $p=0.043$ ), with increased BAL CD8+ T cells ( $45.0 \pm 4.0$  vs  $22.3 \pm 4.0$  %,  $p=0.0006$ ), and reduced BAL CD4:CD8 ratios ( $3.79 \pm 0.76$  vs  $1.16 \pm 0.15$ ,  $p=0.0019$ ). HIV+ AM demonstrated impaired intracellular killing of pneumococci ( $1.3 \pm 0.4$  vs  $0.5 \pm 0.5$  log CFU/mL), a finding reproduced in hMDM treated with gp120 ( $1.2 \pm 0.3$  vs  $0.7 \pm 0.3$  log CFU/mL,  $p=0.019$ ), and associated with reduced apoptosis ( $18.1 \pm 4.8$  vs  $33.5 \pm 7.0$  %,  $p=0.039$ ). However, apoptosis-associated killing was not reduced when hMDM were co-cultured with activated autologous CD8 T cells. gp120 also induced the generation of mitochondrial reactive oxygen species in hMDM (mROS,  $1.86 \pm 0.5$  fold change MFI,  $p=0.016$ ) in hMDM but blunted the induction of mROS with pneumococci ( $p=0.047$ ).

**Conclusions:** The immune environment of the lung fails to normalise during ART with persistence of gp120 and a CD8 lymphocytosis. gp120 causes macrophage oxidative stress and impairs apoptosis-associated killing of pneumococci. gp120 appears to play a role in HIV associated lung disease despite ART.

## 725 The Host Response to the HIV Airway Epithelial Cell Microbiome

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**Background:** Chronic Obstructive Pulmonary Disease (COPD) is an important comorbidity in patients living with HIV. Previous bacterial microbiome studies have shown increased abundance of specific bacteria like *Tropheryma whippelii* in bronchoalveolar lavage samples, but few studies have evaluated the microbiome of airway epithelial cells. Moreover, few studies have determined whether the microbiome can elicit a specific host response.

**Methods:** Two bronchial brush samples were obtained during bronchoscopy from 21 HIV-infected patients. One was used for bacterial microbiome analysis using the Illumina MiSeq platform as well as DNA methylation analysis using the Illumina Infinium 450K Human Methylation array. The other brush was used to evaluate gene expression patterns of the host using the Affymetrix Human Gene ST 2.0 array. Microbiome composition, Shannon diversity, evenness, and operational taxonomic unit (OTU) richness were assessed comparing HIV-infected patients with and without COPD by spirometry, and with and without emphysema by computed tomographic imaging. Weighted gene co-expression network analysis was used to determine the relationship between the bacterial microbiome and host DNA methylation and gene expression patterns.

**Results:** There was no difference in Shannon diversity, evenness, or bacterial community composition between HIV patients with and without COPD or HIV patient with and without emphysema. However, OTU4, OTU15, and OTU38 were able to discriminate between subjects with and without COPD. OTU4 and OTU30 were also able to discriminate between those with and without severe emphysema. 14 gene expression modules correlated significantly to at least one measure of the bacterial microbiome (Table) including modules involved in cilia function, immune response, and antigen presentation. 10 methylation modules correlated significantly to at least one measure of the bacterial microbiome (Table). The turquoise module (representing lung and respiratory tubule development) had the most significant correlations with the microbiome, being negatively correlated with Actinobacteria, Firmicutes and overall OTU richness, and positively correlated with Proteobacteria.

**Conclusions:** Within HIV, certain OTUs are able to distinguish between COPD and non-COPD airway epithelial cells, as well as between those with and without severe emphysema. Microbiome features are additionally related to cilia function, immune response, and lung and respiratory tubule development.

Module	Number of Genes or CpG Sites	Analysis	Pathways Identified	False Discovery Rate-Adjusted P-value
Tan	201	Gene Expression	Lysosome, Immune Response, Plasma Membrane	5.0x10-4
Red	554	Gene Expression	Immune Response, Defense Response, Inflammatory Response, Response to Wounding	1.0x10-12
Midnight Blue	82	Gene Expression	Magnesium Ion Binding	0.05
Green	791	Gene Expression	Cilia	2x10-4
Turquoise	6050	Gene Expression	Intracellular Organelle, Membrane-Enclosed Lumen	2x10-7
Dark Green	43	Gene Expression	None Identified	N/A
Black	452	Gene Expression	Cell to Cell Signaling, Cell Membrane	1.0x10-5
Magenta	365	Gene Expression	Oxidation/Reduction, Microsomes	2.0x10-2
Pink	427	Gene Expression	Immune Response, Immunoglobulin, Antigen Presentation	1.0x10-3
Brown	1274	Gene Expression	Glycoprotein, Plasma Membrane, Immune Response	1.0x10-6
Blue	5675	Gene Expression	Nucleus, Transcription Regulation, Nuclear Lumen	2x10-4
Grey	5861	Gene Expression	Olfactory Transduction	4.9x10-42
Green Yellow	245	Gene Expression	Immunoglobulin, Antigen Processing and Presentation	1x10-4
Light Green	63	Gene Expression	None Identified	N/A
Magenta	2202	Methylation	Blood Vessel Development, Embryonic Development	1x10-2
Orange	266	Methylation	Membrane Fraction, Plasma Membrane, Cell Fraction	0.1
White	176	Methylation	None Identified	0.1
Cyan	1307	Methylation	Regulation of Metabolic Process, Skeletal Development, Cell Migration	1x10-2
Saddlebrown	163	Methylation	None Identified	0.1
Salmon	1403	Methylation	ATP-binding	0.1
Black	3114	Methylation	Pleckstrin Homology, Plasma Membrane, Cellular Morphogenesis	1x10-2
Turquoise	4575	Methylation	Lung Development, Respiratory Tube Development	1.0x10-2
Blue	26951	Methylation	Transcription Regulation, Embryonic Morphogenesis	1x10-2
Brown	20139	Methylation	Plasma Membrane, Cell Adhesion, Glycoprotein	1x10-8

**726 Immunological Signaling During HSV-2 and CMV Vaginal Reactivation at ART Initiation**

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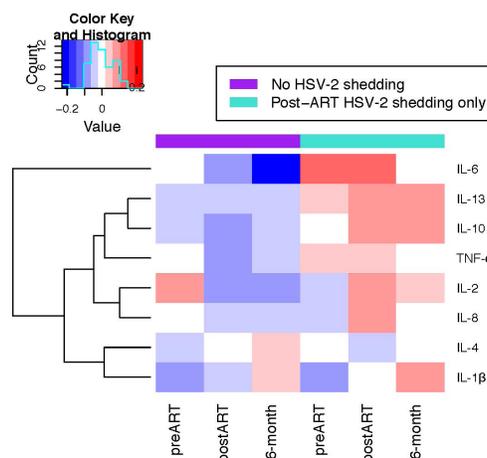
**Background:** Vaginal shedding of herpes simplex virus type 2 (HSV-2) and cytomegalovirus (CMV) was increased in HIV-infected women within the first three to four months after antiretroviral therapy (ART) initiation. The role of vaginal pro-inflammatory cytokine expression on herpetic reactivation was examined in these same women.

**Methods:** HIV and HSV-2 co-infected individuals with a CD4 cell count between 300 and 400 cells/μl were enrolled in a double-blind, randomized placebo-controlled trial of HSV-2 suppression with acyclovir. Participants were randomly assigned to receive placebo or 400mg acyclovir twice daily for 24 months. Self-collected vaginal swab samples from women previously tested for HSV-2 and CMV shedding taken one month prior (-1), at time of ART initiation (0), and months 1, 2, 3, 4 & 6 after ART initiation were screened for presence of pro-inflammatory cytokines IL-1β, -2, -4, -6, -8, -10, -12p70, -13, TNF-α, and IFN-γ (Meso Scale Discovery Inc, Rockville, MD). Cytokines were dichotomized by median value and associations with HSV-2 and CMV shedding were evaluated after adjusting for month of sample collection and ART status. Relationships between cytokine levels and HSV-2 or CMV shedding over time were modeled using Generalized Estimating Equations (GEE). A Poisson model with a log link and robust standard errors was used. Relationships between cytokine levels and shedding were visualized with heat maps using geometric means of cytokine levels.

**Results:** The median values for vaginal IL-1β, IL-2, IL-4, IL-10 and TNF-α were 0 pg/mL; the other medians included IL-6 median=2.95 pg/mL; IL-13 median=9.9 pg/mL; IL-8 median=1,137 pg/mL. Presence of HSV-2 DNA shedding was significantly associated with higher IL-6 (p=0.003) and TNF-α (p=0.010), whereas CMV DNA shedding was associated with higher IL-1β (p=0.015), IL-2 (p=0.007), and IL-6 (p=0.006). For HSV-2 and CMV, women were divided according to shedding status pre- (-1 and 0 months) and post-ART (months 1-4). Women who shed HSV-2 post-ART appeared to have a pattern of higher pro-inflammatory cytokine levels (IL-2, -6, -8 -13, -10, and TNF- α) after HAART initiation (Figure).

**Figure:** Heat maps of cytokine levels for women who did not shed HSV-2 compared to women who did shed post-ART only.

**Conclusions:** The association of HSV-2 and CMV shedding with higher IL-6 levels suggests that herpetic reactivation in the genital tract of African women may be an immune restoration inflammatory syndrome-like event.



**727 Longitudinal Viral Dynamics in Semen of HIV+ Men Before and After Early ART**

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**Background:** Multiple viruses co-infect the male genital tract and most likely influence each other's replication. The longitudinal replication dynamics of HIV and human herpesviruses (HHV) before and after starting early antiretroviral therapy (ART) have not been systematically described.

**Methods:** This study included 423 seminal samples from 196 HIV+ men from the San Diego Primary Infection Cohort. Levels of seminal HIV RNA and HHV DNA (cytomegalovirus [CMV], Epstein-Barr virus [EBV], herpes simplex virus 1 & 2 [HSV], HHV-6, -7 and -8) were measured by RT-PCR. Longitudinal shedding rates were determined by survival analysis. Predictors of seminal HIV shedding were determined using backwards selection in a multivariable GEE model.

**Results:** The first time-point was collected within a median estimated duration of infection (EDI) of 90 days (IQR: 75-166), and 99 individuals had additional time-points over a median follow-up of 119 days (IQR: 36-243). Overall 15% of time-points were sampled on ART and with suppressed plasma HIV RNA. Within the first 48 weeks of EDI, 94% of men had detectable seminal DNA from at least one HHV. Most commonly CMV (85%) and EBV (81%), followed by HHV-7 (35%), HHV-6 (29%), HHV-8 (26%) and HSV (23%). Overall, shedding of seminal HHV was not affected by CD4, HIV RNA, EDI and ART status but did decline with older age ( $p < 0.05$ ). Among shedders with longitudinal sampling ( $\geq 2$  time-points), intermittent shedding patterns (changing shedding status) was less likely for CMV (17%) and EBV (36%) than for HHV-7 (47%), HHV-8 (50%), HHV-6 (60%) and HSV (67%). Seminal HIV RNA was detectable in 97% of men over the first 48 weeks of EDI and was significantly lower when plasma HIV RNA was undetectable (78% vs 33%,  $p < 0.05$ ). Presence of CMV, EBV and HSV (but not CD4, CD8, age and EDI) were independent predictors of seminal HIV shedding after adjusting for plasma HIV and longitudinal measurements. Odds ratios (OR) for associations with seminal HIV shedding were 9.0 (95% CI: 1.1-74.5) for HSV, 1.9 (1.0-3.5) for CMV, 1.9 (1.0-3.7) for EBV, and 2.2 (1.7-2.9) for each increment of  $\log_{10}$  viral load in plasma.

**Conclusions:** Seminal HHV replication was prevalent in our HIV primary infection cohort and was not affected by clinical or demographic parameters with exception of age. Persistent CMV and EBV shedding was common and significantly associated with seminal HIV shedding. HSV shedding was less frequent and mostly intermittent in our cohort, but its association with seminal HIV shedding was the strongest.

## 728 HSV-2 Acquisition Among HIV-1 Infected Adults With Tenofovir-Based ART in ACTG 5175

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**Background:** HSV-2 infection is highly prevalent (50-90%) in HIV-infected persons and associated with increased HIV viral load and greater risk of transmission and disease progression. Tenofovir disoproxil fumarate (TDF) has *in vitro* efficacy against HSV-2 and reduced HSV-2 acquisition in HIV-1 uninfected persons on PrEP. A majority of HIV-infected persons are on TDF-containing antiretroviral treatment (ART); however, the efficacy of TDF to reduce HSV-2 acquisition in HIV-infected persons is unknown.

**Methods:** ACTG 5175 (PEARLS) was an open label randomized trial, which enrolled 1571 ARV naïve HIV-infected persons from India, Brazil, Malawi, South Africa, USA, Peru, Zimbabwe, Haiti, and Thailand from 2005-07 and followed through 2010. Participants were randomized to receive a TDF-containing regimen (TDF, emtricitabine & efavirenz) or a non-TDF regimen (zidovudine, lamivudine, & efavirenz or didanosine, emtricitabine, & atazanavir). We analyzed baseline and exit visit HSV-2 serostatus by Focus HSV-2 EIA with HSV Western blot to confirm indeterminate EIAs (0.9-3.4). An intent to treat analysis was conducted.

**Results:** Of 1567 HIV-infected persons who initiated ART, 53% were male and 51% were <35 years of age; 525 were randomized to an ART regimen with TDF and 1042 to a regimen without TDF. 68 HSV-2 seroconversions occurred: 24 in persons randomized to the TDF regimen (6.4 HSV-2 incidence/100 person-years, p-yrs) and 44 were in persons randomized to a non-TDF regimen (6.6 HSV-2 incidence/100 p-yrs) with a hazard ratio (HR) of 0.89 (95% CI 0.55-1.44;  $p = 0.63$ ). Of 374 p-yrs in the TDF-containing arm, 9 p-yrs were in 27 persons who switched to a non-TDF regimen, and of 663 p-yrs among those randomized to a non-TDF regimen, 161 p-yrs were in 101 persons who switched to the TDF-containing regimen. The unadjusted HR among those who never switched was 1.34 (95% CI 0.7-2.58;  $p = 0.38$ ).

**Conclusions:** In this international, multi-site open label trial of 3 ART regimens, annual HSV-2 incidence was high (average 6.5/100 p-yrs across arms) among HSV-2 uninfected, HIV-infected persons. There was no protective benefit of TDF against HSV-2 acquisition in the randomized comparison, although the high rate of switching to a TDF regimen among those randomized to a non-TDF regimen reduced the ability to detect an effect. Given that our findings conflict with the reduction in HSV-2 acquisition in HIV-uninfected persons on TDF PrEP, further research on whether TDF can prevent HSV-2 acquisition in HIV-infected persons is needed.

## 729 CMV Status Is Associated With CD4/CD8 Restoration in Primary HIV-Infected Patients

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**Background:** The objective was to identify factors associated with the normalization of the CD4/CD8 ratio ( $\geq 1$ ) in patients with a history of primary HIV infection (PHI) and long-term combined antiretroviral therapy (cART).

**Methods:** Cross-sectional study conducted in Lyon, France in HIV patients with a known history of PHI and a minimum of 2 years of effective cART ( $< 200$  copies/ml) without interruption or virological failure. Baseline factors at the time of PHI, type and duration of first and total cART, CMV status, and level of activation markers on CD4 and CD8 cells (CD38 and HLA-DR) at the time of the study were assessed. Patients with hepatitis C or B virus co-infection or with a history of neoplasia or immunosuppressive therapy after the date of PHI were excluded.

**Results:** A total of 83 patients (87% males, median age 37 years [range 18-63]), 85% with a positive CMV serology) were studied. cART was initiated after a median of 10.2 months [0-198] after PHI. Twenty-nine (35%) began cART early within three months after diagnosis of PHI. Before cART, median CD4 nadir was 304 cells/ $\mu$ l [18-642] and viral load 212643 copies/ml [64-10<sup>7</sup>]. The first cART included either a combination of nucleosides with protease inhibitor in 73% or a non-nucleoside analogue or three nucleosides in 27% of cases, respectively, with a similar median duration (13 months). Overall, patients had cART during a median of 7.3 years [2-18.9] with a median of three different lines of cART. Sixty-seven (81%) had more than 500 CD4/ $\mu$ l and 68% achieved a CD4/CD8 ratio  $\geq 1$  at the time of the study. A CD4/CD8 ratio  $\geq 1$  was significantly associated with a lower percentage of HLA-DR+ on CD4 and CD8 cells (9.7 vs 17.6%,  $p < 0.0001$  and 29.9 vs 39.7%,  $p = 0.001$ , respectively). Conversely, age, gender, early initiation of active cART ( $< 3$  months) and type of first cART, were not associated with better CD4/CD8 restoration. The seronegative CMV status was also significantly associated with a higher CD4/CD8 ratio (1.72 vs 1.11;  $p = 0.009$ ) and lower percentage of HLA-DR+ ( $p < 0.001$ ) and CD38+HLA-DR+ ( $p = 0.020$ ) on CD8 cells.

**Conclusions:** In patients with a history of PHI and long-term active cART, a seronegative CMV status was strongly associated with a lower residual immune activation and a better restoration of the CD4/CD8 ratio.

## 730 CD4+ T Cell Dysfunction During Early HIV Infection Might Trigger CMV Shedding

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**Background:** Asymptomatic seminal cytomegalovirus (CMV) replication among ART-treated individuals is associated with increased CD4+ T-cell activation, proliferation and exhaustion as well as higher levels of HIV transcription and persistence. The interplay between persistent CMV replication and antigen-specific immune response (CMV and HIV) during early HIV-infection is unknown.

**Methods:** Paired seminal and blood samples from 28 ART-naïve early HIV-infected CMV-seropositive men who have sex with men (15 CMV shedders and 13 non shedders) were evaluated. Levels of seminal CMV DNA and HIV RNA were measured by RT-PCR, and expression of intracellular interferon (IFN)- $\gamma$  was measured by flow cytometry from peripheral blood mononuclear cells (PBMC) following overnight stimulation with whole CMV and HIV lysate. Levels of programmed death receptor-1 (PD-1) were measured on unstimulated PBMCs. Associations between immunological markers and asymptomatic CMV and HIV replication were determined using Mann-Whitney U tests.

**Results:** Comparison of CMV shedders and non shedders (table 1) of similar age, CD4 count, plasma HIV RNA, CMV IgG titers, and estimated duration of HIV infection showed that individuals shedding seminal CMV had significantly higher HIV RNA levels in semen ( $p < 0.001$ ). The presence of seminal CMV DNA was associated with a decreased CMV-specific response (IFN- $\gamma$  production) in CD4<sup>+</sup> T cells ( $p = 0.088$ ) in peripheral blood but no difference in CD8<sup>+</sup> T-cell IFN- $\gamma$  production. Interestingly, asymptomatic CMV shedding was also associated with a significantly poorer HIV-specific CD8<sup>+</sup> T-cell response ( $p = 0.013$ ). CMV shedders also had significantly higher PD-1 expression on CD8<sup>+</sup> T cells ( $p = 0.011$ ) but no difference in PD-1 expression on CD4<sup>+</sup> T cells.

**Conclusions:** A decreased CMV-specific CD4<sup>+</sup> T-cell response during primary HIV infection is associated with increased frequency of seminal CMV shedding, which in turn may drive CD8<sup>+</sup> T-cell exhaustion and lead to an impaired HIV-specific immune response. These observations could explain the connections between CMV replication and higher levels of seminal HIV shedding, as well as increased cell-associated HIV RNA and proviral HIV DNA levels in blood.

**Table 1: Patient Characteristics and PBMC Profiles**

Total (n=28)	CMV Non-Shedders (n=13)	CMV Shedders (n=15)	p-value <sup>1</sup>
Age (years), mean [CI]	38.54 [33.50 – 43.58]	34.93 [30.44 – 39.42]	0.30
CD4 Count (cells/ $\mu$ L), mean [CI]	593 [423 – 763]	618.2 [428.4 – 808]	0.88
Blood HIV RNA level (copies/mL)	2.32x10 <sup>6</sup> [-2.53x10 <sup>6</sup> – 7.67 x10 <sup>6</sup> ]	3.43 x10 <sup>5</sup> [-1.95 x10 <sup>5</sup> – 8.81 x10 <sup>5</sup> ]	0.61
CMV IgG Titer (IU/mL), mean [CI]	23.81 [17.62 – 29.79]	24.51 [16.16 – 32.86]	0.72
EDI (days), mean [CI]	148.9 [53.42 – 244.4]	205.9 [-3.34 – 415.2]	0.85
Seminal CMV level (copies/mL), mean [CI]	0	4.05x10 <sup>5</sup> [-1.19 x10 <sup>5</sup> – 9.29 x10 <sup>5</sup> ]	<b>&lt;0.0001</b>
Seminal HIV level (copies/mL), mean [CI]	168.4 [-10.58 – 347.3]	57,591 [-51,264 – 166,445]	<b>0.0009</b>
CMV-specific (IFN $\gamma$ ) CD4 <sup>+</sup> T cells <sup>2</sup>	1.007 [0.28 – 1.73]	0.47 [0.12 – 0.81]	<b>0.088</b>
HIV-specific (IFN $\gamma$ ) CD4 <sup>+</sup> T cells <sup>2</sup>	0.084 [-0.014 – 0.18]	0.02 [-0.0077 – 0.050]	0.300
CMV-specific (IFN $\gamma$ ) CD8 <sup>+</sup> T cells <sup>2</sup>	0.15 [-0.011 – 0.31]	0.33 [0.032 – 0.63]	0.36
HIV-specific (IFN $\gamma$ ) CD8 <sup>+</sup> T cells <sup>2</sup>	0.14 [0.018 – 0.27]	0.010 [-0.0096 – 0.030]	<b>0.013</b>
%PD-1 on CD4 <sup>+</sup> T cells <sup>3</sup>	0.29 [0.18 – 0.40]	0.41 [0.25 – 0.58]	0.25
%PD-1 on CD8 <sup>+</sup> T cells <sup>3</sup>	0.21 [-0.12 – 0.54]	0.19 [0.12 – 0.26]	<b>0.011</b>

Confidence Interval (CI); International units (IU); Estimated Date of Infection (EDI); Interferon gamma (IFN $\gamma$ ); Programmed Death Receptor (PD-1); p-value determined by the Mann-Whitney U test; <sup>2</sup>IFN $\gamma$  production normalized to total CD4<sup>+</sup> or CD8<sup>+</sup> T cells and background subtracted; <sup>3</sup>PD-1 expression normalized to total CD4<sup>+</sup> or CD8<sup>+</sup> T cells

### 731 High Anti-CMV IgG Levels Predict HIV Disease Progression

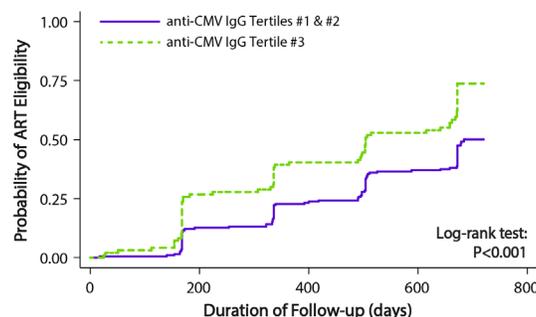
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**Background:** The relationship between the humoral immune response to cytomegalovirus (CMV) and HIV pathogenesis is not well characterized in sub-Saharan Africa. In this secondary analysis of a randomized trial in Rakai, Uganda, we examined correlates of anti-CMV IgG levels and assessed their prognostic value on HIV disease progression and inflammation.

**Methods:** HIV-1/HSV-2 co-infected Ugandan women (N=300) with CD4 counts between 300–400 cells/ $\mu$ L were enrolled in a double-blind, randomized, placebo-controlled trial to examine the effect of acyclovir use on HIV disease progression. The primary outcome was ART eligibility (CD4 count  $\leq$ 250 cells/ $\mu$ L, a WHO Stage IV condition, non-traumatic death, or ART initiation for any reason). Anti-CMV IgG, C-reactive protein (CRP), and soluble CD14 (sCD14) levels were measured on serum samples at baseline, year 1 and year 2 (n=812 visits). Anti-CMV IgG levels were categorized into tertiles, and then dichotomized to compare the 3<sup>rd</sup> tertile to the 1<sup>st</sup> and 2<sup>nd</sup> tertiles in combination. Cox proportional hazard regression models were used to evaluate the predictive value of baseline anti-CMV IgG levels on ART eligibility. Poisson regression with GEE was used to calculate prevalence risk ratios (PRR) to indicate associations with high anti-CMV IgG levels. Correlations were assessed using Spearman's rank-order test.

**Results:** Compared to lower levels of anti-CMV IgG at baseline, women in the 3<sup>rd</sup> tertile had a higher probability of ART eligibility (29.7/100py vs. 53.4/100py,  $P < 0.001$ ). The hazard of reaching ART eligibility was greater for the 3<sup>rd</sup> tertile at baseline independent of study arm, age, body mass index, CRP, sCD14, CD4 count, and HIV viral load (aHR=1.51, 95%CI=1.08-2.10;  $P < 0.016$ ). In a multivariate analysis censored for ART initiation, high anti-CMV IgG levels were associated with high CRP, high HIV viral load, and low CD4 counts ( $P < 0.05$ ). Among 72 women who initiated ART during the study period, anti-CMV IgG levels were significantly higher post-ART initiation (PRR=1.82, 95%CI=1.33-2.49,  $P < 0.001$ ; n=113 visits) than pre-ART (n=95 visits). Log<sub>10</sub> anti-CMV IgG levels correlated with log<sub>10</sub> CRP levels ( $\rho = 0.21$ ,  $P = 0.05$ ) and with log<sub>10</sub> sCD14 levels ( $\rho = 0.21$ ,  $P = 0.045$ ) in fully suppressed women (n=88 visits, N=65).

**Conclusions:** High anti-CMV IgG levels were predictive of HIV disease progression and were associated with increased systemic inflammation. Determinants of elevated anti-CMV IgG levels post-ART initiation require further investigation.



**Figure 1. Kaplan-Meier plot for ART eligibility by baseline anti-CMV IgG levels.**

## 732 CX3CR1+ CD8 T Cells Are Promoted by CMV Coinfection in Treated HIV Infection

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**Background:** Activated CD8 T cells are prominent within atheromas. Their expansion is a hallmark of antiretroviral therapy (ART)-treated HIV infection and is linked to cardiovascular disease risk. We recently showed that co-infection with cytomegalovirus (CMV) is associated with heightened inflammation and is necessary for CD8 T cell expansion in ART-treated HIV-infection. Here we examined the influence of CMV co-infection on the phenotype and function of expanded CD8 T cells in ART-treated HIV infection.

**Methods:** PBMCs were harvested from CMV-seropositive (n=15) or CMV-seronegative (n=10) HIV-infected persons. The activation status, function, and expression of the transcription factors T-bet and Eomes in distinct CD8 T cell maturation subsets were compared by flow cytometry. Memory CD8 T cells subsets were sorted from PBMCs and measured for their ability to produce IFN $\gamma$  after T cell receptor-driven stimulation with anti-CD3/anti-CD28.

**Results:** We identify a population of circulating CCR7<sup>lo</sup> CD8 T cells that express the endothelial-homing receptor for fractalkine (CX3CR1), the thrombin receptor (PAR-1), and the platelet-binding receptor (PSGL-1). The percentage of CD8 T cells expressing CX3CR1 is negatively correlated with the CD4/CD8 ratio ( $\rho=-0.5261$ ,  $P=0.003$ ). Frequencies of circulating CX3CR1+ CD8 T cells are significantly enriched in CMV-seropositive ART-treated HIV-infected subjects (40% of all memory CD8 T cells) when compared to frequencies of CX3CR1+ CD8 T cells in ART-treated HIV+ CMV-seronegative subjects (24%,  $P=0.004$ ). A greater proportion of CCR7<sup>lo</sup>CX3CR1+ CD8 T cells from CMV-seropositive HIV+ donors are CD28-negative (82% vs 47%,  $P=0.005$ ), CD57 positive (47% vs 31%,  $P=0.010$ ), and have a T-bet<sup>hi</sup>Eomes<sup>lo</sup> phenotype (45% vs. 22%,  $P=0.003$ ) compared to findings among CMV-seronegative HIV+ persons. IFN $\gamma$  production following TCR stimulation was concentrated within the CX3CR1+CD57+ population.

**Conclusions:** Here we identify a population of circulating cytokine-producing memory that are negatively correlated with CD4/CD8 ratio, that express endothelium-homing receptors and may contribute to cardiovascular disease risk in ART-treated HIV-infection. CX3CR1+ CD8 T cells are detectable in ART-treated, HIV-positive CMV-seronegative donors but are relatively and absolutely expanded in HIV/CMV co-infection, suggesting a role for inflammation in their development.

## 733 Baseline Sputum and Polyfunctional TB-Specific CD4+ T Cells in HIV-TB Coinfection

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**Background:** Assessing polyfunctionality of T cell function has become increasingly common in the TB field. What this means and how this correlates with disease states and diagnosis needs further exploration. Here we compare CD4+ T cell cytokine profiles and polyfunctionality in response to *M. tuberculosis* (MTB) antigen in relation to diagnostic tests at initial presentation in HIV+ subjects.

**Methods:** 59 HIV+ persons age 14-60 (median 32) with CD4 counts >200 (median 544) were recruited in Kampala, Uganda. Subjects had PTB based on sputum smear and/or culture positivity. Baseline diagnostic sputum smear were graded 0-3 by WHO/ IUATLD categories. All subjects had chest x-ray (CXR). None had prior TB disease or were on ARV. Multicolor flow analysis was performed on PBMC stimulated with PPD, CMV antigen or SEB and frequencies of IL-2, IFN-gamma, TNF-alpha, and MIP1-alpha secreting CD4+ T cells were determined.

**Results:** Subjects with baseline diagnostic sputum smear in lowest category of 0 compared to those with 1 or higher had reduced IFN, IL-2, or MIP after *in vitro* PPD stimulation ( $p\leq 0.03$ ) but were similar to CMV and SEB stimulation. There is increased polyfunctionality to PPD ( $p=0.006$ ) in those subjects with the higher baseline sputum smear grades. This relationship did not hold comparing polyfunctionality and disease extent based on CXR. There was no difference in polyfunctionality between the sputum smear groups with CMV or SEB stimulation. The extent of disease on CXR was at the lowest level in subjects with the lowest baseline sputum smear grade however there was no correlation between those with the lowest sputum smear grade and CD4 count or HIV viral load.

**Conclusions:** These data suggest that subjects with the lowest amount of MTB in their sputum have both quantitatively and qualitatively different cytokine responses to PPD than those with greater levels of MTB burden. They also suggest that these differences are TB specific. There have been some observations in other systems that greater antigen loads provoke a more polyfunctional T cell response. Our data suggest that lower screening sputum smears have a lower load of MTB and generate a less polyfunctional T cells. It is not clear that the higher polyfunctionality associated with higher smear grade has an increased protective benefit since higher smear grades were associated with a greater extent of disease on CXR.

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## 734 Neutrophil-Derived MMP-8 Activity Is Associated With Immunopathology in TB-IRIS

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**Background:** Tuberculosis (TB) is the leading cause of death in HIV-infected patients. Anti-retroviral therapy (ART) reduces mortality but may be complicated by the paradoxical TB-immune reconstitution inflammatory syndrome (TB-IRIS). Diagnostic and targeted treatment strategies for TB-IRIS are lacking. We investigated the hypothesis that matrix metalloproteinases (MMPs), previously implicated in TB tissue destruction, associate with immunopathology in TB-IRIS.

**Methods:** In a longitudinal study conducted at a HIV-TB clinic in Cape Town, HIV-infected ART-naïve patients, with CD4 counts <200 cells/mm<sup>3</sup>, presenting with pulmonary TB, were followed from TB treatment initiation (TBO), for the first 3 months of ART. Serial induced sputum and plasma were collected. MMP, cytokine and matrix degradation product procollagen III N-terminal propeptide (PIIINP) concentrations were quantified (pg/ml) at TBO, ART initiation (ARV0), week 2 (ARV2) and 4 (ARV4) of ART. Chest radiographs (CXR) were scored for disease extent. Patients who developed TB-IRIS were compared with those who did not (non-IRIS controls) using Mann-Whitney U test. Correlations were assessed using Spearman's test.

**Results:** 49 HIV-infected TB patients were recruited. 29 (59%) developed TB-IRIS, after a median of 14 days of ART. Median plasma PIIINP was higher in TB-IRIS patients than non-IRIS controls, at TBO (43600 vs 21651 pg/ml,  $p=0.036$ ), ARV2 (6104 vs 1095 pg/ml,  $p=0.043$ ) and ARV4 (46763 vs 22424 pg/ml,  $p=0.001$ ). Plasma MMPs were elevated in TB-IRIS patients, most significantly MMP-8. Median plasma MMP-8 was higher in TB-IRIS at all timepoints, with the greatest difference observed at TBO (4582 vs 526 pg/ml,  $p=0.002$ ) and at ARV2 (6140 vs 1095 pg/ml,  $p=0.0007$ ). Plasma MMP-8 correlated with plasma PIIINP ( $r=0.435$ ,  $p<0.0001$ ), neutrophil count ( $r=0.617$ ,  $p<0.0001$ ), C-reactive protein ( $r=0.67$ ,  $p<0.0001$ ) and heart rate ( $r=0.262$ ,  $p=0.002$ ), but not CXR score. Plasma MMP-1 and MMP-3 were also higher in TB-IRIS. In TB-IRIS, MMP-8 concentrations were 30-100 fold higher in plasma than in sputum at the corresponding timepoints.

**Conclusions:** MMPs associate with pulmonary tissue destruction in TB. These new findings implicate systemic MMP dysregulation in TB-IRIS pathophysiology, suggesting that neutrophil-derived MMP-8 plays a key role and may be a therapeutic target. MMP-8 and PIIINP are also potential predictive and diagnostic markers of TB-IRIS.

**735 Spatiotemporal Distribution of Pediatric Tuberculosis in Central Durban, South Africa**

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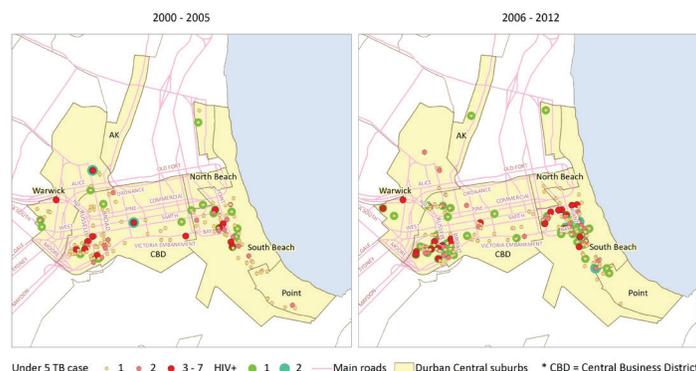
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**Background:** Childhood tuberculosis (TB) is due to recent *M. tuberculosis* infection, and is regarded as a marker of ongoing transmission. Antiretroviral therapy (ART) in HIV+ persons decreases TB risk. This study was conducted to characterize the spatiotemporal distribution of TB in pediatric cases < 5 years old presenting to the TB clinic in central Durban. We hypothesized that following the introduction of ART there would be changes in the spatiotemporal distribution indicating reduced TB transmission.

**Methods:** Using routinely collected data from the Prince Cyril Zulu Centre for Communicable Diseases (PCZCDC) between 2000 and 2012, we mapped cases of pediatric TB (< 5 years old) in central Durban. Address data enabled street level referencing. Cases were mapped by era (pre-ARV, 2000 – 2005 vs post-ARV, 2006 – 2012) and HIV co-infection status.

**Results:** We georeferenced 557 cases of childhood TB to 313 addresses. Of the 135 cases (28%) with HIV results, 59% were co-infected with HIV. Of all cases 93% had pulmonary TB. TB cases were identified in all 6 Durban central suburbs, 2 of which had an aggregation of TB cases. Between 2000 and 2005, 217 TB cases were mapped to 154 addresses, 36 of which had multiple TB cases. Between 2006 and 2012, 340 TB cases were mapped to 225 addresses, 62 of which had multiple TB cases. HIV co-infected patients were identified at 21 addresses in the pre-ARV era and 53 addresses in the post-ARV era.

**Conclusions:** We present evidence of increased pediatric TB cases at specific geographic locations, as well as multiple cases of TB at single addresses in central Durban. An understanding of the spatial distribution of TB data will inform targeted interventions to more effectively control ongoing TB transmission. An increase in TB cases post-ART was not consistent with our hypothesis. The etiology behind the apparent increase in multiple TB cases per address between eras is unclear, but suggests ongoing transmission and warrants further investigation.



**736 Does ART Increase Infectiousness of Smear-Positive Pulmonary Tuberculosis?**

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**Background:** Since the roll-out of antiretroviral treatment (ART) in high HIV/TB burden settings, there have been concerns that ART may increase the infectiousness of tuberculosis in HIV-positive patients by shifting the clinical manifestation of disease to be more similar to HIV-negative patients. In patients stable on ART for more than one year, enough immune recovery may have occurred for them to be more “similar” to HIV-negative individuals. We examine the effect of long-term ART on the prevalence of MTB infection among child contacts of adult smear-positive tuberculosis cases.

**Methods:** A cross-sectional household contact study of smear-positive tuberculosis index cases was conducted in Karonga, Malawi in January 2013–April 2015. Prevalence of tuberculin skin test (TST) positivity was compared between household contacts aged <11 years of HIV-negative, HIV-positive not on ART or HIV-positive on ART <1 year, and HIV-positive on ART for ≥1 year (at tuberculosis diagnosis) index cases. A positive TST was defined as ≥10mm. Data on risk factors for MTB infection were collected using a questionnaire, and analysed using a random effects logistic regression model to account for clustering within household.

**Results:** 416 child contacts (167 index cases), of whom 336 had a TST reading (81%; 150 index cases). Index case HIV/ART status was missing for 20 contacts (6%; 9 index cases). The proportion of index cases with the highest grade of smear-positivity (3+), was 45% (36/80), 39% (9/23), and 45% (17/38) in HIV-negative, HIV-positive on ART ≥1 year, and HIV-positive not on ART or on ART for <1 year respectively. The odds of a positive TST were 2.7 times higher in the contacts of HIV-negative index cases (81/182; aOR 2.7; 95% CI 1.1 – 6.7) and similar for HIV-positive index cases who had been on ART for ≥ 1 year (12/58; aOR 0.9; 95% CI 0.3 – 2.9), compared to contacts of HIV-positive index cases not on ART or on ART for <1 year (18/76).

**Conclusions:** We found an increased prevalence of MTB infection among child contacts of HIV-negative tuberculosis patients compared to contacts of HIV-positive index cases, irrespective of ART status. We found no evidence that HIV-positive index cases on ART for ≥ 1 year at tuberculosis diagnosis were more likely to transmit than other HIV-positive index cases.

Characteristic	Univariable*	Multivariable model** (n=316)	
	OR (95% CI)	aOR (95% CI)	p value
<b>HIV/ART status of index case</b>			
HIV-positive not on ART/ HIV-positive on ART < 1 yr	1	1	0.03
HIV-negative	3.6 (1.3 – 10.0)	2.7 (1.1 – 6.7)	
HIV-positive on ART ≥1 yr	0.9 (0.2 – 3.5)	0.9 (0.3 – 2.9)	
<b>Sensitivity analysis</b>			
HIV-positive not on ART/ HIV-positive on ART < 2 yr	1	1	0.03
HIV-negative	3.4 (1.3 – 8.8)	2.9 (1.2 – 6.7)	
HIV-positive on ART ≥ 2 yr	0.8 (0.2 – 3.2)	1.1 (0.3 – 3.9)	

\*Adjusted for household clustering

\*\*Adjusted for age of child; whether index case mother; age of index case and quality of dwelling structure

**737 Are We Overestimating TB Transmission Among Immigrants and HIV-Positive People?**

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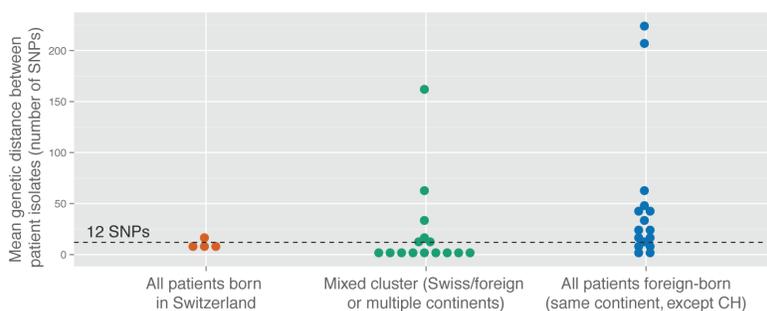
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**Background:** Immigration from high tuberculosis (TB) burden regions and HIV infection are major risk factors for TB in low-incidence countries like Switzerland. We have previously analyzed TB transmission using traditional molecular genotyping methods in a nationwide strain collection. Closely related *Mycobacterium tuberculosis* (Mtb) genotypes, independently imported from regions where they predominate, may lead to an overestimation of recent transmission among immigrants. We used whole genome sequencing (WGS) to analyze transmission clusters in Switzerland with a higher resolution.

**Methods:** We performed WGS on a nationwide collection of 520 Mtb strains isolated in 2000-2008 from HIV-coinfected and HIV-negative TB patients. We defined transmission clusters traditionally as isolates with identical spoligotyping and MIRU-VNTR patterns. We now used WGS data to confirm “true” transmission clusters, defined as isolate pairs separated by a genetic distance of ≤12 single nucleotide polymorphisms (SNP). We used weighted logistic regression adjusted for age, sex, and sputum positivity to identify risk factors for transmission.

**Results:** Only 17/35 (49%) traditionally-defined transmission clusters were confirmed “true” clusters by WGS; the other 18 clusters contained pairs separated by >12 SNPs. Overall, clustering proportion was 17% (90/520 patients, 95% Confidence Interval [CI]: 14-23) using traditional methods, and only 8% (43/520, 95% CI: 7-14) using WGS. Most traditional clusters (75%) involving only Swiss-born individuals were “true” clusters, but only 25% of clusters involving foreign-born patients were confirmed by WGS (Figure). Patients who were HIV-negative (aOR=2.9, 95% CI: 1.2-7.0), male (aOR=2.5, 95% CI: 1.1-5.5) or contact of previous TB cases (aOR=3.1, 95% CI: 1.0-9.6) were more likely to be part of a “true” cluster than others. There was weak evidence for an association between birth countries and transmission (aOR=2.3, 95% CI: 0.9-5.9, comparing Swiss-born patients to others).

**Conclusions:** TB transmission was not more common among immigrants and HIV-positive individuals than others. Traditional genotyping methods lead to an overestimation of recent transmission among foreign-born TB patients, likely due to locally circulating strains independently introduced from TB high-incidence regions. WGS should be applied to identify transmission clusters in low TB incidence settings, particularly in the context of immigration.



**Figure.** Relationship between country of birth of patients within a transmission cluster (defined by MIRU-VNTR) and genetic distance between isolate pairs (based on whole genome sequencing).

**738 Integrated TB/HIV Services for Migrant Miners and Their Families in Lesotho**

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**Background:** Migrant miners, who travel between home in Lesotho and work in South Africa, are a neglected and hard-to-reach population at high risk of TB and HIV acquisition and transmission. Engagement and retention of miners and their family members along the TB/HIV care continuum are suboptimal, and strategies to improve early diagnosis and treatment outcomes in this high-risk group are urgently needed.

**Methods:** In August 2013 we established on-site TB screening, diagnosis and treatment services within regional offices of an employment agency for miners, in 3 border districts of Lesotho where migrant miners congregate to collect deferred pay. Services initially targeted current miners but were extended to include former miners and miners’ family members due to expressed demand. In September 2014 services were expanded to include on-site opt-out HIV testing and counseling for those with a positive TB symptom screen. We abstracted routinely collected data from clinical registers (Table).

**Results:** Between 8/13 and 2/15, 182,776 persons were screened for TB using a symptom questionnaire. Of these, 8,108 (4.4%) screened positive, of which 6,996 (86%) were tested for TB using on-site Xpert MTB/RIF, and 378 (5.4%) were diagnosed with TB, including 23 (6.1%) with rifampicin-resistant TB. Of 71 TB cases who initiated treatment on-site between 3/14 and 8/14, 57 (80%) completed treatment, compared to treatment success of 63% among miners treated at public clinics. With availability of HIV testing on-site, the proportion of presumptive TB cases with documented HIV status increased from 13% (38/291) in 9/14 to 83% (161/193) in 2/15. Overall 32% of presumptive TB cases without a documented HIV status tested for HIV were HIV+. Of note, 35% of TB cases and 41% of newly diagnosed HIV cases were miners’ family members. A third of family members were male, 19% of whom were < 25 years old; in contrast >95% of female family members and miners were ≥ 25 years old.

**Conclusions:** On-site integrated TB/HIV services at a mining employment agency in Lesotho enabled efficient TB screening, diagnosis and management and HIV testing for current and former miners and their family members, and reaching young men. High prevalence of undiagnosed TB was found, with high TB treatment completion noted. HIV prevalence was high among miners and their family members. These findings suggest that this non-traditional venue can offer the opportunity to diagnose and manage TB and HIV in this vulnerable and hard-to-reach population.

INDIVIDUALS REACHED WITH TB SERVICES AT MINING AGENCY				
POPULATION	TB SCREEN POSITIVE	TESTED WITH XPERT MTB/RIF	DIAGNOSED WITH TB	TREATED AT AGENCY
Current Miners	3,070	2,743 (89%)	132 (4.8%)	110 (83%)
Former Miners	2,356	2,052 (87%)	114 (5.6%)	97 (85%)
Family Members	2,682	2,201 (82%)	132 (6.0%)	97 (73%)
<b>TOTAL (N=182,776)*</b>	<b>8,108 (4.4%)</b>	<b>6,996 (86%)</b>	<b>378 (5.4%)</b>	<b>304 (80%)</b>
INDIVIDUALS WITH PRESUMPTIVE TB & UNKNOWN HIV STATUS TESTED FOR HIV AT MINING AGENCY				
POPULATION	TESTED FOR HIV	HIV POSITIVE		
Current Miners (n=814)	175 (29%)	53 (30%)		
Former Miners (n=363)	116 (32%)	30 (26%)		
Family Members (n=593)	140 (24%)	57 (41%)		
<b>TOTAL (1,570)</b>	<b>431 (27%)</b>	<b>140 (32%)</b>		

\*Distribution of current miners, former miners and family members not available for individuals screened

**739 Diagnostic Yield of Household-Level Active Case Finding in Urban Slums in Haiti**Vanessa R. Rivera<sup>1</sup>; Jean W. Pape<sup>2</sup>; Serena P. Koenig<sup>3</sup>; Marc Antoine Jean Juste<sup>2</sup><sup>1</sup>Weill Cornell Med Coll, New York, NY, USA; <sup>2</sup>GHESKIO, Port-au-Prince, Haiti; <sup>3</sup>Brigham and Women's Hosp, Harvard Med Sch, Boston, MA, USA**Background:** Despite major advances in diagnostic and treatment services in settings where TB is endemic, many people with active TB remain undiagnosed. We evaluated the diagnostic yield of TB active case finding (ACF) at the household level; the program was funded by TB REACH.**Methods:** From August 1, 2014 to July 31, 2015, community health workers (CHWs) screened residents in 8 slum communities for cough >2 weeks. Household GPS coordinates were recorded using smart-phone technology for TB mapping purposes. All TB suspects were referred to a TB clinic for HIV and TB screening. All patients with confirmed cough >2 weeks were evaluated for TB with CXR and sputum acid fast bacilli (AFB) smear and Xpert tests. Patients diagnosed with TB were started on treatment within 5 days of initial referral.**Results:** 103,000 individuals were screened for cough through ACF activities. 6,926 (7%) reported cough >2 weeks and were referred for physician evaluation. Cough was confirmed in 3,397 (49%) of the patients referred, and 3,147 (93%) received smear and Xpert testing and were offered HIV-testing. 302 (10%) were HIV-positive, 2,644 (84%) were HIV-negative, and 201 (6%) had indeterminate HIV test results or were not tested for HIV. 90 of 302 HIV-infected patients (30%) were diagnosed with TB; 47 were smear and Xpert positive, 25 were smear negative and Xpert positive, and 18 were smear and Xpert negative but diagnosed with clinical and radiographic criteria. 571 of 2,644 HIV-negative patients (22%) were diagnosed with TB; 408 were smear and Xpert positive, 18 were smear positive and Xpert negative, 104 were smear negative and Xpert positive, and 41 were smear and Xpert negative but diagnosed with clinical and radiographic criteria. 661 patients with cough who received HIV, smear and Xpert testing were diagnosed with TB, and 129 (20%) were smear-negative but Xpert-positive.

TB mapping revealed clusters of higher prevalence of cough and diagnosed TB at the block-level within slum communities.

**Conclusions:** Household-level screening for cough by CHWs was highly effective; 21% of those tested were diagnosed with active TB. A high rate of TB was found among HIV-positive and HIV-negative patients, and Xpert testing resulted in 27% additional diagnoses of bacteriologically-confirmed TB among both groups. Further studies are needed to determine the cost-effectiveness of these strategies.**740 Integration of HIV-TB Screening and Linkage Strengthens Community-Based HIV Care**Ruane V. Barnabas<sup>1</sup>; Heidi van Rooyen<sup>2</sup>; Stephen Asimwe<sup>3</sup>; Torin Schaafsma<sup>1</sup>; Meighan Krows<sup>1</sup>; Alastair van Heerden<sup>4</sup>; Bosco Turyamureeba<sup>3</sup>; James P. Hughes<sup>1</sup>; Jared M. Baeten<sup>1</sup>; Connie M. Celum<sup>1</sup>; for the Linkages Study Team<sup>1</sup>Univ of Washington, Seattle, WA, USA; <sup>2</sup>Human Scis Rsr Council, Durban, South Africa; <sup>3</sup>Kabwohe Clinical Rsr Cntr, Bushenyi, Uganda; <sup>4</sup>Human Scis Rsr Council, Msunduzi, South Africa**Background:** In sub-Saharan Africa the burden of HIV-associated tuberculosis is high; early antiretroviral therapy (ART) combined with isoniazid preventive therapy (IPT) reduces HIV-associated morbidity and mortality. In generalized HIV epidemics in Africa, community-based HIV testing and counseling (HTC) links HIV-positive persons to care; integration of TB symptom screening into HTC can link HIV-positive persons to diagnostic testing for active TB or initiation of IPT and avert disability.**Methods:** We conducted a multisite program of community-based HIV testing and counseling, linkage to HIV care, and standardized WHO TB symptom screening in rural communities in KwaZulu-Natal, South Africa and Sheema district, Uganda. HIV testing was done at home or through mobile units. HIV-positive persons received the TB symptom screening and were referred to local clinics for care and diagnostic testing for active TB. At follow-up visits participant linkage to TB diagnostic testing, treatment for active TB, and IPT was assessed.**Results:** Between June 2013 and February 2015, 15,332 persons received HIV testing and counseling. Among 1,325 HIV-positive persons identified, the median CD4 count was high (486 cells/mL). At enrollment, 976 (74%) participants reported no symptoms of active TB and 157 (12%), 107 (8%), 64 (5%), and 21 (1%) reported 1, 2, 3 and 4 symptoms of TB. Linkage to HIV clinics was high (93%). After 9 months of follow-up, 113/346 (33%) persons with  $\geq 1$  TB symptom had sputum collected for TB testing vs. 266/957 (28%) among participants with no symptoms (RR=1.26, 95% CI 1.07-1.48). Of those tested for TB, 63% (240/379) reported they had received their sputum results. Among participants reporting symptoms at enrollment and tested, 15/345 (4%) were diagnosed with active TB vs. 11/957 (1%) of participants reporting no symptoms (RR=4.11, 95% CI 1.98-8.54). Only 54% (14/26) of HIV infected persons diagnosed with active TB initiated TB treatment. Ten percent (34/338) of participants who reported symptoms and 13% (119/951) of persons without TB symptoms initiated IPT.**Conclusions:** Among asymptomatic HIV-positive persons identified through community-based HIV testing, TB symptom screening increased the likelihood of TB diagnostic testing and TB diagnosis, but linkage to TB diagnostic testing, treatment, and IPT is low. Community-based HIV care requires efficient diagnostic strategies, such as rapid TB testing at HIV diagnosis, and effective linkage strategies to address low uptake of IPT and TB treatment.**741LB WITHDRAWN****742 A Clinical Prediction Rule for the Diagnosis of Tuberculosis in Seriously Ill Adults**Rulan Griesel<sup>1</sup>; Annemie Stewart<sup>2</sup>; Helen van der Plas<sup>3</sup>; Welile Sikhondze<sup>4</sup>; Molebogeng Rangka<sup>5</sup>; Gary Maartens<sup>1</sup>; Marc Mendelson<sup>1</sup><sup>1</sup>Univ of Cape Town, Cape Town, South Africa; <sup>2</sup>Clinical Rsr Cntr, Univ of Cape Town, Cape Town, South Africa; <sup>3</sup>Vincent Pallotti Hosp, Cape Town, South Africa; <sup>4</sup>FIND, Geneva, Switzerland; <sup>5</sup>Univ Coll London, London, UK**Background:** The World Health Organization's (WHO) algorithm for the diagnosis of tuberculosis in seriously ill HIV-infected patients with danger signs (any one of respiratory rate >30/min; heart rate >120/min; temperature >39°C; unable to walk unaided) and cough for  $\geq 14$  days uses chest x-ray and sputum smear results to start empiric antituberculosis therapy. The WHO algorithm preceded the availability of the rapid Xpert MTB/RIF assay. We aimed to develop a clinical prediction rule (CPR) for the diagnosis of tuberculosis by determining an evidence base for the duration of cough, the role of other tuberculosis symptoms, and simple laboratory tests (haemoglobin and white cell count). In addition we determined the diagnostic performance of Xpert MTB/RIF.**Methods:** A prospective cohort study was conducted at 2 secondary level hospitals in Cape Town, South Africa. Inclusion criteria were: HIV-infected, cough (any duration), WHO danger signs, age  $\geq 18$  years, and able to produce spontaneous/induced sputum. Chest x-rays were assessed by a specialist radiologist and categorised as unlikely, possibly or likely tuberculosis. Culture of *M. tuberculosis* from blood or sputum (2 samples sent) was the reference standard for the diagnosis of tuberculosis. In a multivariable model we assessed the ability of the following *a priori* chosen variables to predict the diagnosis of tuberculosis: WHO danger signs; duration of cough; tuberculosis symptoms (fever, night sweats, and weight loss); chest x-ray assessment; haemoglobin; and white cell count. The most predictive variables were used to establish a CPR for the diagnosis of tuberculosis.**Results:** 484 participants were enrolled into the study: median age 36 years; 317 female; median CD4 count was 89 cells/ $\mu$ L (IQR 34-210); and 171 on ART. 256 participants were culture positive for tuberculosis. Sputum smear had a sensitivity of 57.0% and a specificity of 98.7%. Xpert MTB/RIF had a sensitivity of 86.3% and a specificity of 96.1%. The final model included the following variables: cough  $\geq 14$  days, temperature >39°C, being unable to walk unaided, chest x-ray assessment, haemoglobin, and white cell count. Chest x-ray assessment of "likely tuberculosis" and anaemia were the strongest predictors of tuberculosis. The ROC AUC for the CPR was 0.81 (95% CI 0.80-0.82). The CPR is depicted in the table.**Conclusions:** The CPR could facilitate rapid initiation of empiric tuberculosis therapy in seriously ill patients using simple measures. Xpert MTB/RIF performed well in this population.

TABLE CLINICAL PREDICTION RULE AND PROBABILITY OF CULTURE-POSITIVE TB

Variable	Category	Points	Total score	Probability of TB	Sensitivity	Specificity
Temp >39°C	yes	1	≤1	0.1%	100.0%	0.0%
Unable to walk	yes	1	2	0.2%	95.2%	28.8%
TB on x-ray	possible	1	3	0.5%	92.2%	45.4%
	likely	5	4	1.1%	87.2%	59.3%
Cough ≥14 days	yes	1	5	2.5%	78.6%	67.7%
Haemoglobin (g/dL)	3.3 - 8.3	3	6	5.6%	66.0%	80.3%
	8.4 - 10.6	2	7	12.2%	50.8%	90.5%
WCC (x 10 <sup>9</sup> /L)	1 - 6.5	1	8	24.4%	39.1%	93%
	11.2 - 40.4	-2	9	43.0%	31.2%	96.4%
			10	63.7%	19.5%	97.9%
			11	80.4%	5.9%	99.6%
			12	90.5%	0.2%	100.0%

**743 High Mortality Rates in HIV-Infected TB: Suspects Who Rule Out for TB**

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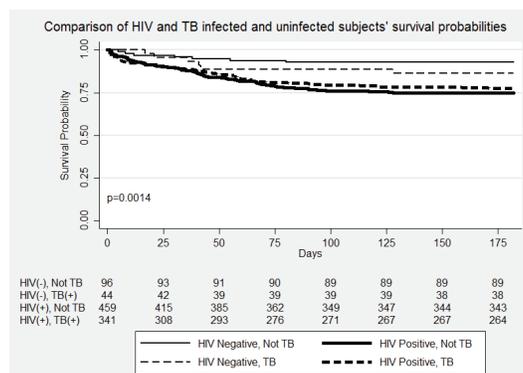
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**Background:** One-third of the 1.1 million TB-HIV co-infected individuals worldwide died in 2013; 78% of the TB-HIV infected live in sub-Saharan Africa (SSA). Increasing proportions of HIV-infected individuals were tested for TB and started on ART which decreased incidence rates of active TB and death. Due to limited diagnostics for other causes of fever and cough, presumptive TB cases who rule-out for TB are often treated empirically for TB and other bacterial pneumonias. We sought to describe the outcomes in this group of patients.

**Methods:** Presumptive TB patients enrolled in 3 TB diagnostic accuracy studies from 2011-2014 were followed for 6 months. TB cases were defined as patients in whom *M. tuberculosis* was cultured from any specimen (sputum or blood), sputum smear microscopy was positive, or if the patient was started on anti-TB treatment with subsequent documented clinical improvement within 2 months of enrollment, diagnosis of active TB within 2 months of enrollment by a non-study clinician. All others were considered not to have TB ('not TB') for this analysis. We used chi-square and Wilcoxon-Mann-Whitney tests on categorical and continuous variables, respectively.

**Results:** 946 presumptive TB patients were enrolled; 2 with indeterminate HIV status were excluded and 804 (85%) were HIV(+). 345/804 (43%) of the HIV(+) and 44/140 (31.4%) of the HIV(-) patients had TB, respectively. Of the HIV(+) patients, 78.4% were hospitalized. Similar proportions of the TB-HIV patients and the 'not TB' HIV patients died after 6 months of follow-up (26.4% vs 26.6%), despite a higher proportion of the "not TB" patients already on ART ( $P=0.0016$ ) and with a higher median CD4 T cell count (59 vs 114 cells/ $\mu$ L,  $P<0.001$ ). Kaplan-Meier curves shown in Figure.

**Conclusions:** Similarly high proportions (26.4 and 26.7%) of TB and 'not TB' patients died among HIV(+) presumptive TB patients. Improving diagnostics for TB and other causes of acute febrile respiratory disease in HIV(+) patients are urgently needed to identify reversible causes of death to improve outcomes.



**744 Mortality in HIV+ Men and Women Investigated for TB at Ethiopian Health Centers**

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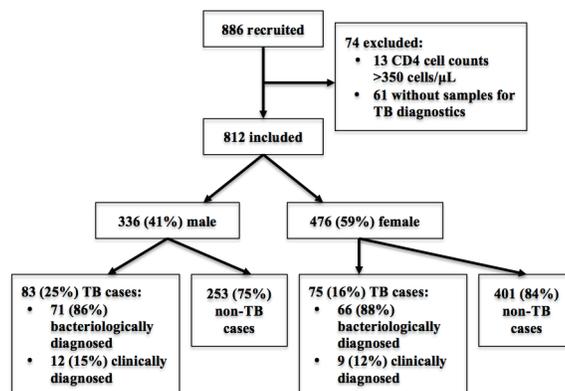
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**Background:** Despite increased access to antiretroviral treatment (ART) in low-income countries HIV-related mortality is high, especially in the first months following ART initiation. We have evaluated the impact of concomitant TB on early mortality and assessed gender-specific predictors of mortality in a cohort of Ethiopian adults subjected to intensified case finding for TB before starting ART.

**Methods:** Prospectively recruited ART-eligible (CD4 <350 and/or WHO stage IV) adults (n=812, 58.6% female) at five Ethiopian health centers were followed for 6 months. All participants were investigated for active TB at study inclusion by sputum culture, Xpert MTB/RIF, and smear microscopy (158/812 (19.5%) were diagnosed with TB, see figure 1). Primary outcome was all-cause mortality. Multivariate Cox models were used to identify predictors of mortality.

**Results:** In total, 37/812 (4.6%) died, among whom 12 (32.4%) had TB. Among TB cases, 8 (66.7%) died before ART start and 4 (33.3%) died after starting ART. For non-TB cases, 10 (40.0%) died before and 15 (60.0%) after ART start. Karnofsky performance score (KPS) and mid-upper arm circumference (MUAC) were associated with mortality in the whole population. However, the associations were different in men and women. In men, only MUAC remained independently associated with mortality, adjusted hazard ratio (aHR) 0.71 (95% CI 0.57-0.88) per centimeter increase. In women KPS <80% was associated with mortality, aHR 10.95 (95% CI 2.33-51.49), as well as presence of cough, aHR 3.98 (95% CI 1.10-14.36). Cough was also associated with mortality for TB cases, aHR 8.30 (95% CI 1.06-65.14), but not for non-TB cases.

**Conclusions:** In HIV-positive adults receiving care at Ethiopian health centers mortality was associated with reduced performance score and malnutrition, with different distribution with regard to gender and TB co-infection at inclusion. These robust variables could be used at clinic registration to identify persons at increased risk of early mortality.



**745 Empiric TB Therapy Versus IPT in HIV-Infected Persons Initiating ART (ACTG A5274 48 w)**

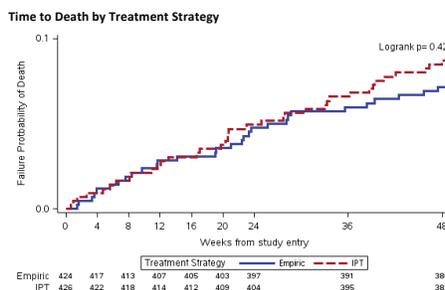
**Johnstone J. Kumwenda<sup>1</sup>**; Amita Gupta<sup>2</sup>; Xin Sung<sup>3</sup>; Miyahara Sachiko<sup>3</sup>; Evelyn Hogg<sup>4</sup>; Lynne Jones<sup>5</sup>; Andrew Zolopa<sup>6</sup>; Gregory P. Bisson<sup>7</sup>; Mina Hosseinipour<sup>8</sup>  
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**Background:** Up to 26% of patients initiating ART in low and middle income countries die within the first year of initiating ART, with TB being a major contributor. Strategies to reduce TB/HIV-associated mortality are urgently needed.

**Methods:** A5274 REMEMBER study is a multi-country randomized clinical trial comparing ART + four-drug empiric TB therapy vs. ART + isoniazid preventive therapy (IPT) in HIV-infected individuals with CD4 counts <50 cells/mm<sup>3</sup>. Participants were screened for TB prior to entry using a symptom screen, locally available diagnostics, and GeneXpert when available. Randomization was stratified by CD4 count (<25 vs. ≥25 cells/mm<sup>3</sup>) and poor prognostic factors (body mass index <18.5, hemoglobin <8 g/dl, and recent hospitalization). The primary endpoint of survival at 24 weeks post-randomization was reported elsewhere and demonstrated no effect of empiric TB treatment on mortality. To evaluate the possible effects of the intervention on longer-term outcomes, we compared the probabilities of death, death or AIDS progression, and confirmed or probable TB by week 48 between arms. Kaplan-Meier method was used to estimate the endpoint probabilities, which were compared with the z-test.

**Results:** We screened 1368 individuals and enrolled 850 (62%) participants. Of 850 enrolled, 53% were male, 90% were black. The median (IQR) age was 36 (30–42) years. The median (IQR) baseline CD4 count was 18 (9, 32) cells/mm<sup>3</sup>. At week 48, there was no statistical difference in mortality between the empiric arm (7.2%; 95% CI: 5.1%, 10.1%) and the IPT arm (8.7%; 95% CI: 6.4%, 11.8%), absolute risk difference 1.6% (95% CI: -2.1%, 5.2%; p=0.41). As in the 24-week analysis, the probability of death or AIDS progression was not significantly different between arms [19.3% (95% CI: 15.8%, 23.4%) for the empiric arm vs. 15.3% (95% CI: 12.2%, 19.1%) for the IPT arm], absolute difference -4.0% (95% CI: -9.1%, 1.1%; p=0.13). At week 48, the empiric arm had more TB compared to the IPT arm (5.6% vs. 2.4%, respectively), absolute risk difference -3.2% (95% CI: -5.9%, -0.5%; p=0.02; unchanged in competing risk analysis).

**Conclusions:** In a high TB burden population of participants with advanced HIV, there was no demonstrable benefit of empiric TB therapy on mortality at 48 weeks. These data support the implementation of enhanced screening for TB prior to ART initiation and the use of IPT, even in persons with advanced HIV residing in high TB burden regions of the world.



**746 TB Treatment Outcomes for HIV/TB Coinfected Children in Resource-Limited Countries**

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**Background:** Diagnosis and treatment of tuberculosis (TB) are challenging in HIV/TB co-infected children. The World Health Organization (WHO) recommends nucleic acid amplification tests (NAAT) for TB diagnosis, and a 4-drug regimen including ethambutol during the intensive phase of treatment (IP). However, many children are diagnosed with TB based on clinical criteria without microbiological confirmation, and ethambutol is sometimes omitted from IP due to concerns about toxicity. We assessed whether TB treatment outcomes differed by mode of diagnosis or IP regimen.

**Methods:** We conducted an observational cohort study among HIV/TB co-infected children (<16 years) enrolled at HIV treatment sites in 5 regions of the International Epidemiologic Databases to Evaluate AIDS (IeDEA) consortium from 2012–2014. Modified Poisson regression was used to estimate the relative risk (RR) of an unfavorable TB outcome (death, treatment failure, default, or unknown).

**Results:** Data were collected from 372 children. Excluding those missing CD4 counts, 295 were included in the main analysis. Median age was 5.7 years (Interquartile Range [IQR] 2–9.6), 46% were female, median CD4 count was 252 (IQR 60–666), 22% had unfavorable TB outcomes, 21% had at least one positive TB test (smear, culture, or NAAT), and 79%

were diagnosed clinically (negative test [37%] or no test performed [42%]). Mode of diagnosis was not associated with unfavorable TB outcomes (RR 1.17; 95% confidence interval [CI] 0.66-2.08). During IP, 74% of children were treated with isoniazid [H], rifampin/rifampicin [R], pyrazinamide [Z], and ethambutol [E], and 26% were treated with HRZ only. IP regimen was not associated with unfavorable TB outcomes (RR 0.82; 95% CI 0.45-1.47). No significant interactions were observed between IP regimen and age (p=0.65) or weight (p=0.99). Secondary analysis including all 372 children similarly did not demonstrate significant associations between mode of diagnosis or IP regimen and TB outcomes.

**Conclusions:** In this population of HIV/TB co-infected children, many were not diagnosed or treated per WHO guidelines. However, neither mode of diagnosis (clinical vs. microbiologic) nor IP regimen (HRZ vs. HRZE) were associated with unfavorable outcomes. Further studies are needed to determine optimal pediatric TB diagnostic and treatment strategies in resource-limited settings as well as to identify predictors of unfavorable TB outcomes in HIV/TB co-infected children.

**Table 1.** Modified Poisson Regression Model for Risk of an Unfavorable TB Outcome

	RR*	95% CI
At least 1 positive TB diagnostic test result (vs. clinical diagnosis)	1.17	0.66-2.08
3-drug HRZ intensive phase regimen (vs. 4-drug HRZE)	0.82	0.45-1.47
Age (per 1 year increase)	1.06	0.96-1.17
Weight (per 1 kg)	0.95	0.90-1.00
Female (vs. Male)	0.84	0.53-1.34
Asia-Pacific (vs. Eastern Africa)	0.52	0.23-1.18
Central Africa (vs. Eastern Africa)	0.41	0.10-1.73
Southern Africa (vs. Eastern Africa)	0.74	0.32-1.68
Western Africa (vs. Eastern Africa)	1.46	0.77-2.74
CD4 Count (per 1 count)	1.00	0.99-1.00

\* The model was adjusted for all covariates in the table.  
Abbreviations: TB, tuberculosis; H, isoniazid; R, rifampicin/rifampin; Z, pyrazinamide; E, ethambutol

**747 Urine LAM Testing in Advanced HIV-Infected Adults in a Trial of Empiric TB Therapy**

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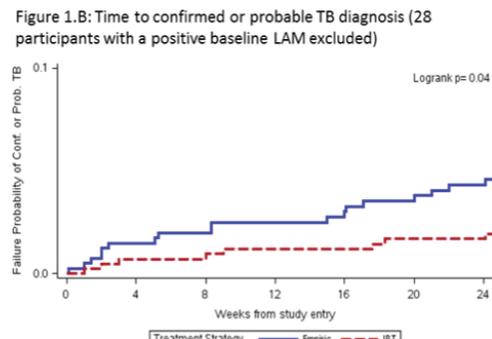
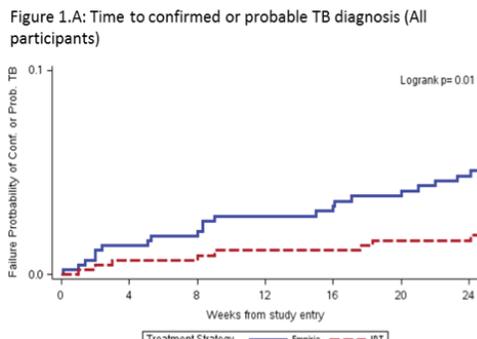
<sup>1</sup>Univ of Pennsylvania, Philadelphia, PA, USA; <sup>2</sup>Johns Hopkins Univ Sch of Med, Baltimore, MD, USA; <sup>3</sup>Harvard Univ, Boston, MA, USA; <sup>4</sup>NIAID, NIH, Bethesda, MD, USA; <sup>5</sup>Frontier Sci & Tech Rsr Fdn, Inc, Amherst, NY, USA; <sup>6</sup>Johns Hopkins Univ, Baltimore, MD, USA; <sup>7</sup>Coll of Med John Hopkins Proj, Blantyre, Malawi; <sup>8</sup>Univ of North Carolina at Chapel Hill, Chapel Hill, NC, USA

**Background:** Urine lipoarabinomannan (LAM) antigen testing is a rapid TB diagnostic but its utility in TB screening and treatment strategies is unclear. We evaluated the diagnostic yield of urine LAM in advanced HIV-infected adults screened out for TB using routine measures, outcomes in those who tested positive, and the effect of excluding LAM+ participants on trial outcomes.

**Methods:** ACTG A5274 is a randomized clinical trial that demonstrated that four-drug empiric TB therapy did not improve 24-week survival and was associated with an increased incidence of TB compared to isoniazid preventive therapy (IPT) (Figure 1A) in HIV-infected individuals initiating ART with CD4 counts <50 cells/mm<sup>3</sup>. Participants were screened for TB prior to entry using a symptom screen, locally available diagnostics, and Xpert MTB-RIF when available. Retrospective testing using the Alere urine LAM antigen assay was performed on stored urine obtained prior to ART initiation (baseline); tests were positive if two readers agreed. We determined the proportion of participants with a positive urine LAM test and the effect of excluding these participants on 24-week survival and TB incidence. Kaplan Meier method was used to estimate the primary endpoint (death or unknown vital status) and TB incidence rates at week 24, and the rates were compared by the z-test. Time to confirmed or probable TB (verified by external review) was compared by the log rank test.

**Results:** Overall, 850 of 1368 candidates were enrolled. Of those screened out, 174 (34%) had suspected TB. Of the 850 enrolled, 53% were male, the median age was 36 years, and the median baseline CD4 count was 18 cells/mm<sup>3</sup>. Of the 850, 566 (67%) provided baseline urine samples that were tested for LAM antigen (283 in each arm); 28 (5%) were positive [21 (7%) and 7 (2%) in the Empiric and IPT arms, respectively]. Of those positive, 1 participant in each arm died and 5 of 21 and 0 of 7 in the Empiric and IPT arms, respectively, developed TB. After excluding these 28 from the analysis, there were 21 primary endpoints (5%) in each arm (p=0.9). The incidence of TB remained higher (4.6% vs. 2%, p=0.04) and the time to TB remained faster in the Empiric arm (p=0.04; Figure 1B; unchanged in competing risk analysis).

**Conclusions:** Addition of urine LAM testing is unlikely to yield a positive test among outpatients with advanced HIV who are systematically screened for TB. Use of these tests in similar settings is unlikely to change the lack of an effect of empiric TB treatment on survival.



**748 Stool Xpert MTB/RIF and Urine LAM for Diagnosing TB in HIV-Infected Kenyan Children**

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**Background:** Pulmonary tuberculosis (TB) is a leading cause of mortality in HIV-infected children. Challenges in obtaining respiratory samples from children and rapid TB/HIV disease progression prior to obtaining culture results can lead to treatment delays. Rapid diagnostic tools from easily obtained specimens are urgently needed.

**Methods:** HIV-infected, antiretroviral therapy (ART)-naïve children under 12 years hospitalized for acute illness were enrolled in a randomized controlled trial (NCT02063880) comparing urgent to post-stabilization ART initiation in Kenya. At enrollment, children provided sputum or gastric aspirate (GA), stool, and urine specimens for TB diagnosis with liquid *Mycobacterium tuberculosis* (MTB) culture, Xpert MTB/RIF (sputum/GA and stool), and urine lateral flow lipoarabinomannan (LAM) testing, respectively. A second sputum/GA sample for culture was obtained within 72 hours of enrollment. We determined the diagnostic performance of stool Xpert and urine LAM (grade ≥1 considered positive) compared to the combined gold standard of sputum/GA culture and Xpert (positive defined as MTB detection in either of the two cultures or sputum/GA Xpert). 95% confidence intervals (CI) were estimated assuming a binomial distribution.

**Results:** Among 141 HIV-infected children, median age was 22 months (interquartile range [IQR]: 10-50) and median CD4 was 15% (IQR: 9-23%). Nine children (6%) had microbiologically-confirmed pulmonary TB (5 positive by culture and Xpert, 3 by culture alone, and 1 by Xpert-alone). Stool Xpert identified 6 of 9 children with confirmed TB (sensitivity: 67% [95% CI: 30-93%]; positive predictive value: [PPV] 100.0% [54-100%], Table 1) and classified 130 children as negative (specificity: 99% [95% CI: 95-100%]; negative predictive value [NPV]: 98% [95% CI: 94-100%]). Among 114 children with a urine sample, LAM identified 3 of 6 confirmed TB cases (sensitivity: 50% [95% CI: 12-88%]; PPV: 25% [95% CI: 6-57%]) and classified 96 of 108 children without confirmed TB as negative (specificity: 90% [95% CI: 81-94%]; NPV: 97% [95% CI: 91-99%]). Diagnostic accuracy results were similar when using the gold standard of culture-alone (Table 1).

**Conclusions:** Stool Xpert had moderate sensitivity and high specificity, PPV and NPV for MTB detection compared to sputum/GA culture and Xpert. Urine LAM appears to have poorer sensitivity and PPV, but was assessed in fewer children. Stool Xpert may be useful for accelerating a TB diagnosis in HIV-infected children.

**Table 1. Diagnostic accuracy of stool Xpert MTB/RIF and urine lipoarabinomannan (LAM) for microbiologically-confirmed pulmonary tuberculosis in HIV-infected Kenyan children**

		Sputum/ Gastric Aspirate Gold Standard Culture and Xpert <sup>a</sup>			Gold Standard Culture <sup>b</sup>		
		Positive/ Total	%	95%CI	Positive/ Total	%	95%CI
Stool Xpert MTB/RIF (n=141)	Sensitivity	6/9	67	(30-93)	5/8	63	(25-92)
	Specificity	130/132 <sup>c</sup>	99	(95-100)	130/133 <sup>c</sup>	98	(94-100)
	PPV	6/6	100	(54-100)	5/6	83	(36-100)
	NPV	130/133	98	(94-100)	130/133	98	(94-100)
Urine LAM (n=114)	Sensitivity	3/6	50	(12-88)	2/5	40	(5-85)
	Specificity	96/108 <sup>d</sup>	90	(81-94)	96/109 <sup>d</sup>	88	(81-94)
	PPV	3/12	25	(6-57)	2/12	17	(2-48)
	NPV	96/99	97	(91-99)	96/99	97	(91-99)

<sup>a</sup> True positive defined as at least one positive *Mycobacterium tuberculosis* (MTB) culture result or a positive sputum/ gastric aspirate Xpert MTB/RIF result

<sup>b</sup> True positive defined as at least one positive MTB culture result

<sup>c</sup> 2 stool Xpert MTB/RIF results were invalid, both of which were negative by gold standard

<sup>d</sup> 3 urinary LAM results were invalid (no purple/gray bar in the control window of the strip), all of which were negative by gold standard

**749 High Sensitivity of Abbott RealTime MTB and MTB RIF/INH Resistance Assays**

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**Background:** A major obstacle to the effective treatment of individuals with Tuberculosis (TB) disease is the accurate identification of *Mycobacterium tuberculosis* (MTB) and drug resistant strains. This study evaluated a novel molecular diagnostic test for TB; the Abbott RealTime MTB (Abbott) and Abbott RealTime MTB RIF/INH Resistance assays (Abbott Resistance).

**Methods:** Of the 100 individuals who provided sputum samples: 17 were diagnosed with rifampicin (Rif) resistant MTB on a non-study Xpert™ MTB/RIF (GeneXpert); 44 were suspected of having drug sensitive TB disease based on the absence of Rif resistance on the non-study Xpert or clinical evaluation, and 39 were healthy controls. 3ml of sputum was collected and the following assays performed: Study Xpert; Abbott (which detects MTB); Abbott resistance (which detects Rif and isoniazid [INH] resistance targeting rpoB, katG and inhA upper stream promoter regions) and Hain GenoType MTBDRplus assay (Hain). Liquid Culture using the Mycobacterial Growth Indicator Tube (MGIT) system (Becton Dickinson) and indirect DST were the gold standards.

**Results:** Diagnostic sensitivity and specificity between Abbott and MGIT was 100% and 89%, respectively (Table). Five of the 6 MGIT/Abbott discordant samples were also positive by either GeneXpert and/or Hain. For the 32 MGIT positive samples, Rif and INH resistance was evaluated by indirect DST and Abbott Resistance. Discrepancies are outlined in the Table, the sensitivity or resistance on Abbott was confirmed by either HAIN and/or GeneXpert. The rpoB probe4 mutation was responsible for the Rif resistance in one discrepant sample and katG 315T mutation was responsible for the sample with INH resistance detected by both HAIN and Abbott Resistance. For Abbott Resistance, 8 (38%) samples had both Rif and INH resistance and four samples (19%) had INH mono-resistance.

**Conclusions:** The Abbott RealTime MTB and Abbott RealTime MTB RIF/INH Resistance assays have a high sensitivity and specificity for detection of MTB and the diagnosis of both Rif and INH resistance. The different resistance patterns observed between the genotypic and phenotypic assays may likely be a result of mutations not targeted by the assay. Although this is a small sample size, INH-mono resistance was detected frequently, indicating the need for a genotypic assay that can detect both Rif and INH resistance.

**Table:** Comparison of the Abbott and Abbott Resistance assays to the gold standard a) MGIT or b) indirect DST, respectively.

Culture			
Abbott	Positive	Negative	Total
Positive	43	6	49
Negative	0	49	49
Total	43	55	98*

\* 2 Culture samples were contaminated and were excluded.

Indirect DST							
Abbott Rif	Resistant	Susceptible	Total	Abbott INH	Resistant	Susceptible	Total
Resistant	15	2	17	Resistant	10	1	11
Susceptible	3	12	15	Susceptible	1	20	21
Total	18	14	32**	Total	11	21	32**

\*\*Of the 43 Culture positive samples, 11 not be compared: 8 below the LOD of the Abbott Resistance, 3 had contaminated DST.

**750 Diagnostic Yield of TB Testing for Patients Who Present for HIV Testing in Haiti**

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**Background:** In resource-poor settings where tuberculosis (TB) is endemic and HIV prevalence is high, failure to diagnosis TB before antiretroviral therapy (ART) initiation among HIV-infected patients is associated with an increased risk of early mortality. We evaluated the diagnostic yield of an integrated TB/HIV symptom screen and the added value of Xpert testing for patients at the time of HIV testing at the GHEKIO Center in Port-au-Prince, Haiti.

**Methods:** We included all adult patients (≥18 years) who presented for HIV testing from August 2014 through July 2015. Patients were screened for cough at the time of HIV testing, and those with cough received evaluation for active TB by CXR, AFB smear, and Xpert testing.

**Results:** 30,316 patients were tested for HIV and screened for cough during the one-year study period—20,655 (68%) were women and the median age was 28, and 3,253 (11%) tested HIV-positive. 1,081 (33%) of the HIV-infected patients reported cough, and 245 (23%) were diagnosed with TB. 89 were smear and Xpert positive, 3 were smear-positive and Xpert negative, 49 were smear-negative and Xpert positive, 13 were smear or Xpert positive but did not have both tests, 11 were smear and/or Xpert-negative but culture-positive, and 80 were smear, Xpert and/or culture negative but diagnosed with symptoms and CXR findings. 5,455 (20%) of 26,814 HIV-negative patients also reported cough, and 1,224 (22%) were diagnosed with TB. 695 were smear and Xpert positive, 45 were smear-positive and Xpert negative, 179 were smear-negative and Xpert positive, 65 were smear or Xpert positive but did not have both tests, 16 were smear and/or Xpert-negative but culture-positive, and 224 were smear, Xpert and/or culture negative but diagnosed with symptoms and CXR findings. 1,469 patients were diagnosed with TB at the time of HIV testing, and 228 patients (16%) were smear-negative but Xpert positive.

**Conclusions:** In countries with high rates of TB and HIV, the early separation of patients with active TB is essential to reducing the transmission of TB. Over one-fifth of patients reported cough at the time of HIV testing, and of these one-quarter of HIV-infected patients were diagnosed with TB. The addition of Xpert testing increased bacteriologically-confirmed (SS/Bac+) diagnoses by 30%. Further studies are warranted to determine the cost-effectiveness of these strategies. 4% of SS/Bac+ patients were smear-positive and Xpert negative—further studies are underway to determine if these represent non-tuberculous mycobacteria.

Method of TB Diagnosis	HIV-positive	HIV-negative
Smear and Xpert-positive	89 (36%)	695 (57%)
Smear or Xpert-positive	13 (5%)	65 (5%)
Smear-positive and Xpert negative	3 (1%)	45 (4%)
Smear-negative and Xpert-positive	49 (20%)	179 (15%)
Culture-positive and/or Smear/Xpert-negative	11 (5%)	16 (1%)
Clinically diagnosed with symptoms and CXR findings	80 (33%)	224 (18%)
<b>Total TB cases Diagnosed</b>	<b>245 (17%)</b>	<b>1,224 (83%)</b>

**751 Association of Tuberculosis With CD4 Recovery and HIV RNA Decline on ART in Europe**

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**Background:** Tuberculosis (TB) may play an important role in immune recovery and HIV replication after antiretroviral treatment (ART) initiation among HIV-infected individuals. We examined the CD4 cell and HIV-RNA viral load changes in TB patients on ART.

**Methods:** We included HIV-positive patients (≥16 years) from 25 cohorts who were ART-naïve and started ART after 2000. Patients with a TB diagnosis >12 months before ART start were excluded. We compared patients with prevalent TB (all forms, diagnosis 2 months before/after ART start) or a recent history of TB (diagnosis 2-12 months before ART start) with patients with no history of TB. We calculated CD4 and HIV-RNA slopes overall and during the first six months after ART start and thereafter using two adjusted linear mixed effects models that included information on origin of patients among other epidemiological variables for each endpoint.

**Results:** We analysed 113,350 patients with a median age of 38 years (interquartile range [IQR] 32-45); 32,185 (28%) were female. At ART start, median CD4 cell count was 254 (IQR 137-371) and HIV-RNA viral load 61,670 (IQR 11,050-198,000). Overall, 1,914

Results are shown in the table:

Overall CD4 cell increases were lower in patients with prevalent (adj. difference -76 cells/μl/year) and recent TB (-32) compared to patients without a TB history. These effect was more pronounced in the first 6 months of ART.

Patients with prevalent TB had slower overall viral load decline (adj. difference 0.16 log<sub>10</sub> copies/mL/year) especially during the first 6 months (0.44), whereas those with recent TB showed a slower decline only during the first 6 months of ART.

Non-European, especially Sub-Saharan Africa origin, was strongly associated with lower CD4 increases and slower HIV-RNA declines, as was female sex. Older age was associated with lower CD4 increases but faster viral load declines.

**Conclusions:** TB had a significant effect on virological and immunological response to ART. While CD4 cell recovery after ART start was worse in patients presenting with TB, viral load decline tended to be slower only in patients with prevalent TB. These unfavorable effects were more pronounced in patients with prevalent disease during the first 6 months of ART and in patients from high-TB incidence countries who may need particular attention in clinical management.

**Table. Associations of CD4 cell count and HIV-RNA viral load changes after starting antiretroviral treatment (ART) in HIV-positive patients with prevalent tuberculosis (TB) at ART start and a recent history of TB compared to patients without a history of TB. Results are presented as adjusted differences of slopes (CD4 cells/μl/year and log<sub>10</sub> copies/mL/year). We calculated CD4 and log<sub>10</sub> HIV-RNA viral load slopes from consecutive measurements 2-6 months apart until 4 years after ART start, and used linear mixed effects models adjusted for age, sex, region of birth, baseline CD4 and RNA, and time-updated CD4 or HIV-RNA. Negative CD4 cell count slope differences indicate a lower increase, and positive HIV-RNA slope differences a slower decline.**

Characteristic	CD4 cell count change		HIV-RNA viral load change	
	diff. (95% CI)	P value	diff. (95% CI)	P value
<b>Prevalent TB at ART start</b>				
Overall	-76 (-90 to -61)	<0.001	0.16 (0.04 to 0.28)	0.01
First six months of ART	-153 (-181 to -127)	<0.001	0.29 (-0.01 to 0.58)	0.06
>6 months after ART	-59 (-75 to -44)	<0.001	0.14 (0.00 to 0.27)	0.045
<b>Recent history of TB</b>				
Overall	-32 (-49 to -14)	<0.001	0.06 (-0.09 to 0.21)	0.4
First six months of ART	-86 (-119 to -53)	<0.001	0.44 (0.08 to 0.79)	0.02
>6 months after ART	-21 (-39 to -3)	0.02	-0.01 (-0.17 to 0.15)	0.9
Female sex	-5 (-10 to -1)	0.017	0.21 (0.18 to 0.25)	<0.001
Age at the start of ART, years	-2.5 (-2.7 to -2.4)	<0.001	-0.008 (-0.010 to -0.006)	<0.001
<b>Origin of birth, compared to Europe</b>				
Sub-Saharan Africa	-76 (-82 to -70)	<0.001	0.19 (0.14 to 0.24)	<0.001
Asia	-40 (-56 to -25)	<0.001	-0.03 (-0.16 to 0.10)	0.6
Central/South America	-23 (-33 to -14)	<0.001	0.09 (0.01 to 0.17)	0.03
North Africa/Middle East	10 (-5 to 25)	0.2	0.24 (0.11 to 0.36)	<0.001

95% CI, 95% confidence interval; ART, antiretroviral therapy; TB, tuberculosis

**752 High TB Risk in HIV-Positive Patients on Second-Line Antiretrovirals in Pune, India**

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**Background:** With the increasing coverage and a 2-4% rate of virologic failure on first line antiretroviral treatment (ART) regimens globally, the number of patients who will need second-line ART containing protease inhibitor (PI) based regimens is increasing. Tuberculosis (TB) continues to be the leading cause of opportunistic infection and death among HIV-infected patients. However data on the TB incidence and mortality among HIV-infected patients receiving second line ART regimens are limited.

**Methods:** Retrospective cohort analysis of 59691 HIV-infected patients was conducted between January 2006 and June 2014 from a large public sector ART center of Byramjee-Jeejeebhoy Medical College-Sassoos General Hospitals (BJMC-SGH), Pune, India. Inclusion criteria included patients receiving PI (boosted atazanavir or lopinavir)-based second line ART regimens. TB was diagnosed either clinically or microbiologically by acid-fast bacillus smear as per the national program guidelines. Study outcomes included estimates of TB incidence and case fatality rates among patients receiving second line ART. We conducted a Poisson regression analysis to assess independent predictors of TB disease.

**Results:** The analyses included 405 patients on second line ART regimens who were followed for a median of 3.96 person-years (interquartile range (IQR), 2.23-5.28). Median age was 35 years (IQR, 31 - 40) and 138 (34%) were females. Median CD4 count at time of initiation of second line ART was 118 (IQR, 45 - 189) cells/cumm. TB incidence was 54.5 per 1000 person-years (PY) 95% confidence interval (CI), 43.5 - 67.4). The median time to TB incidence was 1.92 (IQR, 1.04-2.93) PY. The risk factors for TB included male gender (adjusted incidence rate ratio (AIRR), 3.45, 1.33 - 9.09, p=0.01), and unit decrease in hemoglobin (AIRR, 1.17; 95% CI, 1.04 - 1.32, p = 0.009). All-cause case-fatality was 26.7 (95% CI, 8.7 - 62.3) /1000 PY among TB co-infected patients receiving 2<sup>nd</sup> line ART.

**Conclusions:** Our study documents a high TB incidence and mortality among patients receiving second line PI-based ART. Since the high TB incidence on second line ART pose treatment challenges due to drug interactions between rifampin and PIs, long recommended World Health Organization's TB prevention strategies such as isoniazid prophylaxis need to be prioritized among all HIV-infected patients in India and other high TB burden countries.

**753 TB Outcomes With ATV/r and Two Rifamycin-Containing TB Regimens in HIV/TB in India**

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**Background:** Tuberculosis (TB) is the leading cause of death globally in HIV-infected individuals, and drug interactions can complicate treatment in this population. For those requiring antiretroviral therapy (ART) that includes a boosted protease inhibitor (PI), rifabutin is the recommended rifamycin, although the optimal dose is still in question. Our hypothesis was that HIV/TB co-infected patients treated with a second-line boosted ATV/r ART regimen coupled with a thrice weekly rifabutin-containing TB treatment would have less favorable TB outcomes than those receiving first-line NNRTIs coupled with daily rifampicin-containing TB treatment.

**Methods:** We retrospectively evaluated TB treatment outcomes for two cohorts of HIV/TB co-infected individuals seen at YRG Care Medical Center in Chennai, India, comparing those treated with an atazanavir/ritonavir (ATV/r) ART regimen plus a 150 mg daily or thrice weekly rifabutin-containing TB regimen with those treated with an NNRTI-based ART regimen in conjunction with a daily rifampicin-containing TB regimen.

**Results:** Between 1996 and 2014, 4032 HIV/TB co-infected individuals were treated at YRG CARE, of which 3740 (92.8%) were treated with an NNRTI-based ART regimen and a rifampicin TB regimen (Rifampicin<sup>+</sup>) and 292 (7.2%) an ATV/r-based ART regimen with a rifabutin TB regimen (Rifabutin<sup>+</sup>). Those in the Rifabutin<sup>+</sup> group were less likely to develop relapsed/recurrent TB (relative risk 0.32, 95% CI 0.20-0.49) and had lower all-cause mortality (relative risk 0.45, 95% CI 0.22-0.96) compared to those in the Rifampicin<sup>+</sup> group. In addition, those in the Rifabutin<sup>+</sup> group that received intermittent rifabutin had lower clinical cure rates compared to those who received daily rifabutin (relative risk 0.60, 95% CI 0.48-0.75), although there was no statistically significant difference in rate of relapse/recurrent TB and all-cause mortality between these two subgroups.

**Conclusions:** This large, retrospective study observed that ATT with rifabutin overall was effective, and daily rifabutin dosing may be more effective than intermittently-dosed rifabutin for the treatment of uncomplicated TB in HIV/TB co-infected individuals on a boosted PI-based ART regimen. This study expands our current knowledge about optimal rifabutin dosing with PI-based ART.

**754 Incorporation of Bedaquiline in the South African National TB Programme**

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**Background:** In 2014, 844 extensively drug-resistant (XDR TB) cases were diagnosed in South Africa. Following a successful clinical access programme (December 2012 to February 2015) and drug registration with the national regulatory authority in October 2014, a national framework was compiled to support the introduction of bedaquiline into the South African National TB Programme (SA NTP).

**Methods:** SA NTP guideline indications for bedaquiline (updated June 2015) include at least rifampicin-resistant TB patients with resistance to a fluoroquinolone or/and a second-line injectable drug (pre/XDR TB), both inhA/katG mutations for isoniazid resistance, intolerance or toxicity to standardized second-line regimen (e.g. ototoxicity, renal dysfunction, or psychosis), or surgical intervention. In patients with pre/XDR TB, linezolid is started in combination with bedaquiline. Patients with HIV infection, including those on antiretroviral therapy (ART), are eligible. Guidelines advise ART initiation for all TB patients with HIV infection. Inpatient admission is recommended for first two weeks or until culture conversion if XDR TB. Surveillance for BDQ resistance is done on baseline, 8-week, and 24-week sputum specimens using minimum inhibitory concentration testing by the national TB reference laboratory. Standardized forms for all patients are completed with proposed background second-line regimen, submitted centrally to the NTP, and reviewed by provincial or national clinical committee.

**Results:** From March to end September 2015, 598 patients have been initiated on bedaquiline in 7 of 9 provinces. As of end July 2015, most bedaquiline patients had either preXDR (40%) or XDR TB (39%); 65% were HIV-infected. The most common reason for cases being declined for bedaquiline initiation was patients with insufficient potentially effective drugs in the proposed background regimen.

Provinces that were able to scale-up quickly were those that had access to stock of linezolid, genotypic second-line drug resistance results, and capacity to detect high-frequency hearing loss.

**Conclusions:** Political commitment, national and provincial leadership, facilitation, and monitoring enabled rapid incorporation of clinical trial findings for rifampicin-resistant TB into the SA NTP.

Increased access to capacitated inpatient and outpatient patient management, ECG monitoring, and enhanced pharmacovigilance are needed to continue this rapid expansion.

**755 Treatment Outcomes for HIV/MDR-TB Coinfection in the Era of Antiretroviral Therapy**

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**Background:** The convergence of HIV and multidrug-resistant tuberculosis (MDR-TB) epidemics is a major global health threat. HIV co-infection has been shown to increase the risk of poor treatment outcomes among MDR-TB patients, but few studies have examined whether concomitant administration of antiretroviral therapy (ART) at a programmatic-level could achieve high rates of treatment success among HIV-infected MDR-TB patients. Here, we describe treatment outcomes among MDR-TB patients in Botswana during the period of ART expansion.

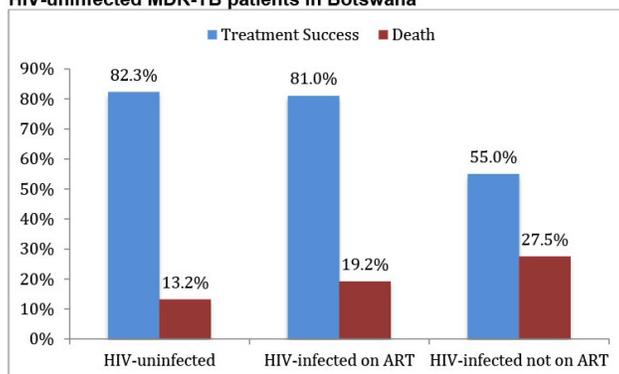
**Methods:** We analyzed data from 5 clinics in Botswana's decentralized MDR-TB treatment program. We included all patients with confirmed MDR-TB who started therapy during 2006-2013. Treatment success was defined as microbiological evidence of cure or completion of the full treatment course. Separate multivariable logistic regression models were

constructed for HIV-infected patients to determine whether ART use during MDR-TB therapy is independently associated the following outcomes: 1) increased treatment success and 2) reduced death.

**Results:** Of the 437 MDR-TB patients included in the analysis, 301 (68.9%) were HIV infected and 261 (86.7%) of those received ART. Overall, 343 (78.5%; 95% confidence interval [CI]=74.4%-82.1%) achieved treatment success, 79 (18.1%; 95% CI=14.8%-22.0%) died, and 15 (3.4%; 95% CI=2.1%-5.6%) failed treatment of defaulted. Treatment success rates were 82.3%, 81%, and 55.0% among patients who were HIV-uninfected, HIV-infected and on ART, and HIV-infected and not on ART, respectively ( $P < 0.001$ ; Figure). Death during treatment occurred among 13.2%, 19.2%, and 27.5% of patients who were HIV-uninfected, HIV-infected and on ART, and HIV-infected and not on ART, respectively ( $P < 0.093$ ; Figure). Among HIV-infected patients, ART use remained independently associated with treatment success (adjusted odds ratio [aOR]=3.5; 95% CI=1.5-8.0) after controlling for age, baseline CD4 count, number of drugs in the original regimen, and year of treatment initiation. The effect of ART use on preventing death did not reach statistical significance in multivariable analysis (aOR=0.6; 95% CI=0.3-1.5).

**Conclusions:** High rates of treatment success can be achieved programmatically among HIV-infected MDR-TB patients on ART, similar to that among HIV-uninfected patients. HIV-infected MDR-TB patients continue to experience unacceptably high rates of death, particularly among patients not on ART.

**Figure. Treatment success and death among HIV-infected and HIV-uninfected MDR-TB patients in Botswana**



#### 756 Hearing Loss and Laboratory Adverse Events in Patients With MDR TB and HIV on ART

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Multidrug-resistant tuberculosis (MDR TB) is associated with poor outcomes and high mortality in patients with HIV. Concurrent treatment for both diseases is recommended, but there are concerns about potentially additive toxicities, given the known and frequent side effects from MDR TB therapy and HIV treatment individually, as well as organ toxicity from HIV itself. South Africa has one of the world's worst epidemics of MDR TB and more than 80% of patients are HIV co-infected. Current guidelines are based on expert opinion, given the lack of high-quality prospective data.

**Methods:** We conducted an observational study of patients with culture-confirmed MDR TB with and without HIV co-infection. Subjects were followed monthly to assess treatment response and adverse effects. Audiology was performed monthly during the intensive phase; safety labs were performed every month throughout therapy and thyroid stimulating hormone (TSH) was tested every 3 months. Abnormal laboratory results were graded using the DAIDS Toxicity Table. We calculated the proportion of subjects experiencing hearing loss, and changes in creatinine, ALT, TSH, and potassium.

**Results:** Among the 206 enrolled subjects, 150 were HIV-infected, all of whom received antiretroviral therapy (ART). 131 (64%) were female, and the median age was 33 years (IQR 26-41). 56% of subjects experienced some degree of hearing loss (grade  $\geq 1$ ) and 9% developed severe (grade  $\geq 3$ ) loss. 40% developed hypothyroidism necessitating levothyroxine replacement therapy. Abnormalities of creatinine, ALT and potassium were common (23%, 20%, & 49% of subjects, respectively), but most were Grade 1 and resolved spontaneously. Grade  $\geq 3$  abnormalities were seen in only 5%, 4% and 5% of subjects respectively. None of the laboratory AEs examined were more common or more severe in subjects co-infected with HIV and receiving concurrent ART.

**Conclusions:** Hearing loss and laboratory AEs are common among all MDR TB patients, yet co-infection with HIV and the addition of ART does not significantly increase their frequency or severity, despite the frequent co-administration of kanamycin and tenofovir. Given the high incidence of hearing loss and hypothyroidism, programs should ensure that proper monitoring, as recommended, occurs for all patients, regardless of HIV status. Although nephrotoxicity, hepatitis, and hyper/hypokalemia occurred, these events were rarely severe and were not more common in patients with HIV receiving concurrent ART.

#### 757 Burden of Tuberculosis in HIV+ Pregnant & Postpartum Women in Cape Town, South Africa

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**Background:** Tuberculosis (TB) is a major concern in HIV+ individuals and antiretroviral therapy (ART) programmes but little attention is paid to TB in the context of pregnancy and PMTCT services. We investigated the burden of tuberculosis before, during and after pregnancy in a cohort of HIV+ women accessing antenatal care services under Option B+.

**Methods:** Consecutive women making their first visit at a primary care antenatal clinic completed a brief questionnaire, underwent CD4 enumeration, and had an ultrasound for pregnancy dating. TB diagnoses were via passive detection by routine public sector services based on symptom screening with or without bacteriologic confirmation. TB diagnoses were obtained through TB notification data for the period extending from 18 months before the estimated date of conception to 6 months postpartum. In analysis Poisson methods were used to compare the incidence of TB pre-conception, during pregnancy and postpartum per 100 person-years (py).

**Results:** Among 1507 HIV+ women (mean age, 29 years), 989 initiated ART in pregnancy (66%; median CD4 377 cells/uL, IQR 250-543) and 34% (n=518; median CD4 391 cells/uL, IQR 271-527) were already on ART at the time of conception. Overall incidence of TB pre-conception, during pregnancy and postpartum was 2.4 (95% CI, 1.8-3.2), 1.1 (95% CI, 0.6-1.9) and 1.4 (95% CI, 0.7-2.7) per 100py, respectively. 79% of cases were first TB episodes, with 21% retreatment cases; retreatment cases accounted for 18%, 23% and 40% of cases in the pre-conception, pregnancy and postpartum periods. 78% of TB cases were pulmonary (66% bacteriologically confirmed) and 22% were extrapulmonary (18% bacteriologically confirmed); this did not vary by pregnancy status. Overall TB incidence was doubled in women with CD4 cell count <200 cells/uL (IRR 1.96; p=0.007). After

adjusting for age, CD4 cell count and ART status, the incidence of TB was significantly reduced during pregnancy compared to pre-conception (IRR, 0.46; 95% CI, 0.25-0.84) and was lowest in women on ART in pregnancy (IRR, 0.38;  $p=0.021$ ) and increased slightly postpartum (IRR, 1.50;  $p=0.417$ ).

**Conclusions:** TB incidence appears reduced in HIV+ women during pregnancy compared to the pre-conception period but the absolute incidence remains high in this setting. While intensified case detection interventions in PMTCT services warrant consideration, expanded access to ART in pregnancy under Option B+ may further reduce the burden of TB in pregnant and postpartum women.

#### 758 Pregnancy Intensifies the IFN-gamma Suppression of HIV in TB-Infected Indian Women

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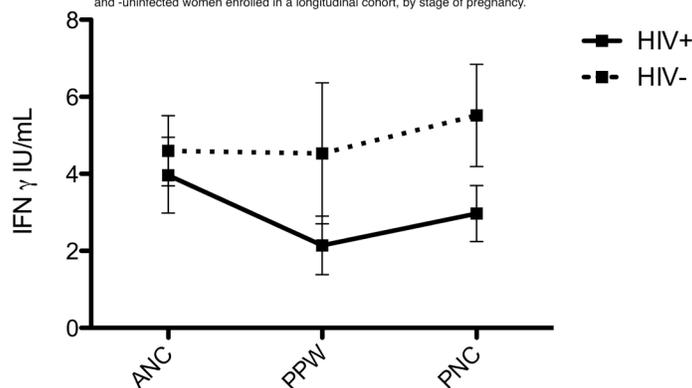
**Background:** Tuberculosis (TB) is more likely to occur immediately postpartum than at any other time in a woman's life. Low interferon gamma (IFN-g) has been associated with increased risk of TB. The aim of this study was to determine if immune changes in pregnancy and postpartum affect the IFN-g response to *M. tuberculosis* (MTB) antigens.

**Methods:** Screening for latent TB with an IFN-g release assay (QuantiFERON<sup>®</sup> Gold in tube, QGIT) was performed during 2<sup>nd</sup>/3<sup>rd</sup> trimester of pregnancy, at delivery and 3 months postpartum in HIV-infected and -uninfected pregnant women at a government hospital in Pune, India. A subset enrolled during pregnancy was followed longitudinally with testing repeated at delivery and postpartum. Sociodemographic and medical history data were also collected. Concentrations of IFN-g were compared using the Wilcoxon ranksum test. Logistic regression was performed to assess predictors of maximal IFN-g response (defined as 10 IU/mL) to MTB antigens.

**Results:** Valid QGIT results were available for 310 women during pregnancy, 426 women at delivery and 209 postpartum women. Among those with a positive QGIT, HIV-infected women produced a lower median IFN-g than HIV-uninfected women at all time points (median 2.7 vs 3.2 IU/mL in pregnancy, 1.8 vs 1.9 IU/mL at delivery). This was statistically significant postpartum (2.62 in HIV-infected vs 6.02 IU/mL in HIV-uninfected,  $p=0.01$ ). The 21 HIV-uninfected and 55 HIV-infected women in the longitudinal cohort showed the same trend (see Figure 1). HIV-infected women trended towards a significant decrease in IFN-g between pregnancy and delivery ( $p=0.07$ ). HIV-uninfected women had a significant increase in IFN-g production between delivery and postpartum ( $p=0.0002$ ) while HIV-infected women did not ( $p=0.19$ ). Having a maximal IFN-g response to MTB antigens was associated with not having HIV (1.6, CI 0.9-2.9,  $p=0.09$ ) and being postpartum (OR 3.5, CI 2-6.2,  $p<0.05$ ).

**Conclusions:** IFN-g reached a nadir for both HIV-infected and -uninfected women at delivery; HIV-uninfected women had a significant increase in IFN-g production postpartum while HIV-infected women did not. Lower IFN-g production during pregnancy, especially in those with HIV, may facilitate progression to active TB, though symptoms may not appear until postpartum, when IFN-g production increases again. TB diagnostics development should take these dynamic immune changes during pregnancy into account.

Figure 1. Comparison of IFN-g production among QGIT-positive HIV-infected and -uninfected women enrolled in a longitudinal cohort, by stage of pregnancy.



#### 759 Evaluation of Provider-Initiated Cryptococcal Antigen Screening, South Africa

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**Background:** Cryptococcal antigen (CrAg) screening can identify persons at risk for disseminated cryptococcal disease (DCD); pre-emptive treatment can prevent disease progression. In August 2012, the Western Cape Province (WC) implemented provider-initiated CrAg screening among antiretroviral therapy (ART)-naïve patients with CD4 count <100 cells/μl followed by fluconazole treatment for asymptomatic CrAg-positive patients. We evaluated the implementation and effectiveness of this program.

**Methods:** We used National Health Laboratory Service data for CD4 count and CrAg test results, provincial HIV program data for ART status, nationwide DCD surveillance program data for patient outcome, and medical records of CrAg-positive patients for fluconazole prescriptions. We assessed the proportion of eligible patients screened for CrAg, and treated pre-emptively, and the prevalence of CrAg positivity among those screened during September 1, 2012 – August 31, 2013. We compared incidence of DCD among eligible patients who were screened vs. not screened.

**Results:** Of 4,395 eligible patients, 26.6% (n=1170) were screened. The proportion of patients screened increased from 15.9% in September 2012 to 36.6% in August 2013. The prevalence of CrAg positivity was 2.1% (24/1170). Treatment data were available for 13/24 CrAg-positive patients, 9 of whom were treated with any fluconazole. There were 9 (0.8%) incident cases of DCD among the 1170 patients who were screened for CrAg, all of which occurred among those who screened CrAg-negative but had a substantial delay in starting ART vs. 49 (1.5%) incident cases among the 3225 eligible patients who were not screened ( $p=0.07$ ). Median time between CD4 count and DCD was 103 days (range: 35–497) in the screened group vs 155 days (range: 17–589) in the group not screened. A significantly higher proportion of those who were CrAg screened were also prescribed ART (72.9%, 853/1170) compared with those who were not screened (48.3%, 1556/3225) ( $p<0.001$ ).

**Conclusions:** The penetrance of provider-initiated CrAg screening in the WC was poor. Although not statistically significant, there were half as many DCD cases in the CrAg-screened group compared with those not screened. CrAg screening along with prompt ART initiation can reduce the DCD burden, but needs to be implemented well. Laboratory-based reflex screening, where CrAg testing is automatically performed on CD4 test remnant blood, would enhance screening, and should be considered where possible.

## 760 Maximizing Detection and Improving Outcomes of Cryptococcosis in Rural Tanzania

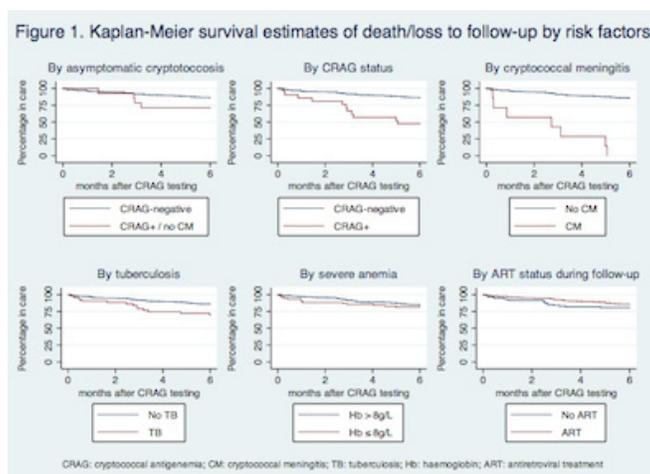
**Diana Faini**<sup>1</sup>; Aneth V. Kalinjuma<sup>2</sup>; Julie Neborak<sup>3</sup>; Alexa King<sup>3</sup>; Dorcas Mnzava<sup>2</sup>; Tracy Glass<sup>4</sup>; Hansjakob Furrer<sup>5</sup>; Christoph Hatz<sup>4</sup>; David R. Boulware<sup>3</sup>; Emilio Letang<sup>4</sup>  
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**Background:** The WHO recommends pre-antiretroviral treatment (ART) CD4-targeted cryptococcal antigen (CRAG) screening in sub-Saharan Africa. Implementing this strategy only in outpatient settings may underestimate the true CRAG prevalence and decrease its impact.

**Methods:** In October 2013, lab-reflex CRAG screening was implemented at the St. Francis Referral Hospital, Ifakara, Tanzania for all HIV+ hospitalized patients and outpatients with CD4  $\leq$ 150/ $\mu$ L. The impact on CRAG detection and outcome was assessed. Cox regression identified predictors of death/loss to follow-up (LFU) at 6 months.

**Results:** Of 1976 persons registered from 10/2013 to 07/2015, 500 (25%) ART-naive had CD4  $\leq$ 150/ $\mu$ L and were CRAG screened, contributing 2965 persons-month follow-up. Median age was 39 years (IQR 33-46), median CD4 count was 58 cells/ $\mu$ L (IQR 23-100), and 12% (59/500) had tuberculosis. CRAG prevalence was 6.4% (32/500) and 7.7% (30/376) with CD4 counts  $\leq$ 150 and  $\leq$ 100 cells/ $\mu$ L respectively, 1.7-fold higher than the 2008-2012 outpatient prevalence in the same cohort (3.7%  $\leq$ 150cells/ $\mu$ L,  $p=0.021$ ). Inpatients ( $n=82$ ) had a CRAG prevalence of 12% vs. 5.3% in outpatients ( $p=0.02$ ), and accounted for 31% of all CRAG+. Median time from HIV to CRAG testing was 1 day (IQR 0-6). A lumbar puncture was done on the same day of CRAG testing in 97% (31/32) CRAG+, and 39% (12/31) had cryptococcal meningitis (CM), 17% of whom (2/12) without neurologic symptoms. Fluconazole tailored for CM presence was started in 81% (26/32) of CRAG+ and ART in 72% CRAG+ (23/32) and 76% (382/500) overall. Known 6-month mortality for those recruited before 02/2015 ( $n=361$ ) did not differ between CRAG-negative and CRAG+ without CM (9% vs.7%,  $p=0.9$ ), yet was 86% (6/7) among CM patients ( $p<0.001$ ). LFU was 31% (104/340), 29% (4/14), and 14% (1/7) respectively. Independent predictors of death/LFU at 6 months were CRAG+ (adjusted hazard ratio (aHR) 3.2, 95% CI 1.2-8.2), CM (aHR 5.5, 95% CI 1.7-18), no ART initiation (aHR 2.2, 95% CI 1.5-3.4), tuberculosis (aHR 1.8, 95% CI 1.03-3.2), and hemoglobin (aHR 1.2 per 1 g/dL decrease, 95% CI 1.1-1.3) (Fig.1).

**Conclusions:** Implementation of lab-reflex CRAG screening resulted in an increased and rapid detection of CRAG and CM. Mortality was highest for CM patients but did not vary between CRAG+ without CM treated with pre-emptive fluconazole and CRAG-negative patients. These results support the urgent adoption of the WHO guidelines for CRAG screening in Africa and its implementation both in inpatient and outpatient settings.



## 761 Neurocognitive Function in HIV-Infected Persons With Cryptococcal Antigenemia

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**Background:** HIV-infected persons with detectable cryptococcal antigen (CRAG) in blood have increased mortality compared with HIV-infected persons who are CRAG-negative. This study examined neurocognitive function among persons with asymptomatic cryptococcal antigenemia.

**Methods:** Participants from three prospective HIV cohorts underwent neurocognitive testing at time of antiretroviral therapy (ART) initiation. Cohorts included persons with cryptococcal meningitis ( $n=90$ ), asymptomatic CRAG+ ( $n=87$ ), and HIV-infected persons without central nervous system infection(s) ( $n=125$ ). Asymptomatic CRAG+ participants receiving preemptive fluconazole also had neurocognitive testing additionally performed 4 weeks after ART initiation. Z-scores for each neurocognitive test were calculated relative to an HIV-negative Ugandan population with a composite quantitative neurocognitive performance Z-score (QNPZ-8) created from eight tested domains. Neurocognitive function was measured pre-ART for all three cohorts, and additionally 4 weeks after ART initiation among asymptomatic CRAG+ participants.

**Results:** Cryptococcal meningitis and asymptomatic CRAG+ participants had lower median CD4 counts (17 and 26 cells/mL, respectively) than the HIV-infected control cohort (233 cells/mL) as well as lower Karnofsky performance status (60 and 70 vs. 90, respectively). The composite QNPZ-8 for asymptomatic CRAG+ (-1.80 Z-score) fell between the cryptococcal meningitis cohort (-2.22 Z-score,  $P=0.02$ ) and HIV-infected controls (-1.36,  $P=0.003$ ). Overall neurocognitive function improved after four weeks of ART among the asymptomatic CRAG+ cohort (QNPZ-8 increased to -1.0,  $P<0.001$ ) to be within one standard deviation of population norms and similar to other HIV-infected persons.

**Conclusions:** Significant deficits in neurocognitive function were identified in asymptomatic CRAG+ persons with advanced HIV/AIDS even without signs or sequelae of meningitis. Neurocognitive function in this group improves over time after initiation of pre-emptive fluconazole treatment and ART.

## 762 Immunologic Discrimination of Cryptococcal IRIS From Culture-Positive Relapse

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**Background:** Immune Reconstitution Inflammatory Syndrome (IRIS) may complicate antiretroviral therapy (ART). Whereas ART promotes HIV viral suppression and CD4<sup>+</sup> T cell increases on a population level, the utility of these measurements as objective diagnostic criteria for IRIS remains unclear for an individual. We determined whether CD4 and plasma HIV RNA measurements are diagnostically useful to help differentiate paradoxical IRIS from culture-positive relapse.

**Methods:** In prospective cohorts with cryptococcal meningitis enrolled from 2006-2012, 70 HIV+ persons had 75 recurrent symptomatic meningitis episodes, due to paradoxical IRIS ( $n=62$ ) or culture-positive relapse ( $n=13$ ). IRIS was defined as per the INSH consensus case definition, with CSF culture status being the primary determinant of the causative event. We compared CD4 count change, plasma HIV viral load change, and CSF cytokines ( $n=50$ ) between IRIS vs relapse.

**Results:** 62 paradoxical cryptococcal-IRIS events did not consistently present with substantial reconstitution of CD4+ T cell counts (61% increased by  $\geq 25$  cells/mL), nor did these CD4 values distinguish IRIS from 13 culture-positive relapse events (54% with increases of  $\geq 25$  cells/mL). All IRIS cases had excellent, appropriate virologic responses, yet 50% (6/12) with culture-positive relapse did not have HIV viral suppression ( $>40,000$  copies/mL min). The median CSF WBC counts did not differ at time of cryptococcal-IRIS event (median 25; IQR, 5 to 85, max 240 cells/mL) versus relapse (median 15; IQR,  $<5$  to 40, max 1150 cells/mL) ( $P=.53$ ). Immune parameters which distinguished IRIS at event included 3-4 fold greater CSF levels of: Th1 cytokine IFN-g ( $P=.006$ ), Th2 cytokine IL-4 ( $P=.011$ ); pro-inflammatory IL-17 ( $P=.038$ ). Persons with culture-positive relapse did have markedly higher CSF IL-13 levels (relapse: median 253 (IQR, 63-471) pg/mL vs. IRIS: 6.9 (IQR, 2.4-17) pg/mL,  $P=.006$ ) and non-statistically higher GM-CSF levels ( $P=.17$ ).

**Conclusions:** CD4 counts, plasma HIV RNA, nor CSF WBCs at event onset did not consistently discriminated IRIS from relapse. The distinct immunologic signatures that did distinguish the two clinical scenarios, included 35-fold higher CSF IL-13 levels in relapse, a Th2 cytokine which is non-protective and associated with uncontrolled cryptococcal infection in murine models. In IRIS, multiple T-cell cytokines were increased (IFN-g, IL-4, IL-17) at event. In resource-limited settings, those with CSF culture-positive relapse should be targeted for viral load testing.

## 763 Acute Kidney Injury and Urine Biomarkers in HIV-Associated Cryptococcal Meningitis

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**Background:** Cryptococcal meningitis is a major cause of mortality in HIV-infected persons. Amphotericin B deoxycholate remains key to effective treatment despite its toxicities, including acute kidney injury (AKI). Current AKI definitions are based on serum creatinine or urine output, both late indicators of kidney injury. Biomarkers that could predict AKI earlier could be useful in directing amphotericin management. Neutrophil gelatinase-associated lipocalin (NGAL), is an early marker of ischemic AKI. Urinary Cystatin C (CysC) is a marker of tubular dysfunction. Tissue inhibitor of metalloproteinases-2 (TIMP-2) can predict AKI in critically ill patients. We assessed these urine biomarkers in patients with cryptococcosis treated with amphotericin.

**Methods:** Participants were prospectively enrolled into the Cryptococcal Optimal ART Timing (COAT) trial from 2010 to 2012 and were treated with daily amphotericin 0.7-1.0 mg/kg and fluconazole for 2 weeks. Baseline and follow up serum creatinine concentrations were measured. Estimated glomerular filtration rate (GFR) was calculated using the Modification of Diet in Renal Disease (MDRD) study equation, and AKI defined as decrease in GFR to  $<60$  mL/min/1.73m<sup>2</sup> within 4 weeks of diagnosis. We measured NGAL, CysC and TIMP-2 among 130 participants with stored urine samples collected a median of 4 days (IQR: 1- 4) from diagnosis. We explored AKI incidence, risk factors for incident AKI, and associations with mortality using univariate and multivariate Cox proportional hazards models.

**Results:** Among 130 participants, median age was 35 years (IQR: 30-40), 52% (n=68) were men, median CD4 T cell count was 21 cells/mL (IQR: 9-74), and 44% (n=57) died within 12 months. Four patients (3%) had GFR $<60$  at baseline. Incident AKI occurred in 42% (53/126) with GFR $<60$ . Incident AKI was independently associated with mortality (adjusted hazard ratio [aHR]=3.5, 95%CI: 1.95-6.1). Persons developing AKI had higher baseline CD4 T cell counts (49 vs. 14 cells/mL,  $p<0.01$ ). Higher urinary TIMP-2 was associated with development of AKI in univariate ( $p=0.02$ ) and multivariate analysis (aHR=1.4, 95%CI: 1.02-1.94 for each doubling of TIMP-2) but TIMP-2 was not associated with mortality.

**Conclusions:** AKI occurred in 42% of HIV-infected patients treated with amphotericin B deoxycholate for cryptococcal meningitis, and AKI was associated with mortality. TIMP-2 shows promise as a urine marker for antecedent prediction of amphotericin-associated AKI.

Table 1: Urinary Biomarkers in HIV-associated Cryptococcal Meningitis associated with Incident Acute Kidney Injury (GFR  $<60$ )

Urine Biomarker	Univariate Analyses		Adjusted Analyses <sup>2</sup>	
	Hazard Ratio <sup>1</sup> (95% CI)	P-value	Hazard Ratio <sup>1</sup> (95% CI)	P-value
Cystatin C (CysC)	1.04 (0.88, 1.23)	0.62	1.05 (0.88, 1.25)	0.56
Neutrophil gelatinase-associated lipocalin (NGAL)	1.06 (0.91, 1.24)	0.46	1.04 (0.88, 1.22)	0.66
Tissue inhibitor of metalloproteinases-2 (TIMP2)	1.47 (1.08, 2.00)	0.02	1.41 (1.02, 1.94)	0.04
Urine Protein	2.28 (1.05, 4.94)	0.04	2.35 (1.02, 5.42)	0.05
Urine Creatinine	1.04 (0.82, 1.32)	0.74	1.08 (0.84, 1.38)	0.56
Protein/Creatinine Ratio	0.83 (0.38, 1.80)	0.63	0.64 (0.28, 1.46)	0.29

<sup>1</sup>Proportional Hazards Regression Analyses using log<sub>e</sub> transformed biomarker data. The hazard ratio presents the risk per doubling of the biomarker for developing incident acute kidney injury (GFR $<60$  mL/min/1.73 m<sup>2</sup>).

<sup>2</sup>Each biomarker in an individual model and adjusted for antiretroviral treatment (ART) group (early ART or deferred ART group), age, gender, decreased level of consciousness at diagnosis, and CSF quantitative culture at diagnosis.

## 764 Pulmonary Aspergillosis May Be Common in AIDS With Smear Negative Tuberculosis

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**Background:** Smear-negative tuberculosis (TB) is common in AIDS in the developing world. WHO diagnostic guidelines require persistent productive cough plus abnormal chest x-ray and failure to respond to antibiotics. Microbiological proof of TB infection is not required. Subacute invasive aspergillosis (SAIA) also occurs in AIDS, with a clinical and radiological presentation that is very similar to tuberculosis. Autopsy studies of AIDS patients show aspergillosis occurs in 3% of all AIDS deaths, but ante-mortem diagnosis occurs in less than 10% of these cases. Measurement of *Aspergillus*-specific IgG is key to the diagnosis of SAIA, but rarely available in areas of high tuberculosis prevalence. The frequency of SAIA in patients presenting with AIDS and apparent smear-negative tuberculosis is not known. The Siemens *Aspergillus*-specific IgG test has a sensitivity of 96% and specificity of 98% for the diagnosis of chronic pulmonary aspergillosis using a diagnostic cut-off of 10 mg/L (1). We have used this assay to investigate levels of *Aspergillus*-specific IgG in patients with AIDS and smear negative tuberculosis.

**Methods:** We tested sera from 100 healthy Ugandan controls and 39 HIV infected persons admitted to Mulago Hospital, Kampala, Uganda with apparent smear negative tuberculosis, with chronic cough and an abnormal chest X-ray, but no evidence of tuberculosis or other diagnosis after thorough investigation including sputum culture, GeneXpert PCR testing and bronchoscopy.

**Results:** The mean patient age was 35 years and 59% of patients were female. Mean CD4 count was 109 cells/mL and 44% of patients had CD4 count  $<50$  cells/mL. Raised *Aspergillus*-specific IgG was present in 2% of healthy controls, but 26% of patients (95% CI 14 – 41%,  $p=0.000$ ). 40% of those with a positive test died within 2 months of sampling.

**Conclusions:** The presence of raised *Aspergillus*-specific IgG in the context of chronic cough, abnormal chest X-ray and exclusion of other conditions is highly suggestive of either subacute or chronic pulmonary aspergillosis. SAIA is probably occurring frequently in Ugandan patients with AIDS and being misdiagnosed as smear-negative tuberculosis. Further prospective studies with CT scanning, plus effective fungal culture and serology should be performed to investigate this possibility.

Reference

1 - Page ID, Richardson M, Denning DW. Comparison of six *Aspergillus*-specific IgG assays for the diagnosis of chronic pulmonary aspergillosis (CPA). 2015. Journal of Infection. In press.

**765 Safety of Stopping Primary *T. gondii* Prophylaxis With Suppressed Viremia and CD4>100**

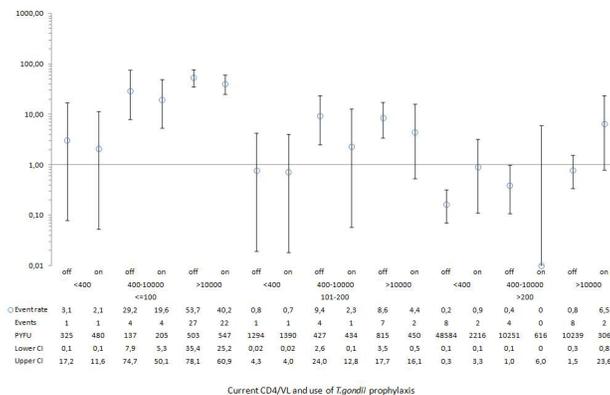
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**Background:** Current guidelines recommend that primary *Toxoplasma gondii* prophylaxis can be safely discontinued in HIV and *T. gondii* co-infected patients with suppressed viraemia on antiretroviral therapy (ART) and a CD4 cell count >200 cells/mm<sup>3</sup>. Whether such a policy can be extended to patients with CD4 cell counts between 100-200 cells/mm<sup>3</sup> is unknown.

**Methods:** The Collaboration of Observational HIV Epidemiological Research in Europe (COHERE) included data from from 10 European cohorts on 11,015 HIV and *T. gondii* co-infected patients who started taking ART after 1997. *T. gondii* infection was considered present if patients were *T. gondii* IgG antibody seropositive. Central nervous system (CNS) toxoplasmosis was diagnosed according to the 1993 CDC case definition. Multivariate Poisson regression models were used to model incidence rate ratios (IRRs) of CNS toxoplasmosis.

**Results:** There were 99 CNS toxoplasmosis episodes during 79,220 person-years of follow-up (PYFU). The incidence of CNS toxoplasmosis stratified by current CD4 cell count, viral load (VL), and use of prophylaxis is shown in the figure. Among patients who had a current CD4 cell count of 100-200 cells/mm<sup>3</sup> and a VL<400 copies/mL, incidence of CNS toxoplasmosis was 0.7 episodes per 1000 PYFU (95% CI, 0.02-4.0; 1 event during 1,390 PYFU) in those receiving *T. gondii* prophylaxis and 0.8 episodes per 1,000 PYFU (95% CI, 0.02-4.3; 1 event during 1,294 PYFU), in those who stopped prophylaxis. The incidence of CNS toxoplasmosis in virologically suppressed patients on ART (VL<400 copies/mL) with CD4 >200 cells/mm<sup>3</sup> on or off primary *T. gondii* prophylaxis was 0.9 (95% CI, 0.1-3.3) and 0.2 (95% CI, 0.1-0.3) episodes per 1,000 PYFU, respectively. The predictors of CNS toxoplasmosis among patients with a current CD4 cell count above 100 cells/mm<sup>3</sup> were: previous AIDS diagnosis (IRR, 2.86; 95% CI, 1.40-5.86; P=.004); doubling CD4 cell count/year (IRR, 0.30; 95% CI, 0.19-0.47; P<.001); and, detectable viral load (IRR, 4.25; 95% CI, 1.97-9.16; P<.001), whereas primary prophylaxis was not a significant predictor (IRR, 0.79; 95% CI, 0.33-1.87; P=.59).

**Conclusions:** The incidence of primary CNS toxoplasmosis among virologically suppressed patients on ART and who had CD4 cell counts >100 cells/mm<sup>3</sup> was very low regardless of prophylaxis use. Extending current guidelines for safely discontinuing primary *T. gondii* prophylaxis to patients with CD4 counts of 100-200 cells/mm<sup>3</sup> and suppressed VL would be appropriate.



**766 WITHDRAWN**

**767 The Cerebrospinal Fluid TPPA for Neurosyphilis Diagnosis**

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**Background:** There is no single sensitive and specific test for neurosyphilis (NS) diagnosis. While the cerebrospinal fluid (CSF)-Venereal Disease Research Laboratory (VDRL) test is specific, it lacks sensitivity. In contrast, the CSF- fluorescent treponemal antibody-absorption (FTA-ABS) test is sensitive, but lacks specificity. The CSF FTA-ABS test is not available in many areas. The CSF-*Treponema pallidum* particle agglutination assay (TPPA) is an alternative to the CSF-FTA-ABS, but little information is available regarding its diagnostic performance. A titer cut-off of ≥1:320 improves the NS diagnostic performance of the CSF-*Treponema pallidum* hemagglutination test, a treponemal test that is similar to the TPPA.

**Methods:** CSF-FTA-ABS and CSF-TPPA were determined in a research laboratory using standard methods for a convenience sample of 192 patients with syphilis enrolled in a study of CSF abnormalities in syphilis. CSF white blood cell (WBC) concentration and CSF-VDRL reactivity were determined in a clinical laboratory. Participants underwent a structured medical history and neurological examination. Kappa statistic, sensitivity and specificity were calculated using standard formulas. Differences were estimated by two sample test of proportion using Stata version 11.2.

**Results:** 186 (97%) participants were male and 159 (83%) were HIV-infected. Median (IQR) age was 37 (32-43) years and median serum RPR titer was 1:64 (1:32-1:256). 134 (70%) had early syphilis, 61 (32%) had a reactive CSF-VDRL, 69 (36%) had >20 WBCs/ul CSF and 40 (22%) of 183 without pre-existing abnormalities had vision or hearing loss. CSF FTA-ABS was reactive in 94 (49%), CSF-TPPA was reactive in 103 (54%), and CSF-TPPA was  $\geq 1:320$  in 58 (30%). Agreement between CSF-FTA-ABS and CSF-TPPA was good ( $\kappa=0.68$ ). Sensitivities and specificities of CSF-FTA-ABS for laboratory and clinical definitions of neurosyphilis were higher than for CSF-TPPA, but these differences were not statistically significant. For clinically defined neurosyphilis, sensitivities of CSF-FTA-ABS and CSF-TPPA were significantly higher than for CSF-VDRL ( $P=0.0006$  and  $P<0.0001$ ). The specificity and sensitivity of a CSF-TPPA titer  $\geq 1:320$  did not differ significantly from CSF-VDRL (Table).

**Conclusions:** In our study sample, the NS diagnostic performance of the CSF-TPPA is similar to the CSF-FTA-ABS test, and CSF-TPPA  $\geq 1:320$  can rule-in the diagnosis of NS. These results should be independently replicated.

	Neurosyphilis Definition: Reactive CSF-VDRL		Neurosyphilis Definition: Vision or Hearing Loss	
	% Specificity (95% CI)	% Sensitivity (95% CI)	% Specificity (95% CI)	% Sensitivity (95% CI)
CSF-FTA-ABS	74 (66-82)	98 (95-100)	59 (51-67)	78 (65-90)
CSF-TPPA	66 (57-74)	95 (90-100)	52 (44-60)	78 (65-90)
CSF-VDRL	--	--	78 (71-84)	68 (53-82)
CSF-TPPA $\geq 1:320$	86 (80-92)	66 (54-78)	75 (68-82)	50 (35-65)

95% CI, 95% confidence interval

**768 Invasive Pneumococcal Disease Among HIV-Infected Individuals in Toronto, Canada**

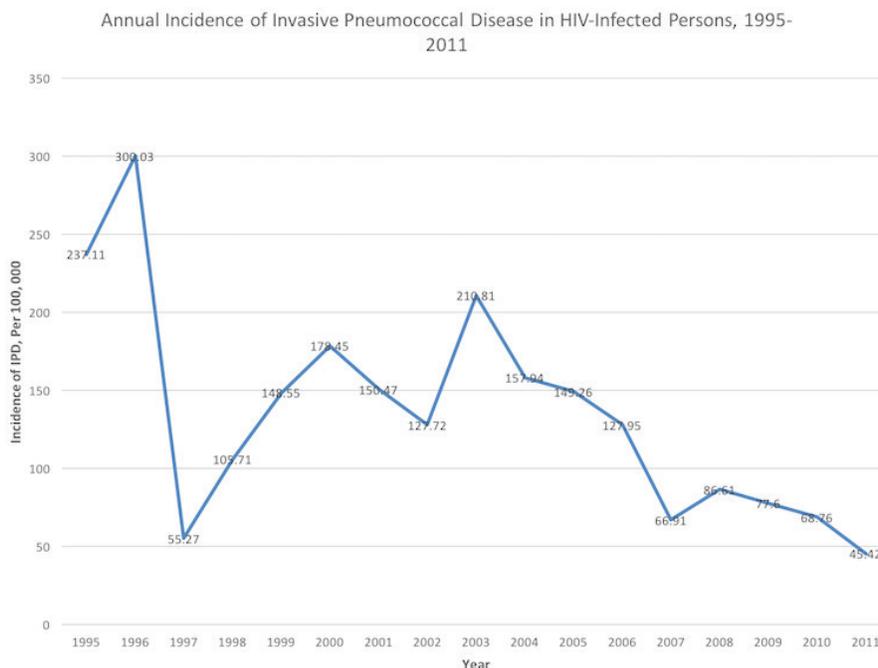
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**Background:** Invasive pneumococcal disease (IPD) is an important cause of morbidity and mortality in HIV-infected persons. Those with HIV are at higher risk of IPD than the general population; however, incidence has declined with antiretroviral therapy. This study examined trends in incidence, serotype distribution and antibiotic susceptibility over time to assess the impact of prophylaxis and childhood immunization.

**Methods:** Population-based surveillance for IPD has been ongoing in metropolitan Toronto and Peel region since 1995. Clinical information is collected by patient interview and chart review. One isolate per episode is serotyped and antimicrobial susceptibility testing is performed. Annual incidence from 1995 to 2011 was calculated using HIV population prevalence data.

**Results:** HIV-infected persons accounted for 316/8446 (3.74%) of IPD episodes from 1995 to 2015. Median age was 41.7 years (IQR 35.7-48.9); 85.8% of episodes occurred in men. The most common site of infection was pneumonia (84.5%), followed by bacteremia without focus (8.9%). From available data, 102/232 (44.0%) had received pneumococcal vaccine  $\geq 2$  weeks prior to infection, and 52/239 (21.8%) were on trimethoprim-sulfamethoxazole (TMP/SMX) prophylaxis. Annual incidence of IPD decreased over time (Figure 1). Case-fatality peaked at 16.5% between 2000-2004, then decreased to 4.9% in 2005-2009 and 5.2% in 2009-2015. Vaccination status did not affect case-fatality ( $p=0.2$ ). Over the study period, there was no change in the proportion of episodes due to PPV-23 serotypes (24.4%); there was a decrease in PCV7 serotypes (54.8% to 17.3%) and an increase in PCV13/not PCV7 (16.1% to 30.8%) and non-conjugate vaccine serotypes (6.5% to 28.9%). Serotype distribution since 2009 has remained stable with 51.9% attributable to non-conjugate vaccine serotypes, 30.8% to PCV-13 serotypes and 17.3% to PCV-7 serotypes. Resistance to erythromycin, penicillin and ceftriaxone increased over time, but remained low for amoxicillin and levofloxacin. TMP/SMX prophylaxis was associated with resistance to TMP/SMX ( $p<0.0001$ ), but not penicillin ( $p=0.13$ ).

**Conclusions:** HIV-positive persons accounted for 3.7% of IPD over a 20-year period; incidence decreased over time, particularly after introduction of a publicly-funded PCV for children in 2005. Guidelines now recommend PCV13 for immunocompromised persons; ongoing surveillance will be important to assess the impact of this recommendation on incidence, serotype distribution and mortality for those with HIV.



**769 Aetiology and Outcome of Community-Acquired Pneumonia in HIV-Infected Malawian Adults**

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**Background:** Pneumonia is the commonest reason for adult hospitalisation in Malawi, but there are few data describing its aetiology either locally or in comparable sub-Saharan African settings. We aimed to describe the aetiology of pneumonia amongst hospitalised HIV-infected adults with acute community-acquired pneumonia (CAP) and its relationship to antiretroviral therapy (ART) use and outcome.

**Methods:** Blood, urine, sputum, nasopharyngeal aspirate (NPA) and pleural fluid specimens were collected from adults ( $\geq 18$  years) with clinically-defined CAP and symptoms for  $\leq 14$  days admitted to a large central hospital in Blantyre, Malawi. Bacterial aetiologies were defined by blood culture and additionally for *Streptococcus pneumoniae* using an immunochromatographic assay for urinary polysaccharide antigen. *Mycobacterium tuberculosis* was identified by acid-fast bacilli sputum (and pleural fluid) microscopy, mycobacterial culture and Xpert MTB/RIF assay. Influenza, other respiratory viruses and atypical bacterial pathogens were identified using a multiplex RT-PCR assay on NPA. Patients were followed to 30 days.

**Results:** We recruited 459 adults between May 2013 and January 2015 of whom 355 (78%) were HIV-infected (220 (62%) males; median age 35 years (interquartile range (IQR): 30-41); median CD4 count 99 cells/cm<sup>3</sup> (IQR: 44-193). HIV-infection was newly diagnosed in 124 (35%). 189 (83%) of known HIV-infected individuals were on ART. 30-day mortality was 16% (54/342). An organism was identified in 267 (75%). *S. pneumoniae* (68 (19%)), *Mycobacterium tuberculosis* (64 (18%)) and influenza (30 (8.5%)) were the most common organisms. Infection with *M. tuberculosis* was independently associated with higher mortality (aOR: 2.51; 95% CI: 1.17-5.36). Co-infection, mainly with non-influenza respiratory viruses, was common. 65%, 45% and 50% of patients with *S. pneumoniae*, *M. tuberculosis* and influenza, respectively, had at least one co-infection; rates of co-infection did not vary with ART use or CD4 count. Co-infection was not associated with worsened outcome.

**Conclusions:** The major burden of hospitalised pneumonia in Malawi remains in HIV-infected patients with advanced immunosuppression. In addition to *S. pneumoniae* and influenza, tuberculosis is a major cause of pneumonia, even amongst patients with reported short symptom duration and is associated with increased mortality. Co-infection, typically with respiratory viruses, is common and larger studies are required to better understand prognostic significance.

**770 No Increased Risk of HIV Incidence During Pregnancy**

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**Background:** There is inconclusive evidence regarding whether women are at greater risk for acquiring HIV during pregnancy. We examined HIV incidence in women enrolled in a randomized clinical trial during pregnant and non-pregnant person time.

**Methods:** Data came from the Methods for Improving Reproductive Health in Africa (MIRA) study which was conducted in South Africa and Zimbabwe from 2003-2006. Women 18-50 years with at least one follow-up visit within 6 months of enrollment were analyzed. All study visits included HIV and pregnancy testing, as well as self-report of intravaginal practices and sexual risk behaviors. The analysis examined person-time between study visits and estimated HIV incidence using person years (py) at risk during pregnant and non-pregnant periods (non-pregnant periods were classified by self-reported hormonal contraceptive (HC) use). Cox proportional hazards models were fitted using pregnancy status as a time-varying exposure, demographic characteristics as time-fixed covariates and sexual risk behaviors as time-varying covariates (non-pregnant/no HC use was the referent group).

**Results:** 4,549 women from the MIRA study were included in this analysis (92% of all enrolled), 766 (17%) of whom had a lab-confirmed pregnancy. Median follow-up time was 18 months [interquartile range (IQR): 12-24] and median age was 27 years. There were 240 incident HIV cases overall, 16 occurred during pregnant periods. HIV incidence was 3.9/100py overall; during pregnancy, incidence was 3.8/100py, when women were not pregnant incidence was 4.8/100py with injectable HC use, 4.4/100py with no HC and 2.6/100py with oral HC. In unadjusted and adjusted models, pregnancy was not found to increase HIV incidence compared to when women were not pregnant and not using HC (unadjusted hazard ratio (HR) 0.8, 95%CI 0.5-1.3; adjusted 0.7, 95%CI 0.4-1.2). Sex without a condom (HR 1.3; 95%CI 1.0-1.7) and suspecting or knowing a male partner had other sexual partners (HR 1.3, 95%CI 1.0-1.6) were both found to be predictors of HIV incidence in multivariable models.

**Conclusions:** We did not find higher incidence of HIV during pregnancy compared to periods when women were not pregnant however continued high incidence of HIV was observed among pregnant women and is known to increase the risk of mother-to-child transmission of HIV. These findings highlight the need for greater efforts to prevent HIV infection in pregnant women.

**771 Effect of Pregnancy on Response to Antiretroviral Therapy Among African Women**

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**Background:** While most recent evidence does not support a role for pregnancy in accelerating HIV disease progression, very little information is available on the effects of incident pregnancy on response to antiretroviral therapy (ART). Hormonal, immune and behavioral changes during pregnancy may influence response to ART. We sought to explore the effects of incident pregnancy (after ART initiation) on virologic, immunologic, and clinical response to ART.

**Methods:** Data were collected from HIV-infected women participating in 3 prospective studies (Partners in Prevention HSV/HIV Transmission Study, Couples Observation Study, and Partners PrEP Study) from seven countries in Africa from 2004 to 2012. Women were included in this analysis if they were  $\leq 45$  years of age, were started on ART during the study and were not pregnant at ART initiation. Pregnancy was treated as a time-dependent variable to include all pregnancies occurring after ART initiation. Virologic failure was defined as a viral load (VL) greater than 400 copies/ml  $\geq 6$  months after ART initiation and viral suppression as viral load  $< 400$  copies/ml. Multivariable Cox proportional hazards models assessed the association of pregnancy and time to viral suppression, virologic failure, WHO clinical stage III/IV and death. Linear mixed effects models assessed the association of pregnancy and CD4 count and VL. All analyses were adjusted for confounders, including pre-ART CD4 count and plasma VL.

**Results:** A total of 1041 women were followed, contributing 1097.6 person-years of follow-up. One-hundred ten women became pregnant at least once after ART initiation. Median CD4 count prior to ART initiation was 276 cells/mm<sup>3</sup> (IQR, 209-375); median pre-ART viral load was 17,511 copies/ml (IQR, 2480-69286). Pregnancy was not associated with difference in time to virologic failure (adjusted HR, 0.68, 95% CI, 0.37-1.22), time to viral suppression (adjusted HR, 1.21, 95% CI, 0.82-1.77), time to WHO Clinical stage III or IV (adjusted HR, 0.79, 95% CI, 0.19-3.30) or time to death (adjusted HR, 2.04 (95% CI, 0.25-16.8)). Incident pregnancy was associated with an adjusted mean decrease in CD4 count of 47.7 cells/mm<sup>3</sup> ( $p < 0.001$ ) over the course of follow-up, but not with difference in viral load ( $p = 0.06$ ).

**Conclusions:** For HIV-infected women on ART, incident pregnancy does not have an effect on virologic or clinical HIV disease progression. A modest decrease in CD4+ T cell count could be due to physiologic effects of pregnancy.

**772 Trends in Hospitalizations of Pregnant HIV-Infected Women in the USA: 2004-2011**Alexander Ewing<sup>1</sup>; Hema Datwani<sup>2</sup>; Lisa Flowers<sup>1</sup>; Sascha Ellington<sup>1</sup>; Denise Jamieson<sup>1</sup>; Athena P. Kourtis<sup>1</sup><sup>1</sup>CDC, Atlanta, GA, USA; <sup>2</sup>CDC, Philadelphia, PA, USA

**Background:** Although patterns of hospitalizations of HIV-infected pregnant women in the U.S. have changed with the introduction of combination antiretroviral therapy (cART), increased rates of adverse outcomes among HIV-infected, compared with uninfected, women have persisted. There is a lack of recent evidence on the hospitalization burden among HIV-infected pregnant women in the U.S. and of estimates of deliveries among such women.

**Methods:** Using hospital discharge data from the Nationwide Inpatient Sample's years 2004, 2007 and 2011, we compared the numbers, demographic characteristics and morbidity outcomes of hospitalizations of pregnant women by HIV status in the U.S. Multivariate logistic regression was used to examine time trends, adjusting for confounders. Analyses were weighted to produce national estimates.

**Results:** In 2011, there were 4,751 estimated pregnancy hospitalizations, including 3,855 delivery hospitalizations for HIV-infected pregnant women, unchanged since 2004. In 2011, compared with those of HIV-uninfected women, pregnancy hospitalizations of HIV-infected women, on average, were longer, incurred higher hospital charges, were more likely to be in the South, and to be covered by public insurance. They also had higher odds of many adverse outcomes, including preterm delivery (aOR: 1.46 [95% CI: 1.19-1.79]), preeclampsia/hypertensive disorders of pregnancy (aOR: 1.44 [1.16-1.80]), bacterial infections (aOR: 2.90 [1.88-4.47]) and viral/mycotic/parasitic infections (aOR: 5.56 [4.21, 7.35]). From 2004-2011, prevalence of hospitalizations with gestational diabetes and pre-eclampsia/hypertensive disorders of pregnancy increased for both HIV-infected and uninfected women. Prevalence of bacterial infections increased only among hospitalizations of HIV-infected pregnant women.

**Conclusions:** The numbers of hospitalizations during pregnancy and of deliveries have not increased for HIV-infected women since 2004. Pregnancy hospitalizations of HIV-infected women remain more medically complex than those of HIV-uninfected women. An increasing trend in infections among the hospitalizations of HIV-infected pregnant women and increasing trends in gestational diabetes and hypertensive disorders of pregnancy among both HIV-infected and uninfected women warrant further attention.

**773 Cost-Effectiveness of Cotrimoxazole Among HIV+ Pregnant Women in Malarious Regions**

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**Background:** Malaria is a leading cause of morbidity and mortality among HIV+ pregnant women in East Africa. The pregnancies of at least 1 million women are complicated by co-infection of malaria and HIV annually. Current guidelines recommend either cotrimoxazole (CTX) or intermittent preventive treatment (IPTp) for HIV+ pregnant women to prevent malaria and its complications. While daily CTX makes its schedule simple, concerns remain over adherence and discontinuation of CTX at high CD4 counts. We assess the cost-effectiveness of CTX compared with IPTp to prevent malaria among HIV+ pregnant women.

**Methods:** We constructed an individual-level microsimulation model of malaria and HIV among pregnant women, matched by CD4 count, and antiretroviral therapy and malaria statuses to the Malawi population. Cohorts of 10,000 HIV+ pregnant women were simulated for a 40-week pregnancy period. We compared 3 strategies: (i) 0/1-doses IPTp, (ii) 3-dose IPTp, and (iii) daily CTX. Our primary outcomes include maternal malaria episodes, anemia, low birth weight, and neonatal mortality. We estimated disability-adjusted life-years (DALYs) averted for the CTX and 3-dose IPTp strategies compared with the 0/1-doses IPTp. Costs were estimated based on data from observational studies and published literatures. Sensitivity analyses assessed the effect of adherence to CTX, its efficacy in preventing malaria, and the risk of malaria.

**Results:** Compared with 0/1-doses IPTp, 3-dose IPTp averted 43.6 DALYs per 100 pregnant women at an estimated cost of \$70.3, an incremental cost-effectiveness ratio (ICER) of \$1.61. The CTX strategy was associated with additional 15.8 DALYs averted per 100 women compared with 3-dose IPTp at an incremental cost of \$16.8, an ICER of \$1.06. The CTX strategy was less effective than 3-dose IPTp when more than 1.8% of pregnant women dropped out of CTX every week, less than 46% of women taking CTX for the duration of pregnancy. When the risk of malaria was 40% lower than the base case scenario, the DALYs and estimated costs from CTX and 3-dose IPTp strategies were not significantly different: both averted 43.4 additional DALYs compared with 0/1-doses IPTp. One-way sensitivity analyses on CTX efficacy, down to 50% of base case efficacy, did not substantially affect the primary findings.

**Conclusions:** In malarious regions, daily CTX to HIV+ pregnant women regardless of CD4 count is more effective than IPTp, and highly cost-effective by standard measures as long as more than half of women adhere to daily dosing.

**774 Intracellular Atazanavir Concentrations Remain Stable During Pregnancy in HIV Women**Emanuele Focà<sup>1</sup>; Andrea Calcagno<sup>2</sup>; Andrea Bonito<sup>1</sup>; Marco Simiele<sup>2</sup>; Elisabetta Domeneghini<sup>1</sup>; Antonio D'Avolio<sup>2</sup>; Maria Antonietta Forleo<sup>1</sup>; Giovanni Di Perri<sup>2</sup>; Eugenia Quiros Roldan<sup>1</sup>; Stefano Bonora<sup>2</sup><sup>1</sup>Univ of Brescia, Brescia, Italy; <sup>2</sup>Univ of Torino, Torino, Italy

**Background:** Several physiological changes occurring in the third trimester may impact the pharmacokinetics of administered drugs. Significant reductions in protease inhibitors (PIs) plasma concentration have been described and the correct dose is unclear in HIV-positive pregnant patients. Acting at the intracellular level, PIs intracellular (peripheral blood mononuclear cells, PBMC) concentrations may be relevant and they have not been studied during pregnancy.

**Methods:** HIV-positive pregnant patients treated with atazanavir/ritonavir (300/100 mg, ATV/r) plus either tenofovir/emtricitabine or abacavir/lamivudine were prospectively enrolled after signing a written informed consent. ATV and ritonavir (RTV) plasma and intracellular (IC, intra PBMCs) were measured at every visit [second (2T) and third trimester (3T) and within 3 months post-partum (PP)] using validated HPLC/MS-MS methods (with direct evaluation of cells volume). Data are described as medians (interquartile ranges) and through non-parametric tests.

**Results:** 20 patients were enrolled; median age and body mass index were 31.4 years (25.8-35.6) and 24.7 Kg/m<sup>2</sup> (23-32.5). All patients were on treatment with plasma HIV RNA <50 copies/mL; CD4+ T-lymphocytes were 738 /uL (529-833). ATV plasma concentrations were 526 ng/mL (334-1066), 474 ng/mL (317-969) and 740 ng/mL (589-1132) during 2T (n=12), 3T (n=14) and PP (n=15); respective ICs were 723 ng/mL (569-1615), 762 ng/mL (382-1141) and 555 ng/mL (364-1930). RTV plasma concentrations were 35 ng/mL (29-57), 25 ng/mL (12-53) and 65 ng/mL (23-137) during 2T, 3T and PP; respective ICs were 1146 ng/mL (964-1676), 804 ng/mL (526-1511) and 986 ng/mL (613-1461). ATV intra/plasma ratios were 1.25 (1.12-1.89), 1.36 (0.61-2.68) and 1.05 (0.52-2.37); RTV intra/plasma ratios were 33.19 (22.75-46.39), 29.21 (14.80-63.12) and 16.6 (7.19-1.36). ATV ICs concentrations and intra/plasma ratios showed non-significant changes over time (Wilcoxon's p>0.05) while RTV intra/plasma ratios were lower during the third trimester and postpartum (p=0.03 and p=0.05).

**Conclusions:** Intracellular ATV exposure was unchanged during second and third trimester supporting the standard ATV/RTV 300/100 mg dosing throughout pregnancy.

**775 Pharmacokinetics of Increased Dose Darunavir During Late Pregnancy and Postpartum**Alice Stek<sup>1</sup>; Brookie M. Best<sup>2</sup>; Edmund Capparelli<sup>2</sup>; Jijia Wang<sup>3</sup>; David E. Shapiro<sup>4</sup>; Tim R. Cressey<sup>5</sup>; Elizabeth Smith<sup>6</sup>; Regis Kreitchmann<sup>7</sup>; Nahida Chakhtoura<sup>8</sup>; Mark Mirochnick<sup>9</sup><sup>1</sup>Univ of Southern California, Los Angeles, CA, USA; <sup>2</sup>Univ of California San Diego, San Diego, CA, USA; <sup>3</sup>Harvard Sch of PH, Boston, MA, USA; <sup>4</sup>Cntr for Biostatistics in AIDS Rsr, Harvard Sch of PH, Boston, MA, USA; <sup>5</sup>Harvard Sch of PH, Boston, MA, USA; <sup>6</sup>NIAID, NIH, Bethesda, MD, USA; <sup>7</sup>Santa Casa de Misericordia de Porto Alegre, Porto Alegre, Brazil; <sup>8</sup>Eunice Kennedy Shriver NICHD, Bethesda, MD, USA; <sup>9</sup>Boston Univ Sch of Med, Boston, MA, USA

**Background:** IMPAACT P1026s previously demonstrated that during pregnancy darunavir (DRV) exposure was reduced by 26% compared to postpartum with DRV/ritonavir (RTV) 600/100 mg bid. We hypothesized maternal plasma DRV exposure during pregnancy would increase with DRV/RTV 800/100 mg bid.

**Methods:** IMPAACT P1026s is an ongoing, non-blinded study of antiretroviral pharmacokinetics (PK) in pregnant women that includes a cohort in the USA taking DRV/RTV 800/100 mg bid. Intensive steady-state 12 hour PK profiles were performed during the 2<sup>nd</sup> trimester (2T), 3<sup>rd</sup> trimester (3T) and 2-6 weeks postpartum (PP). DRV and RTV plasma

concentrations were measured using HPLC; DRV detection limit was 0.09 mcg/mL. Target DRV exposure was 43.6 mcg<sup>2</sup>hr/mL, ≥ 70% of median AUC in non-pregnant adults on 600/100 mg.

**Results:** PK data are available for 17 women (10 Black, 7 Hispanic). At 3T, median age (range) was 28.3 (18.5–41.8) yr, weight 80.7 (53.3–133.5) kg, gestational age (GA) 33.3 (30.1–34.9) wk, median duration of DRV/r use was 123 (3–316) wk. Median CD4 was 451/mm<sup>3</sup> (128–1140) in 3T and 469/mm<sup>3</sup> (220–1164) PP. VL was <400 in 3T and delivery in 15/16 women and in 12/15 women PP. Median (range) GA at delivery was 39.0 (32.6–41.0) wk; birthweight 3390 (1950–3970) g. 8 infants are confirmed HIV negative; for 9 status is pending. Table presents DRV and RTV PK data for these subjects and, for comparison, DRV PK data from our previous P1026s cohort receiving DRV/RTV 600/100 mg. Median DRV AUC with 800 mg dose during 3T was 36% lower than with 600 mg dose PP (not statistically significant, p=0.14). Geometric mean of the individual women's 3T/PP ratios was 0.65 (90% confidence interval (CI) 0.54–0.78; p<0.001). C max and C last were significantly lower, and oral clearance was higher, in 3T compared to PP. While 63% (95% CI 35–85%) of the women met our AUC target in 3T, 93% (95% CI 68–100%) met the target PP, but this difference was not statistically significant.

**Conclusions:** These data confirm that DRV exposure is decreased during the third trimester of pregnancy. Increasing the DRV dose to 800 mg during pregnancy failed to increase DRV exposure compared to 600 mg. While viral suppression was good in our subjects, if achieving DRV exposure during pregnancy equivalent to that in nonpregnant adults is desired, other strategies, such as increasing the RTV dose or using an alternative booster, will need to be investigated.

DRV and RTV PK parameters, presented as median (interquartile range) or number (%)

PK parameter	DRV 800 mg BID 2 <sup>nd</sup> and 3 <sup>rd</sup> trim, 600 mg BID PP			RTV 100 mg BID			DRV 600 mg BID (from prior cohort P1026)	
	2nd trim (n=4)	3rd trim (n=16)	PP (n=15)	2nd trim (n=4)	3rd trim (n=16)	PP (n=15)	3rd trim (n=34)	PP (n=27)
AUC (ug <sup>2</sup> hr/mL)	68.6 (55.5-80.2)	51.8 (41.8-61.8)	80.9 (69.6-108.1)	7.5 (5.4-10.0)	5.7 (3.8-8.7)	5.9 (5.5-11.2)	45.9* (29.3- 50.5)	61.7 (49.7-80.6)
Met AUC target/total	4/4 (100%)	10/16 (63%)	14/15 (93%)	-	-	-	19/34* (56%)	22/27 (81%)
C max (ug/mL)	8.5 (7.9-8.7)	5.9* (5.4-8.5)	8.8 (7.9-10.3)	0.81 (0.62-1.23)	0.78 (0.55-0.98)	0.88 (0.71-1.43)	5.5* (4.4-7.1)	7.8 (6.4-9.5)
C last (ug/mL)	3.43 (0.8-2.0)	2.5* (2.1-3.1)	4.0 (3.3-5.2)	0.48 (0.31-0.56)	0.36 (0.19-0.42)	0.37 (0.12-0.64)	2.2 (1.7-3.3)	2.51 (2.0-3.3)
Cl/F (L/hr)	11.2 (10.0-12.7)	15.4* (13.0-19.2)	7.4 (5.6-8.6)	13.8 (10.3-20.1)	17.6 (11.6-25.2)	14.6 (9.0-17.7)	13.1* (11.4-20.5)	9.5 (7.4-11.7)

\*p<0.05 3<sup>rd</sup> trim compared with postpartum, Wilcoxon signed-rank test

776 High Risk of Liver Enzyme Elevation in Pregnant Women Receiving Protease Inhibitors

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**Background:** Antiretroviral therapy (ART) is recommended for all HIV-infected pregnant women and is highly effective in preventing vertical transmission of HIV. High rates of liver enzyme elevation (LEE) during pregnancy and some cases of severe hepatotoxicity have been reported, but the causes remain unclear. Surveillance including sequential liver function tests is recommended for all HIV+ pregnant women in France. Our objective was to estimate the prevalence, causes and risk factors of LEE in the national prospective French Perinatal Cohort.

**Methods:** We studied the 6264 HIV+ pregnant women treated with ART, enrolled in the French Perinatal Cohort between 2005 and 2014, who had ≥1 normal measure of liver enzymes during pregnancy. Adjusted hazard ratio (aHR) for the association of LEE with ART were estimated using multivariate Cox models with ART as time-dependent variable, separately for women on ART at conception and for those starting ART during pregnancy.

**Results:** Liver enzyme elevation (grade ≥1) was observed in 17.4% (n=1087), grade 3-4 in 2%. Two third occurred in the third trimester. Among women with LEE, 13% had active hepatitis B or C, 7% preeclampsia, 11% intrahepatic cholestasis of pregnancy, and less than 1% other identified causes (sickle cell disease, malaria or bile duct obstruction). More than 2/3 of LEE were unexplained: some of them were the reason for hospitalizations (n=53), cesarean sections (14), inductions of labor (3), and changes in ART regimens (36). Unexplained LEE was significantly associated with higher risk of preterm births; p<0.001.

In the women already on ART at conception, the risk of unexplained LEE was lower with NNRTI-based regimens (n=633) than with ritonavir-boosted protease inhibitors (PI/r n=1557): aHR=0.48 [0.29-0.79]. The risk was similar with boosted and non-boosted PIs (n=127): aHRs = 0.80 [95%CI 0.37-1.72] with no difference among the various PI drugs. Among the women initiating ART during pregnancy, most regimens included PIs (89%). Compared with lopinavir/r (the most prescribed PI), LEE were less frequent for nelfinavir, tended to be higher for darunavir/r, and similar for atazanavir/r (Table1). The few women who initiated NNRTI, NRTI monotherapy or dual therapy as first treatment were excluded from this analysis.

**Conclusions:** The rate of LEE among HIV-infected women is high and impacts obstetrical care management. In most case, the cause remained undetermined. Our results suggest a possible role of PIs which needs further investigation.

Table 1. Association between protease inhibitors and liver enzyme elevations (grade 1-4) among women initiating PI-based ART during pregnancy in the ANRS-French Perinatal Cohort.

Protease Inhibitors (time dependent variables)	n	Bivariable Cox model			Multivariable Cox model		
		HR	95%CI	p	aHR	95%CI	p
Darunavir/r	80	1.5	[0.9-2.7]	0.008	1.7	[0.9-3.1]	0.01
Fosamprenavir/r	24	2.1	[0.9-5.1]		1.9	[0.7-5.2]	
Atazanavir/r	110	0.9	[0.5-1.7]		0.9	[0.5-1.7]	
Indinavir/r	55	0.9	[0.4-2.2]		0.7	[0.2-2.2]	
Saquinavir/r	126	1.3	[0.8-2.1]		1.5	[0.9-2.3]	
Lopinavir/r	1638	1	ref		1	ref	
Nelfinavir	198	0.3	[0.1-0.6]		0.3	[0.1-0.7]	

PI: protease inhibitor, ART: antiretroviral therapy, n: number in the first ART-regimen during pregnancy; CI : confidence interval; /r: ritonavir-boosted; aHR: Hazard ratio adjusted for time of initiation, nucleoside transcriptase inhibitors used concomitantly, alcohol use, body mass index, CD4 count and viral load at delivery, twin pregnancy, and parity.

### 777 Pharmacokinetics of Daily Nevirapine in Neonates at High Risk of HIV Acquisition

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**Background:** Infants born to HIV-infected women with no or a short duration of antiretroviral treatment during pregnancy are at high-risk of perinatal transmission. Nevirapine (NVP) is a key component of antiretroviral (ARV) prophylaxis for infants at high risk of intrapartum HIV infection. We developed a population pharmacokinetic (PK) model to describe NVP concentrations in infants from birth through the first 2 weeks of life.

**Methods:** Infants were enrolled in an adaptive single-arm, multicenter trial in Thailand assessing 'Perinatal Antiretroviral Intensification' to prevent mother-to-child transmission of HIV in pregnant women with <8 weeks of triple ARV treatment prior to delivery (ClinicalTrials.gov NCT01511237). Intensification consisted of maternal single-dose NVP (sd-NVP) during labor and an infant 2 week course of AZT+3TC+ NVP, followed by AZT+3TC for 2 weeks. NVP dosing was 2 mg/kg for 7 days, then 4 mg/kg for 7 days. Infant blood samples were drawn from the umbilical cord, on the first day of life and at 2 weeks. NVP population PK parameters were estimated using non-linear mixed-effects regression models. Monte Carlo simulations were performed to estimate the probability of achieving target NVP trough concentrations (C<sub>24</sub>) for prophylaxis (>0.10 mg/L) and for therapeutic efficacy (>3.0 mg/L).

**Results:** Sixty two infants (56% male) were included. At birth, median (range) gestational age was 38.6 (35.7-41.7) weeks and weight was 2.9 (2.3-3.7) kg. NVP concentrations were best described by a one compartment PK model. Body weight influenced oral clearance (CL/F) and volume of distribution (Vd/F). Population estimates of NVP CL/F and Vd/F were 3.67 L/h and 0.144 L, respectively. Based on simulations for a 3 kg infant, without maternal sd-NVP, >88% would have a NVP C<sub>24</sub> >0.1 mg/L after 24 hours through 2 weeks. Predictions using the WHO recommended 15 mg once daily dose, >92% of infants have a NVP C<sub>24</sub> >0.1 mg/L after 24 hours. For NVP-based therapy, assuming linear kinetics, a 6 mg/kg twice daily dose produced a C<sub>24</sub> >3.0 mg/L in 72% of infants at 48 hours and 76% at 2 weeks. With 8 mg/kg twice daily the C<sub>24</sub> was predicted to be >3.0 mg/L in 81% of infants at 48 hours and 86% at 2 weeks.

**Conclusions:** The escalating NVP dose in PHPT-5 and the WHO single dose approach rapidly achieve and maintain target prophylactic concentrations over the first 2 weeks of life. Therapeutic NVP doses of 6 to 8 mg/kg twice daily should be studied in infants initiating treatment within the first few days of life.

### 778 Haematological Toxicity and Neonatal Prophylaxis in Infants at High MTCT Risk

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**Background:** Guidelines recommend use of combination neonatal prophylaxis (CNP) with 2 or 3 drugs in specific high risk situations. Data on safety of neonatal prophylaxis (NP), particularly CNP, are limited. Our aim was to identify whether NP type is associated with 1) severe or potentially life-threatening anaemia or neutropenia in the first 6 months of life and 2) haemoglobin (Hb) level and neutrophil count (NC) at ages 0-18 months.

**Methods:** An individual patient-data meta-analysis was conducted within 6 cohorts. Infants born 01/01/96 to 30/06/10, at high risk for acquiring HIV (defined according to US Guidelines) and with HIV status, Hb and NC measures available were included. CNP was defined as ≥2 antiretrovirals given for PMTCT. Hb and NC were graded using 2004 DAIDS paediatric toxicity tables. Adjusted logistic regression models assessing risk of grade 3-4 anaemia / neutropenia in the first 6 months of life included cohort, period, infant HIV and in utero ART exposure *a priori*. Mixture models of 1) Hb levels and 2) log<sub>10</sub>-transformed NC were conducted to explore associations with NP type at ages 0-18 months.

**Results:** Of the 1836 infants (969 male), 468 (25%) were preterm (<37 weeks), 1149 (63%) had been exposed to antenatal cART and 398 (22%) received CNP (126 with 3 drugs). Median duration was 5.7 weeks for 1 drug NP and 5.9 weeks for CNP. Overall, 117 (6.7%) infants had Grade 3-4 anaemia at age 0-6 months and 140 (9.1%) had Grade 3-4 neutropenia (Table). Grade 3-4 anaemia was not associated with NP type (AOR 1.09 for 1 drug and 1.70 for 3 drugs vs 2 drug NP, *p*>0.05), but was with preterm delivery (AOR 1.86 for 33-36 and 2.68 for ≤32 weeks vs term) and in utero ART exposure (data not shown). Infants receiving no NP had increased risk of grade 3-4 neutropenia (AOR 3.26 vs 2 drug NP, *p*=0.04), as did severe preterm (≤32w) infants (AOR 2.55 vs term, *p*=0.006). Overall 7746 Hb and NC results were available for 1836 infants up to age 18 months; no significant differences in predicted Hb levels or NC were apparent by NP type (Hb: coefficient -0.189 [95%CI -0.38, 0.007], *p*=0.060; NC: 0.021 [95%CI -0.012, 0.055], *p*=0.218).

**Conclusions:** A small proportion of infants experienced grade 3-4 haematological toxicity in their first 6 months of life; risk of anaemia was not associated with type of NP exposure in adjusted analysis, whilst among infants receiving NP, there was no significant difference in risk of neutropenia by number of NP drugs.

	One drug NP		Two drugs NP		Three drugs NP	
	Grade 0-2	Grade 3-4	Grade 0-2	Grade 3-4	Grade 0-2	Grade 3-4
<b>Anaemia (n=1737); N (%)</b>						
All <sup>*</sup>	1185 (93.1)	88 (6.9)	247 (93.2)	18 (6.8)	110 (90.9)	11 (9.1)
≤32 weeks GA	41 (87.2)	6 (12.8)	15 (93.8)	1 (6.3)	10 (83.3)	2 (16.7)
33-36 weeks GA	536 (91.2)	52 (8.8)	78 (89.7)	9 (10.3)	46 (86.8)	7 (13.2)
≥37 weeks GA	592 (95.2)	30 (4.8)	145 (95.8)	8 (5.2)	50 (96.2)	2 (3.8)
<b>Neutropenia (n=1544); N (%)</b>						
All <sup>**</sup>	1050 (90.5)	110 (9.5)	197 (92.5)	16 (7.5)	90 (86.5)	14 (13.5)
≤32 weeks GA	34 (85.0)	6 (15.0)	13 (81.3)	3 (18.7)	6 (60.0)	4 (40.0)
33-36 weeks GA	492 (90.6)	51 (9.4)	69 (92.0)	6 (8.0)	36 (87.8)	5 (12.2)
≥37 weeks GA	510 (90.6)	53 (9.4)	109 (94.0)	7 (6.0)	45 (91.8)	4 (8.2)

GA (gestational age); <sup>\*</sup>29 infants missing GA; <sup>\*\*</sup>24 infants missing GA

### 779 Maternal CMV Urinary Shedding in HIV-Infected Women and Congenital CMV Infection

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**Background:** Congenital cytomegalovirus (CMV), a cause of hearing loss and developmental delay, may be more prevalent in HIV-exposed infants. We evaluated CMV urinary shedding in HIV-infected pregnant women to determine if HIV-exposed infants born to mothers with CMV shedding were at increased risk for congenital CMV or HIV.

**Methods:** The study population was a subset of mother-infant pairs enrolled in NICHD HPTN 040 (perinatal trial with women identified with HIV during labor with no antiretroviral use in pregnancy) for whom maternal and infant urines were available. One mL aliquots of maternal urines were tested with qualitative Real-Time PCR for CMV DNA; those with positive results were tested by quantitative CMV PCR. Infant urines were similarly tested with mother-infant pair results correlated.

**Results:** Urine specimens were available for 260 women, the majority (85.4%) from the Americas (Brazil, Argentina, U.S.) as opposed to South Africa (14.6%). Twenty-four women (9.2%) had detectable CMV viremia by qualitative PCR. Most women (91.6%) had viremia with low level detectable CMV (<200 copies/mL) while two had 236 and 53,524 CMV copies/mL urine respectively. Ten (3.8%) infants had CMV detected in their urine at the time of birth (range of 367-592,274 copies/mL). Among mothers with detectable urinary CMV at the time of delivery, 20.8% (5/24) had infants with congenital CMV. In contrast, only five of 236 infants (2.1%) born to mothers with undetectable urinary CMV had congenital CMV (p=0.0008). The two women with higher levels of CMV viremia had infants with congenital CMV. Mean CD4 counts were similar between women with CMV viremia versus those without (441.8 vs. 481.1 cells/mm<sup>3</sup>, p=0.92). Women with CMV viremia had a higher mean HIV viral load in magnitude, but of no statistical significance (94,664 vs 70,906.8 copies/mL, p=0.91). These women were also more likely to transmit HIV to their infants (29.2% vs. 8.1%, p=0.005). Infant death was more frequent in children of women with CMV viremia as opposed to no CMV shedding (4.2% vs. 0.85%, p=0.25). Women with CMV viremia were 5 times more likely to transmit HIV (OR=4.68, 95%CI: 1.73-12.69) and 12 times more likely to transmit CMV (OR=12.16, 95%CI:3.23-45.73).

**Conclusions:** Urinary CMV shedding is more frequent than expected in HIV-infected pregnant women not on antiretrovirals. Maternal CMV viremia at the time of birth is a significant risk factor for CMV and/or HIV transmission to infants and a potential marker for adverse infant outcomes.

Maternal CMV in Urine	CMV Transmission Rate	HIV Transmission Rate	Infant Death Rate	Mean Maternal CD4 Count (cells/mm <sup>3</sup> )	Mean Maternal HIV Viral Load (copies/mL)
<b>CMV Positive</b> N=24 (9.2%)	5/24 (20.8%)	7/24 (29.2%)	1/24 (4.2%)	441.8 (SD 178.6) range (128-778)	94,664 (SD 202,185.2) range (94-718,900)
<b>CMV Negative</b> N=236 (90.8%)	5/236 (2.1%)	19/235 (8.1%)	2/236 (0.85%)	481.1 (SD 311.1) range (28-2160)	70,906.8 (SD 253,893.1) range (0-2,700,000)
<b>Total</b> N=260	10/260 (3.8%)	26/260 (10%)	3/260 (1.2%)	477.4 (SD 301.1) range (28-2160)	73,108.3 (SD 249,313.4) range (0-2,700,000)
<b>p-value*</b>	0.0008	0.0049	0.2530	0.9239	0.9134

\* Fisher's exact test for categorical variables or Kruskal-Wallis test for continuous variables  
SD: Standard deviation

**780 TLR9 Variant Is Associated With Earlier HIV, EBV, and CMV Acquisition in Infants**

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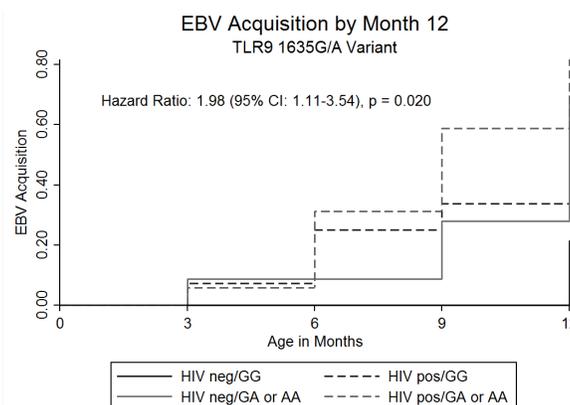
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**Background:** Cytomegalovirus (CMV) and Epstein-Barr (EBV) virus are acquired by the majority of African HIV-infected infants during the first year of life. Both viruses establish persistent infection with prolonged systemic viremia. CMV is associated with an increased risk of HIV progression, morbidity, mortality and developmental impairment. EBV is an important contributor to lymphoma in the setting of HIV. We recently reported that a single-nucleotide polymorphism in Toll-like Receptor 9 (TLR9) is associated with an increased risk of HIV acquisition in Kenyan infants. Because TLR9 recognizes unmethylated CpG sequences in viral DNA, we hypothesized that this variant may also affect risk of CMV and EBV acquisition and viral replication.

**Methods:** HIV, CMV and EBV outcomes were assessed longitudinally from birth to 1 year of age among HIV-infected and exposed infants from a Kenyan perinatal cohort. HIV infection was diagnosed using PCR for HIV DNA or the GenProbe Assay for HIV RNA. CMV and EBV DNA were measured from plasma specimens using real-time PCR, with serologic confirmation of DNA-negatives. Infants were genotyped for the TLR9 1635A/G (rs352140) polymorphism. Pearson's Chi squared tests, Cox proportional hazards, and linear regression were performed to assess the association of the 1635A/G variant with acquisition, peak viral levels, and viral suppression.

**Results:** CMV and TLR genotype data were available in 37 HIV-infected infants. More than 80% of CMV infections occurred before 3 months of age. At 1 month of age, 42% of infants with the 1635 AA or AG genotypes, and 11% of infants with 1635 GG genotype had acquired CMV (p=0.03). EBV and TLR genotype data were available in 104 HIV-exposed and infected infants. The probability of EBV infection at 12 months was 61% among HIV-infected and 19% among exposed infants. Infants with one or more copies of the 1635A allele showed an increased risk of EBV acquisition during the first year of life, adjusting for HIV status (HR=1.98, 95%CI=1.11, 3.54). No associations were found between TLR9 polymorphisms and CMV or EBV peak viral loads, or with time to CMV or EBV suppression (p>0.05 for all).

**Conclusions:** The TLR9 1635A/G variant was associated with earlier acquisition of CMV and EBV, and in a prior analysis with earlier acquisition of HIV, suggesting a key role for this locus in defense against viral infection. Determining the functional phenotype of this TLR9 variant may inform novel therapeutic or vaccination strategies.



**781 HCMV Induces CCR5 Expression, Activation and HIV Infection at Feto-Maternal Interface**Erica L. Johnson<sup>1</sup>; Sahithi Boggavarapu<sup>2</sup>; Elan S. Johnson<sup>2</sup>; Asim Lal<sup>2</sup>; Parth Agrawal<sup>2</sup>; Rana Chakraborty<sup>2</sup><sup>1</sup>Emory Univ Sch of Med, Atlanta, GA, USA; <sup>2</sup>Emory Univ, Atlanta, GA, USA

**Background:** Despite effective administration of combination antiretroviral therapy to HIV-1-infected pregnant women, mother-to-child transmission (MTCT) of HIV-1 remains a significant global public health concern. Co-pathogens that pose threats to the developing fetus include human cytomegalovirus (HCMV), may also facilitate *in utero* transmission of HIV-1 in populations where co-infection is common. HCMV infects up to 90% of reproductive-age women in developing and developed countries. We hypothesize that HCMV promotes *in utero* transmission through increased immune activation, local inflammation, and maternal stimulation of fetal/placental macrophages (or Hofbauer cells [HC]) to increase susceptibility to HIV-1. Strong associations between *in utero* HCMV infection and a higher incidence of MTCT of HIV-1 have been documented; however, the mechanisms that increase MTCT remain unknown.

**Methods:** With written informed consent, placenta/cord blood were collected from 15 HIV-1/Hep B/HCMV seronegative women (>18 years) at Emory Midtown Hospital in Atlanta, GA. In this study, myeloid subsets were exposed to HCMV *in vitro*, followed by HIV-1BaL. Cells were also treated with IFN- $\alpha$ /IFN- $\beta$ . Viral replication was determined by HIV-1 p24 ELISA, qPCR, FACS, and Western blot analysis determined the expression of CCR5, activation markers, and antiviral ISGs. Cytokines and IFNs were measured by ELISA. Data were analyzed by using Student's t-test and Mann-Whitney test.

**Results:** HCMV exposure upregulated expression of the HIV-1 co-receptor CCR5, along with activation markers (CD16, CD80 and HLA-DR) on HCs. HCMV significantly induced the secretion of proinflammatory cytokines (IL-6 and TNF- $\alpha$ ) by HCs, which enhanced their susceptibility to HIV-1 infection. We also showed type-I IFNs alone potently restrict HIV-1 replication in HCs, however HCMV infection inhibits Stat2 activation, which may override type-I IFN-induced protection to promote HIV-1 infection.

**Conclusions:** These data suggest that activation and local inflammation induced by maternal HCMV *in utero* alter CCR5 expression in macrophages at the feto-maternal interface to promote MTCT of HIV-1. We also present evidence that HCMV inhibits type-I IFN-dependent Stat2 signaling in the placenta, which may disrupt the intrinsic antiviral response in this compartment. Identifying biological determinants of MTCT provides a sentinel foundation upon which to design strategies for prenatal prophylaxis and the development of effective immunotherapies.

**782 Population-Level Declines in Vertical HIV Transmission With Changing PMTCT Guidelines**Jean Maritz<sup>1</sup>; Nei-Yuan Hsiao<sup>2</sup>; Wolfgang Preiser<sup>1</sup>; Landon Myer<sup>2</sup><sup>1</sup>Stellenbosch Univ, Cape Town, South Africa; <sup>2</sup>Univ of Cape Town, Cape Town, South Africa

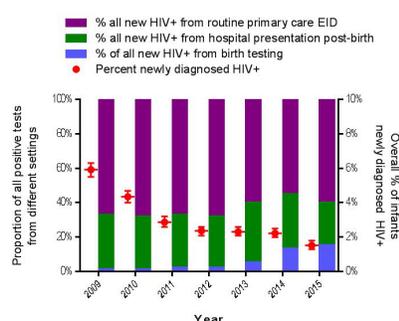
**Background:** Despite major shifts in policies for prevention of mother-to-child HIV transmission (PMTCT) there are few population-level data on the changing epidemiology of perinatal HIV in South Africa (SA).

**Methods:** We examined data on all HIV PCR tests conducted by the SA National Health Laboratory Service in children  $\leq 6$  months of age in the Western Cape province between January 2009 and August 2015. During this period, PMTCT policies moved from zidovudine monotherapy with antiretroviral therapy (ART) for pregnant women based on CD4+ thresholds of  $\leq 200$  cells/ $\mu$ L before July 2010, to  $\leq 350$  cells/ $\mu$ L by June 2013, to ART for all HIV+ pregnant women ("Option B+") from July 2013. Breastfeeding guidance shifted to support exclusive breastfeeding with nevirapine prophylaxis for the first 6 months of life from 2011. Analyses examined changes in HIV PCR testing (i) annually as well as (ii) by PMTCT policy period (with time lags to allow for delivery and postpartum testing), and (iii) distinguished positive tests from birth testing (conducted at  $\leq 7$  days of age) from routine early infant diagnostic (EID) testing at primary care clinics, from hospital-based testing associated with symptomatic clinical presentations.

**Results:** Overall 99,684 children underwent HIV PCR testing. Among all children tested at  $\leq 6$  months of age, the HIV PCR positivity rate dropped from 5.9% in 2009 to 1.5% in 2015 (Figure); the rate of positive tests after implementation of "Option B+" (2.0%) was significantly lower than the period when ART thresholds were based on  $\leq 350$  cells/ $\mu$ L (2.5%;  $p < 0.001$ ). The mean age at first HIV PCR test decreased from 59.1 days in 2009 to 44.7 days in 2015 ( $p < 0.001$ ); approximately half of this change was explained by increasing numbers of HIV PCR tests conducted at birth during this period, which grew from  $< 1\%$  to 14% of all tests conducted (Figure). Among tests conducted beyond 7 days of age, a growing proportion took place as part of routine EID testing over time rather than hospital-based testing ( $p < 0.001$ ). Despite increases in birth testing, during 2014-2015 only 13% of all newly diagnosed HIV+ infants were identified through birth testing; the new infections were identified either through routine EID (56%) or hospital-based testing (31%).

**Conclusions:** There have been major reductions in perinatal HIV infections with changing PMTCT policies in this setting. Despite the rise of birth testing, the majority of HIV+ infants are still identified through routine EID programmes.

Figure. Proportion of all positive HIV PCR tests from different clinical settings and decreasing HIV positivity rate in the Western Cape province, South Africa, 2009 – 2015

**783 Introduction of Birth Testing Into the South African National Consolidated Guidelines**Ahmad Haeri Mazanderani<sup>1</sup>; Tendesayi Kufa-Chakezha<sup>1</sup>; Gayle Sherman<sup>2</sup><sup>1</sup>NICD, Johannesburg, South Africa; <sup>2</sup>Univ of the Witwatersrand, Johannesburg, South Africa

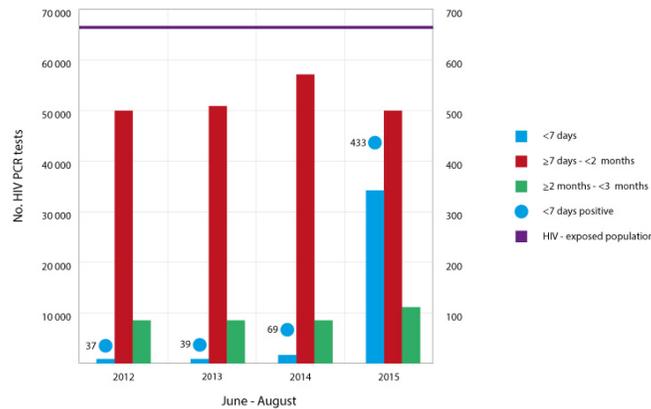
**Background:** Birth testing of all HIV-exposed infants was introduced into the South African National Consolidated Guidelines on 1 June 2015. Prior to this, routine HIV PCR testing was performed in infants at 6 weeks of age, with earlier testing in symptomatic infants. In 2014, when the estimated 6-week transmission rate was 1.8%, targeted birth testing of high risk neonates was introduced. We describe at a national level, the number and results of HIV PCR tests done at  $< 7$  days,  $< 2$  months, and  $< 3$  months of age during the period 01 June to 31 August 2015 and compare with the same period for 2012-2014 to determine immediate uptake of birth testing following implementation of new guidelines.

**Methods:** HIV PCR test data from 2012-2015 was extracted from the Corporate Data Warehouse of the National Health Laboratory Service, a central data repository of all registered test-sets within the public health sector in South Africa. Data was extracted by year, month, age and result. Birth testing coverage was calculated as a proportion of HIV exposed neonates tested for HIV at age  $< 7$  days over the number of HIV exposed neonates requiring testing (live births x maternal HIV seroprevalence). The estimated intra-uterine HIV transmission rate for South Africa was determined by calculating the positivity rate of all HIV PCR specimens in neonates aged  $< 7$  days.

**Results:** For the 3 months since the implementation of birth testing there is a substantial increase in the number of HIV PCR tests done at <7 days amounting to 35 559 tests from a baseline of 1091 in 2013 and 2355 in 2014 when targeted birth testing was introduced. This trend is reflected in all 9 provinces with the national birth testing coverage estimated at 61% for August 2015. The number of HIV PCR positive tests during the first week of life increased to 433 compared with 69 positive tests for the same period in 2014. The national intra-uterine transmission rate is estimated at 1.2%. The decrease in testing at <2 months (by 6880 tests) and increase at <3 months (by 3172 tests) likely reflects transition from 6 week to 10 week testing.

**Conclusions:** These results suggest that universal HIV PCR testing at birth for all HIV-exposed neonates can be achieved in South Africa and will assist in earlier detection of intra-uterine HIV infected infants. Priorities remain successful linkage into care and monitoring outcomes of neonates who test HIV PCR positive at birth, and to ensure repeat testing at 10 weeks for neonates who test HIV PCR negative at birth.

Introduction of Birth Testing into the South African National Consolidated Guidelines



784 Infant Cotrimoxazole Prophylaxis Associated With Commensal Bacterial Resistance

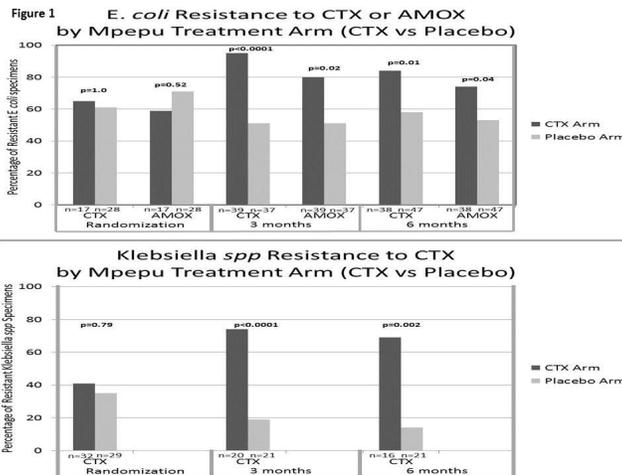
**Kathleen Powis<sup>1</sup>**; Sajini Souda<sup>2</sup>; Shahin Lockman<sup>3</sup>; Gbolahan Ajibola<sup>4</sup>; Kara Bennett<sup>5</sup>; Florence Chilisa<sup>6</sup>; Michael Hughes<sup>6</sup>; Sikhulile Moyo<sup>7</sup>; Joseph Makhema<sup>4</sup>; Roger L. Shapiro<sup>6</sup>  
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**Background:** Cotrimoxazole (CTX) prophylaxis reduces morbidity and mortality in HIV-infected children, even in settings with high background rates of CTX-resistant pathogens. However, with declining mother-to-child HIV transmission globally, the risks and benefits of infant empiric CTX prophylaxis need to be re-evaluated, including the potential for higher rates of resistance to CTX and other antibiotics following CTX prophylaxis.

**Methods:** The Mpepu Study randomized HIV-exposed infants to either CTX or placebo starting between 14-34 days of life and continued through 15 months of age. In 2014-2015, stool was collected from a subset of participating infants at randomization and at 3 and 6 months of age, and stored at -70°C prior to culture. In specimens that grew *E. coli* or *Klebsiella spp*, antibiotic susceptibility testing by Kirby Bauer method was done for CTX (CTX 25µg) in Mueller Hinton agar. Amoxicillin (Amox 10µg) testing was also performed for *E. coli* isolates. Clinical & Laboratory Standards Institute guidelines for disc diffusion were used to classify resistant isolates. Fisher's exact testing was used to compare resistance by randomization arm (CTX/placebo) and to evaluate clinical outcomes.

**Results:** A total of 380 stool samples from 221 infants were cultured: 116 at randomization, 152 at 3 months, and 112 at 6 months. Two hundred and six samples grew *E. coli*, 139 samples grew *Klebsiella spp*, and 101 samples grew other species. Resistance to both *E. coli* and *Klebsiella spp* was common at the randomization visit and did not differ by study arm (Figure 1). At 3 and 6 months, infants randomized to CTX were significantly more likely to have *E. coli* and *Klebsiella spp* resistant to CTX (all comparisons p<0.01), and *E. coli* isolates were also more likely to be resistant to Amox (all comparisons p < 0.05). Of 78 infants with CTX resistant *E. coli* and/or *Klebsiella spp* at 3 and/or 6 months, 1% died, 4% had grade 3/4 diarrheal illness, and 1% had grade 3/4 pneumonia in the first 12 months. Clinical comparison to CTX-sensitive infants was precluded, as there were only 8 such infants.

**Conclusions:** In the setting of high baseline CTX- and Amox-bacterial resistance, infant CTX prophylaxis further increased both CTX- and Amox-resistant isolates of commensal gastrointestinal bacterial. There were few adverse clinical events among infants with resistance. Additional research is needed to determine the longer-term clinical, microbiologic, and public health impact of resistance selected by CTX prophylaxis.



## 785 Low Uptake of Routine Infant Diagnostic Testing Following HIV PCR Testing at Birth

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**Background:** There is growing emphasis on testing HIV-exposed infants at birth to detect intrauterine infections. However concerns have been raised that implementation of birth testing may reduce uptake of routine early infant diagnostic testing (EID) at around 6 weeks of age. As there are no data on this question, we examined the association between birth PCR and subsequent routine EID testing in the Western Cape province of South Africa (SA), a setting where 6-week EID test coverage is estimated to reach >70% of all HIV-exposed infants.

**Methods:** Data on all HIV PCR tests conducted in the province (approximately 14 000 per year) were accessed from the SA National Health Laboratory Service. Infants with birth PCR (defined as testing in the first 7 days of life) and subsequent EID testing (8 to 182 days of age) were linked probabilistically using Finegrained Record Integration and Linkage Tool (Atlanta, USA); a range of sensitivity analyses were used to maximise linkage of birth tests to subsequent EID testing. Two periods of birth testing policies were compared: discretionary testing by clinicians (DT; Jan 2009- March 2014) and testing of all 'high risk' pregnancies based on duration of maternal ART, documented HIV viraemia, and related factors (HR; April 2014-June 2015).

**Results:** Overall 3322 newborns received birth testing (80% within three days of life), comprising 3% of all HIV PCR tests conducted in infants. The number of tests increased >20-fold after the start of HR testing policies. Throughout most birth tests were undertaken in obstetric hospitals, though the proportion of birth tests from primary care facilities increased from 11% under DT to 33% under HR testing (Table). Birth PCR positivity rates decreased from 6% in 2009 to 1.6% in 2015 ( $p=0.001$ ). Of children with negative birth PCR results, only 49% had any evidence of a follow-up EID test. This proportion decreased to 43% when restricted to a window around prescribed EID testing, but was stable over time with no difference in follow-up testing rates or mean age at retesting under DT vs HR testing policies ( $p=0.506$  and  $0.112$ , respectively).

**Conclusions:** Changing birth testing policies have led to dramatic increases in birth tests. However follow-up EID testing rates in infants testing PCR negative at birth are substantially lower than local estimates of 6-week EID coverage. These data suggest that implementation of birth testing will require particular care to avoid undermining postpartum EID services.

**Table.** Numbers of HIV PCR tests conducted at birth, birth test results, and follow-up early infant diagnosis (EID) testing rates across the Western Cape province, South Africa, under different birth testing policies, 2009-2015. Figures represent numbers and percentages.

Period dates	All children with birth tests	Clinician discretion birth testing policy	'High risk' birth testing policy	p-value
	Jan 2009 - June 2015	Jan 2009 to Mar 2014	Apr 2014 to Jun 2015	
Number of birth tests conducted	3322	1094	2228	
Mean birth tests conducted per month	43	17	159	
Number of facilities with birth testing	113	44	113	
Test location:				
Obstetric hospital	2459 (74)	975 (89)	1484 (67)	0.001
Primary care clinic	863 (26)	119 (11)	744 (33)	
Test result:				
Negative	3179 (95.7)	1027 (93.9)	2152 (96.6)	
Indeterminate	40 (1.2)	19 (1.7)	21 (0.9)	0.001
Positive	103 (3.1)	48 (4.4)	55 (2.5)	
Children with negative birth test linked to any repeat test from 8-182 days of age	1547 (49)	491 (48)	1056 (49)	0.506
Children with negative birth tests linked to any repeat test from 28-112 days of age (denoting EID)	1368 (43)	386 (38)	982 (46)	0.249
Mean (SD) in days age at repeat testing	51.7 (0.6)	50.3 (1.2)	52.3 (0.7)	0.112

## 786 The Value of Confirmatory Testing in Early Infant HIV Diagnosis (EID) Programs

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**Background:** EID assays have high specificity, but positive predictive value is poor when MTCT risk is low. HIV-infected infants treated with ART may lose detectable antibody, RNA, and DNA. Uninfected infants who start ART after false positive (FP) results are therefore difficult to identify. Confirmatory testing is recommended to avoid ART in uninfected infants; while common in South Africa, it is not routine in many settings. We projected clinical outcomes, FP (incorrect) ART initiations, costs, and cost-effectiveness of EID programs with and without confirmatory testing.

**Methods:** We used the CEPAC-Pediatric model to simulate 6-week EID testing for a cohort of infants born to HIV-infected mothers. We used clinical, cost, and EID assay data from South Africa, as an example (laboratory-based nucleic acid assay: specificity 98.8%; result-return time 1 month; cost \$25). We simulated no EID (comparator), EID with confirmation (ART initiation immediately after first positive test; cessation if negative confirmatory test), and EID without confirmation (ART initiation after single positive test). For uninfected infants with FP results, we assumed 25 years of HIV care and ART, and excluded toxicity and stigma. We calculated incremental cost-effectiveness ratios (ICERs) in \$/year of life saved (YLS).

**Results:** Projected MTCT risk at 6 weeks of age was 3.0%. EID programs with confirmation markedly increased life expectancy for HIV-infected infants, from 21.1 years (no EID) to 26.3 years (Table). Of every 1000 ART initiations, 6 were FP, accounting for 0.2% of cohort lifetime HIV care costs (total lifetime costs: \$1,770/infant tested). The ICER of EID with confirmation compared to no EID was \$1,200/YLS (<0.2x South Africa's per-capita GDP of \$6,500). EID without confirmation led to similar clinical outcomes, but at greater lifetime costs (\$2,090/infant tested); 297 of every 1000 ART initiations were FP, accounting for 14.3% of lifetime HIV care costs. Results were sensitive to assay specificity, MTCT risk, ART delay for confirmatory testing, and duration of ART after FP results.

**Conclusions:** Without confirmatory EID testing, nearly 30% of infants testing positive and initiating ART could be truly HIV-uninfected in low-MTCT settings, and care for FP infants could comprise a substantial fraction of HIV program costs. Confirmation of positive EID results is cost-saving compared to EID without confirmation in South Africa, and is critical before ART initiation in all settings.

**Table: Model-based projections of the impact of false positive early infant diagnosis results in South Africa**

	Life expectancy (HIV-infected infants, years)	False positives per 1000 ART initiations	Lifetime cost per HIV-exposed infant (\$)ª	Proportion of total lifetime costs due to care for false positives	ICER (\$ per year of life saved)ᵇ
No EID	21.1	N/A	\$1,430	N/A	---
6 week EID with confirmatory testing	26.3	6	\$1,770	0.2%	1,200
6 week EID without confirmatory testing ¸	26.3	297	\$2,090	14.3%	Dominatedᵈ

ICER: incremental cost-effectiveness ratio; N/A: not applicable  
 a. Costs are in 2013 US dollars (\$).  
 b. ICERs are calculated from life expectancy and lifetime costs for all HIV-exposed infants, including both HIV-infected and HIV-uninfected infants, discounted at 3%/year. Discounted results not shown in Table.  
 c. We simulate ART initiation after the first positive EID assay result is received, with ART cessation if a confirmatory assay is subsequently negative. Because HIV-infected infants do not delay ART initiation for a confirmatory test result, the projected life expectancy for both EID strategies is similar.  
 d. Dominated: a strategy that is more expensive and either equally effective or less effective than an alternative strategy, reflecting an inefficient use of healthcare resources.

**787 Diagnostic Accuracy of Cepheid Xpert HIV-1 Qual for Early Infant Diagnosis**

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**Background:** Among HIV-exposed infants, delays in early infant diagnosis (EID) result in high morbidity and mortality. The World Health Organization recommends testing of HIV-exposed infants at 6 weeks for EID. In many resource-limited settings, testing is available only in centralized laboratories, resulting in delays in diagnosis. Cepheid recently released Xpert HIV-1 Qual (Xpert), the first near point-of-care test for EID. The diagnostic accuracy of this new test was evaluated in a central laboratory in Harare, Zimbabwe.

**Methods:** Paired testing of dried blood spot samples from 446 HIV-exposed infants and children ≤18 months was performed using Xpert and Roche Cobas AmpliPrep/Cobas TaqMan (Roche), with Roche serving as the comparator. Samples were collected between January and August 2015 and tested in a central laboratory in Harare, Zimbabwe. Archived HIV PCR-positive samples were used to enable sensitivity to be estimated with reasonable precision.

**Results:** Of the pairs of samples tested, 174/446 (39.0%) were positive on Roche. The median age at testing was 6.9 weeks (interquartile range: 6.1 – 15.3 weeks). Of those with information on feeding, 317/403 (78.7%) were breastfed exclusively. The sensitivity of Xpert was 96.0% (95% confidence interval [CI]: 91.9 – 98.4%) and the specificity was 100% (95% CI: 98.7 – 100%).

**Conclusions:** Xpert HIV-1 Qual is a promising test for use at the point of care in resource-limited settings. These findings need to be confirmed by similar studies with decentralized point-of-care testing under conditions of intended use.

**788 Point-of-Care p24 Infant Testing May Increase Patient Yield Despite Low Sensitivity**

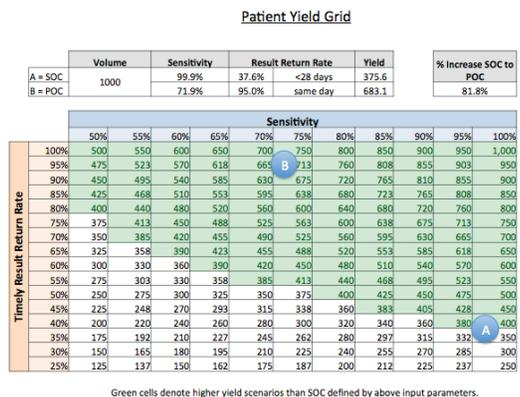
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**Background:** Poor access to early infant HIV diagnosis (EID) is a primary cause for the antiretroviral treatment (ART) gap in HIV-infected children. A point-of-care (POC) test for EID may help overcome this challenge by eliminating the need for centralized nucleic acid testing (NAT) and enabling prompt referral to ART. We evaluated the accuracy of a POC p24 HIV Antigen Test in primary health clinics (PHC) in Mozambique and estimated its impact on patient yield.

**Methods:** POC p24 and laboratory-based NAT for EID were conducted in 879 HIV-exposed infants under 18 months of age that were enrolled consecutively at three peri-urban PHC in Maputo City. Lancet-drawn blood specimens were tested on site by nurses using a prototype POC test for p24 antigen detection. The same blood draw was used to create dried blood spots for laboratory testing. Sensitivity, specificity and predictive values were calculated for the POC assay. The potential impact of the POC assay on patient yield was estimated based on a grid (Figure 1) mapping the intersection of sensitivity and timely result return rates. We used an estimate of 95% for same-day results observed in POC environments and national EID program data indicating 37.6% of results returned within 28 days for standard of care (SOC).

**Results:** The sensitivity and specificity of POC p24 EID testing were 71.9%; (95% confidence interval [CI]: 58.5-83.0%) and 99.6% (95% CI: 98.9-99.9%), respectively. Overall agreement was high (Cohen Kappa = 0.80; 95% CI: 0.71-0.89). Positive 81.2% (95% CI: 72.9-89.5%) and negative 98.8% (95% CI: 98.3- 99.3%) test agreements were also high. The predictive value of a positive test was 93.2% (95% CI: 81.3-98.6%) and the predictive value of a negative test was 97.9% (95% CI: 96.8-98.8%). When compared to SOC, the use of this POC p24 test could link up to 81% more patients to timely care in Mozambique. Return rates of SOC would have to improve to 68.3% before SOC yields would surpass those of this POC test.

**Conclusions:** This prototype p24 assay was feasible for near-to-patient use in PHC but showed a low sensitivity for detecting HIV-infected infants. POC technologies that perform below ideal thresholds may still be valuable diagnostic tools given their potentially significant impact on linkage to care.



**789 Malawi's Option B+ 2011-2015: The Impact of Rapid ART Decentralization**

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**Background:** In July 2011, Malawi began offering lifelong ART to all HIV-infected pregnant and breastfeeding women, regardless of clinical or immunological stage (Option B+). To scale up this policy, Malawi fully integrated PMTCT and ART services and decentralized services to all facilities. Critical enablers included highly simplified and standardized clinical guidelines aiming to eliminate barriers to ART services at peripheral sites; an established system of quarterly supervision to all sites to ensure basic quality of service and guideline adherence; integrated M&E tools; and nurse-based ART initiation and follow up.

**Methods:** Quarterly supervision includes collection of verified ART cohort reports from all sites. This data is entered into a central database at MOH to create the Quarterly HIV Program Reports. We analyzed this site-level data to describe annual program performance 1 year before and 4 years after introduction of Option B+.

**Results:** Within 4 years, the number of active ART sites increased from 303 to 714, and the proportion of antenatal clinics providing ART services increased from 37% in June 2012 to 98% in June 2015. The percent of ART sites which were rural (55% to 73%), health centers (47% to 69%) or enrolling fewer than 50 new ART patients per quarter (52% to 73%) all increased from 2011 to 2015. Total annual ART initiations (73,805 to 107,181), ART initiations among pregnant women (4,850 to 25,851) and patient transfers between sites (16,978 to 26,514) all increased as a result of decentralization. Between July 2014 and June 2015, 94% of sites enrolled both Option B+ and general ART patients. ART coverage among known HIV-infected pregnant women increased from 22% to 95%. The proportion of sites with 12 month retention >80% declined from 56% in the year prior to Option B+ to 49% four years after. In June 2015, 7% of sites were scored as 'in need of urgent clinical mentoring', compared to 22% in June 2012.

**Conclusions:** In Malawi, full decentralization of ART and integration with PMTCT successfully led to a rapid increase in patients initiating ART and ART coverage among pregnant women. Integration was evident in the high proportion of sites enrolling both Option B+ and general ART patients. Through quarterly supervision, service delivery models and service quality at sites improved over time. Routine service data are known to underestimate ART retention by about 10% due to unrecorded patient transfers or visits. Actual 12 month retention has remained stable or decreased slightly.

## 790 Optimizing PMTCT Outcomes in Rural North-Central Nigeria: A Cluster-Randomized Study

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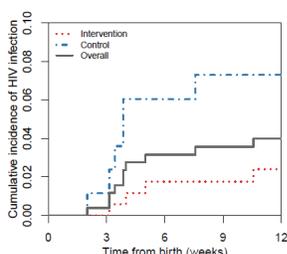
**Background:** Antiretroviral therapy (ART) and retention in care are essential for prevention of mother-to-child HIV transmission (PMTCT). In a randomized trial, we assessed the impact of a family-focused, integrated PMTCT care package on maternal ART initiation, post-partum mother-infant pair retention and infant HIV infection.

**Methods:** We pair-matched 12 sites, randomizing clinic-pairs to intervention vs. control (SOC) arms. Standard-of-care (SOC) services included: group provision of health information; opt-out HIV testing with same-day results; infant feeding counseling; referral to hub centers for CD4/treatment initiation; home-based care services; infant prophylaxis and early infant diagnosis. Intervention sites received SOC plus (a) Point-of-care CD4+cell count testing; (b) de-evolution of decentralized PMTCT tasks to trained lower-cadre providers; (c) integrated mother-infant care; and (d) active male partner and community involvement. A generalized linear mixed effects model with random effect for matched clinic-pairs was used to determine intervention effects for maternal ART initiation and retention of mother-infant pairs at 6- and 12-weeks postpartum.

**Results:** We enrolled 369 participants (n=172, intervention; n= 197, SOC). Participants were comparable across arms for marital status, time of HIV diagnosis, and distance to facility. Enrolment median CD4+ cell count was higher in intervention mothers than in SOC (424 cells/ $\mu$ L (IQR: 268-606) vs. 314 cells/ $\mu$ L (IQR: 245-406), p<0.001). Most participants were WHO clinical stage 1 (98%), of high functional status (working/ambulatory, 99%), and delivered vaginally (96%). After adjusting for age, education, travel time to facility, employment, maternal ethnicity, and time of HIV diagnosis, intervention mothers were significantly more likely to initiate ART than SOC (Relative Risk, RR [95% Confidence Interval, CI]= 3.3 [1.4-7.8]). Mother-infant pairs in the intervention arm had comparatively higher likelihood than SOC arm pairs of being retained in care at 6- and 12-weeks postpartum (RR=9.1 [5.2-15.9] and 10.3 [5.4-19.7], respectively). At the 12 week visit, 2.4% (95% CI: 0.9-6.3%) of infants tested HIV-positive in the intervention arm vs. 7.3% (95% CI: 3.3-15.6%) in the control arm.

**Conclusions:** This integrated, family-focused service package improved maternal ART uptake and retention and reduced infant HIV infection.

Figure: Cumulative incidence of infant HIV infection during the first 12 weeks by trial arm in 12 rural sites, Niger state, Nigeria



## 791 Randomized Trial of a Lay Counselor-Led Combination Intervention for PMTCT Retention

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**Background:** Despite simplification of prevention of mother-to-transmission (PMTCT) services, retention of HIV positive (HIV+) mothers and infants across the PMTCT-pediatric care continuum remains problematic. A variety of interventions have been proposed to improve retention but rigorous assessment of their efficacy to improve maternal and child outcomes are scarce.

**Methods:** The Maternal-Infant Retention for Health (MIR4Health) study was conducted at 10 PMTCT sites in Nyanza, Kenya, between Sept 2013 –Sept 2015, to evaluate the effectiveness of a combination package of lay counselor administrated evidence-based interventions (Active Patient Follow-up/APFU) compared with the Standard of Care (SOC) on mother-infant retention. HIV+ pregnant women starting antenatal care were randomized to APFU (lay counselor administered individualized health education, home visits, phone and short message service appointment reminders, physical tracing immediately after missed clinic visits, and individualized retention and adherence support) vs. routine PMTCT/postnatal HIV care as per national guidelines. Retention of mother-infant pairs was defined as documented clinic attendance of mother and infant at 6 months (mos) postpartum  $\pm$  3 mos. Intent-to-treat analysis was used to assess the difference in retention between arms. Further analysis was done excluding women with pregnancy complications, neonatal/infant death, and transfer-out to compare lost-to-follow-up (LTFU).

**Results:** 340 HIV+ pregnant women were randomized to APFU (170) or SOC (170): 106 (31%) were known HIV+ (58 APFU, 48 SOC arms); median gestational age 24 weeks (IQR 17-28); median CD4+ 426 cells/mm<sup>3</sup> (IQR 274-601). A total of 142 (83.5%) APFU women had a documented live birth vs. 130 (76.5%) SOC women (p=0.10). At 6 mos postpartum, 130 mother-infant pairs were retained in the APFU arm vs. 112 the SOC arm. APFU subjects were 16% more likely to be retained at 6 mos postpartum compared to SOC (RR=1.16, 95% CI: 1.01-1.33; p=0.03). After excluding pregnancy complications, transfers and neonatal/infant deaths, 10.3% of the APFU arm and 18.8% of the SOC arm were LTFU (RR=0.55, 95% CI: 0.30-0.99; p=0.04). There were 3 infants testing HIV DNA PCR positive in the APFU arm and 6 in the SOC arm (p=0.25).

**Conclusions:** Engaging lay workers to provide a combination package of evidence-based interventions improved retention and reduced loss to follow-up among mother-infant pairs in a high prevalence community in Nyanza, Kenya.

**792 Continuity of Care Among Pregnant Women Lost to Follow-up After Initiating ART**

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**Background:** African countries are implementing Option B+, but high loss to follow-up (LTF) among pregnant women on antiretroviral therapy (ART) threatens program success and mother/infant lives. Due to the lack of linked data, LTF estimates do not account for unreported transfers. We hypothesize that “clinic shopping” and rural-urban travel after delivery may inflate LTF estimates. To test this, we traced lost patients using a national lab database in South Africa to assess continuity of care and update LTF estimates.

**Methods:** We included all HIV+ women initiating ART during pregnancy at seven clinics in Gauteng Province from 1 Jan 2012 to 31 July 2013 and considered LTF (no visit >3 mo; n=312). Using name and date of birth, we manually searched the National Health Laboratory Service database. Records were categorized as from the initiation site or a new facility. Continued HIV care was defined as accessing care after ART initiation at a new facility shown by ≥1 CD4 or viral load test on record, or any record from a new ART clinic. “Clinic shoppers” were defined as seeking care at a new ART facility within Gauteng.

**Results:** At ART initiation, median age was 29 years (IQR:25-33) and CD4+ cell value was 268 cells/μL (200-340). Median time between initiation and last clinic visit was 112 days (29-268). Records were missing—including from the initiation site—for 115 (36.9%) women. Of the 197 located, 97 (49.2%) continued HIV care at a new facility. Most (71.1%) were clinic shoppers; 28.9% sought care in other provinces. Overall median time out of care was 406 days (238-734). Compared to women accessing care in other provinces, clinic shoppers stayed out of care longer (median 530 days, IQR:332-808 vs. 269, IQR:72-409, p<0.01) and median CD4 upon care reentry trended lower (317 cells/μL, IQR:159-610 vs. 499, IQR:213-571). Considering all 97 women as engaged in care, cohort LTF drops from 38.1% to 26.3%.

**Conclusions:** We found substantial continued care among women considered LTF after initiating ART during pregnancy, both within the same city and in other provinces. This highlights the difficulty of producing accurate estimates of retention in care and underscores the need for a unique identifier and a national, linked health database. We also found that women are suspending care for extended periods of time with consequent immunosuppression. More must be learned as to how women choose HIV facilities, access care, and travel around the time of delivery; continuum of care estimates may be overly pessimistic.

**793 Disclosure and Knowledge Are Associated With Retention in Malawi’s Option B+ Program**

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**Background:** There are limited data on factors associated with retention in Option B+. We sought to explore characteristics of women retained in Option B+ in Central Malawi, with a focus on the role of HIV disclosure, pre-ART education, and knowledge around the importance of Option B+ for maternal and child health.

**Methods:** We performed a case-control study of HIV-infected women in Malawi initiated on ART under Option B+. Cases were enrolled if they met criteria for default from Option B+ (out of ART for >60 days) and controls were enrolled in ~3:1 ratio if they were retained for at least 12 months. We surveyed sociodemographic characteristics, HIV disclosure (participant to partner and participant aware of partner status), self-report about receiving pre-ART education, and Option B+ knowledge using six questions. Univariate logistic regression was performed to determine factors associated with retention. A multiple logistic regression model was used to evaluate HIV disclosure and Option B+ knowledge while adjusting for age, schooling, and travel time to clinic.

**Results:** We enrolled 50 cases and 153 controls. Median age was 30 years (IQR 25-34) and the majority (82%) initiated ART during pregnancy at a median gestational age of 24 weeks (IQR, 16-28). 91% of the cases (39/43) who started ART during pregnancy defaulted by 3 months postpartum. HIV disclosure to the primary sex partner was more common among women retained in care (100% versus 78%, p<0.001). Odds of retention were significantly higher among women with: age > 25 years (OR 2.44), completion of primary school (OR 3.06), awareness of partner HIV status (OR 5.20), pre-ART education (OR 6.17), higher number of correct answers to Option B+ knowledge questions (OR 1.82), and one or more methods of support while taking ART (OR 3.65) (Table). Pre-ART education and knowledge were significantly correlated (r = 0.43, p<0.001). Travel time of > 3 hours to clinic and later gestational age at ART initiation were associated with significantly reduced odds of retention (OR 0.13 and 0.95, respectively). In multivariate analysis, awareness of partner HIV status (OR 4.07, 95%CI 1.51, 10.94, p=0.02) and Option B+ knowledge (OR 1.60, 95%CI 1.15, 2.23, p=0.004) remained associated with retention.

**Conclusions:** Interventions that address partner disclosure and strengthen pre-ART education around the benefits of ART for maternal and child health should be evaluated as strategies to improve retention in Malawi’s Option B+ program.

**Table: Univariate regression for the outcome of retention in Option B+ n=203\***

Variable*	Cases† (n=50)	Controls‡ (n=153)	Odds Ratio (95% CI)	P-value
Age				
18- 25	21 (43)	36 (24)	1.00 (Reference)	
> 25	28 (57)	117 (76)	2.44 (1.24, 4.81)	0.01
Education				
Less than primary school	44 (88)	108 (71)	1.00 (Reference)	
Primary school and beyond	6 (12)	45 (29)	3.06 (1.22, 7.69)	0.009
Duration to travel to ART clinic				
≤ 1 hour	12 (24)	82 (54)	1.00 (Reference)	
> 1 hour up to 3 hours	27 (54)	61 (40)	0.33 (0.15, 0.70)	
> 3 hours	11 (22)	10 (6)	0.13 (0.05, 0.37)	<0.001
Diagnosed with HIV during the most recent pregnancy?				
No	4 (8)	24 (16)	1.00 (Reference)	
Yes	46 (92)	129 (84)	0.47 (0.15, 1.43)	0.15
ART initiation on the same day as HIV diagnosis?				
No	2 (4)	13 (9)	1.00 (Reference)	
Yes	44 (88)	127 (81)	0.44 (0.10, 2.03)	0.26
Status at ART initiation				
Pregnancy	43 (86)	123 (80)	1.00 (Reference)	
Breastfeeding	7 (14)	30 (20)	1.50 (0.61, 3.66)	0.36
Gestational age in weeks at ART start among those pregnant, Median (IQR)	26 (16-32)	24 (16-28)	0.95 (0.91, 0.99)	0.01
Participant disclosed HIV status to primary sex partner?				
No	8 (16)	0 (0)	N.C.*	<0.001*
Yes	28 (56)	109 (71)		
Not applicable	14 (28)	44 (29)		
Participant aware of partner’s HIV status?				
No	17 (47)	16 (15)	1.00 (Reference)	
Yes	19 (33)	93 (85)	5.20 (2.24, 12.07)	
Not applicable	14 (28)	44 (28.8)	3.34 (1.34, 8.30)	0.001
Received pre-ART education*				
No	28 (56)	26 (17)	1.00 (Reference)	
Yes	22 (44)	126 (83)	6.17 (3.06, 12.43)	<0.001
Correctly answered Option B+ knowledge questions (out of 6 questions), Median (IQR)	4 (3-4)	4 (4-5)	1.82 (1.35, 2.45)	<0.001
Number of knowledge questions answered correctly				
0-2	11 (22)	12 (8)	1.00 (Reference)	
3-4	30 (60)	86 (56)	2.63 (1.05, 6.58)	
5-6	9 (18)	55 (36)	5.60 (1.90, 16.49)	0.006
Received pre-ART education*				
No	28 (56)	26 (17)	1.00 (Reference)	
Yes	22 (44)	126 (83)	6.17 (3.06, 12.43)	<0.001
Received support while taking ART†				
No	6 (13)	6 (4)	1.00 (Reference)	
Yes	40 (87)	146 (96)	3.65 (1.12, 11.93)	0.04
One or more side effect from ART†				
No	9 (20)	43 (28)	1.00 (Reference)	
Yes	36 (80)	110 (72)	0.64 (0.28, 1.44)	0.27

\*All variables reported as N (%) unless otherwise noted  
 †Cases defined by Malawi National definition of default = out of ART for more than 60 days; Controls were required to be in care for at least 12 months  
 ‡Not calculated; 100% of controls reported disclosure; \*Calculated with chi-square test  
 †Based on self-report of learning about five different Option B+ topics prior to starting ART  
 ‡Support was defined by use of support groups at the clinic or in the community, reminders for appointments, peer mentoring, or other counseling  
 †Side effects included dizziness, difficulty sleeping, sadness/depression, and weakness/generally unwell

**794 Initiating cART in Pregnancy: Impact on HIV RNA Decay**

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**Background:** cART reduces HIV mother-to-child transmission, allowing vaginal delivery if viral load is low. cART is evolving and the optimal regimen for initiating treatment in pregnancy uncertain.

**Methods:** Routine clinical care data from nine London and Brighton sites, 2001 to 2015, were anonymised and collated. Demographics, baseline and subsequent HIV plasma loads, delivery information and ART were collected retrospectively. Essentially women started cART during this pregnancy and had HIV load re-measured after 14 days (+/-3 days). Elite

controllers and women with an undetectable HIV load on day 14 (+/-3 days) were excluded. Time to undetectable was inferred from the mid-point between the last detected and first undetectable HIV loads. Data analysis was performed in SPSS.

**Results:** 160 women with median age 30 (range 17-42) years; 83% Black African/Caribbean, 12% white; HIV was acquired heterosexually in 96% and by IDU in 2%. Four had hepatitis virus co-infection. Baseline CD4 count and viral load were 313 (30-1136) cells/ml and 17290 (548-588020) cp/ml respectively. cART was commenced at 22 (5-39) weeks gestational age (GA). In the first 14 days of therapy, HIV half-life (T/2) was 2.5 (1.4-5.3) days and 105 (66%) achieved an undetectable HIV load at 36 weeks GA, 130 (81%) by delivery. Data were analysed by third agent: PI (n=114; 71%), NNRTI (n= 31; 18%) or Integrase Inhibitors (II) (n=7; 4%) giving HIV T/2 of 2.6; 2.3 and 1.5 days, whilst time to undetectable were 42, 41 and 27 days respectively. There were significant differences in T/2 by class (p = 0.001) and by individual agent (p = 0.02) but not for time to undetectable. T/2 was significantly slower for Atazanavir (ATZ) than for Nevirapine, Saquinavir and Lopinavir (Table). In logistic regression analysis ATZ (p 0.02) and GA at starting cART (p 0.03) were associated with HIV load at delivery. GA being 4 weeks later in those with a detectable HIV load at delivery whilst ATZ was associated with high suppression rate.

**Conclusions:** A range of cART combinations were effective in achieving undetectable HIV load at 36 weeks GA and at delivery. Correlation between initial decay and viral load at delivery was poor: ATZ had both a slower initial viral decay and a high rate of viral suppression. The limited data on II suggest they perform well in pregnancy and may be useful for high HIV load, especially if treatment is initiated after 20 weeks GA.

	Total number	Viral load (copies/ml)	CD4 (cells/ $\mu$ l)	GA* at start of HAART	HIV half-life, T/2 (days)	Days to <LDL**	At 36 weeks, % < LDL	At delivery, % < LDL
Atazanavir	48	40,201	349	20.3	2.7	43	81	96
Lopinavir	47	49,113	359	24.7	2.3	47	51	70
Nevirapine	25	55,813	204	19.6	2.3	41	64	72
Saquinavir	13	31,118	313	22.4	2.6	29	77	77
Darunavir	8	152,502	306	18.8	2.8	76	38	87
Trizivir	5	59,48	407	21.5	2.6	52	100	100
Efavirenz	5	24,608	346	17.5	2.3	69	50	80
PI	114	42,524	358	22.1	2.6	42	68	82
NNRTI	31	49,509	209	18.9	2.3	41	61	74
Integrase	7	227,556	203	21.7	1.5	27	57	100

Values quoted in table are median figures  
 \*GA = gestational age  
 \*\*LDL = Lower Detection Limit (VL < 50 copies/ml)

**795 Viraemic Episodes Occur Frequently in Postpartum South African Women on ART**

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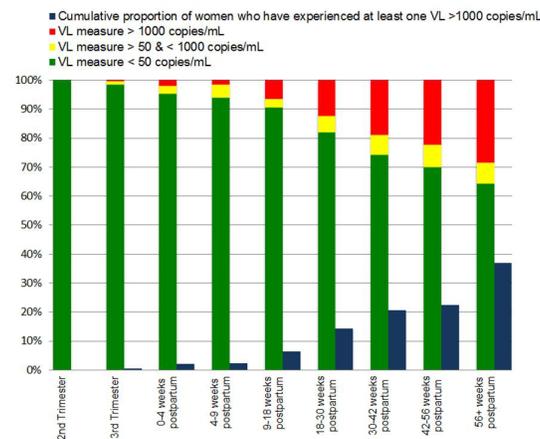
**Background:** With growing numbers of pregnant women starting antiretroviral (ART) there are significant concerns regarding adherence, especially during the postpartum (PP) period. Viral load (VL) monitoring for pregnant and PP women is promoted in international guidelines to identify nonadherence and treatment failure, but little is known about the frequency and magnitude of elevated VL in this group.

**Methods:** From April 2013-May 2014, we recruited a cohort of pregnant women initiating ART from a public sector primary care antenatal clinic in Cape Town, South Africa. Separate from routine HIV care, up to 9 VLs were conducted at regular intervals from pre-initiation through 12 months PP. In analysis, VL taken after reaching viral suppression (VS, <50 copies/mL [c/mL]) were divided into major (>1000c/mL) and minor (>50 and ≤1000c/mL) viraemic episodes (VE). Mixed-effects Poisson models were used to examine the incidence rates (IR) and rate ratios (IRR) of VE over time by maternal characteristics.

**Results:** Among 607 women (median age, 28 years; median gestation at initiation, 21 weeks; median CD4, 345 cells/uL, median pre-ART VL, 3.99 log<sub>10</sub> c/mL, 4% previous defaulting on ART), 86% (n=523) achieved VS and are included. After VS, 2636 VL tests were conducted over a total of 5092 woman-months (wm) of observation (707 wm antenatally, 4385 wm PP); 56% and 39% of women were breastfeeding at 6 and 12 months PP. Overall, 117 major and 56 minor VE were observed (IR, 2.3 and 1.1 per 100wm, respectively; p<0.001); 60% of major VE involved sustained viraemia>1000c/mL (≥2 consecutive measures). Peak viraemia post-VS (median, 3.79 log<sub>10</sub> c/mL) was linearly correlated with pre-ART VL (p<0.001). From initial VS to 12m PP, 70% of women maintained VS consistently while 22% experienced at least 1 major VE and the vast majority of VE (98%) occurred PP (Figure). Major VE after VS was independently associated with younger age (IRR, 0.90 per year; p<0.001), previous defaulting on ART (IRR, 3.28 vs no previous ARVs; p=0.030), and PP follow-up (IRR, 7.85 vs antenatal sampling; p<0.001). Among PP women, VE frequency increased with increasing duration of ART use (IRR, 1.16 per month after VS; p<0.001).

**Conclusions:** Viremia occurs frequently after VS in women initiating ART in pregnancy. VL monitoring postpartum is likely to have substantial yield within ART programs; however the relative contributions of nonadherence versus viral resistance, and in turn the most appropriate intervention strategies, require urgent attention.

Figure. Results of 2636 serial viral load (VL) tests in 523 HIV-infected women initiating ART in pregnancy following viral suppression, by timing of testing antenatally or postpartum.



**796 Viral Suppression and Retention 2-5 Years After ART Initiation in Pregnancy in Uganda**

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**Background:** Recent studies have demonstrated that up to 40% of women are lost to follow-up after initiating antiretroviral therapy (ART) during pregnancy in Option B+ programs. Data are limited on long-term virologic outcomes and retention in care, particularly after cessation of breastfeeding.

**Methods:** We evaluated retention in care and viral suppression (VS) 2-5 years postpartum among previously ART-naïve women who initiated ART during pregnancy (Option B+) at 12-28 weeks gestation in a study (PROMOTE, NCT00993031) in rural Uganda. Participants breastfed and were followed for up to 1 year postpartum, then referred to clinics in surrounding communities. A random sample (n=200) was invited to participate in a cross-sectional follow-up study after completing the trial, including a questionnaire and pregnancy and HIV viral load (VL) testing. Retention in care was defined as having attended an HIV clinic in the last 90 days. Logistic regression models were used to examine factors associated with VS (VL ≤400 copies/ml).

**Results:** One hundred fifty women (75%) were successfully contacted for follow-up. Median months postpartum was 46 (IQR 37-52) and median CD4 count was 664 cells/mm<sup>3</sup> (IQR 476-870). Of the 150 contacted, 131 (87.3%) were on ART (78 on EFV, 35 on NVP, 18 on LPV). Fifty-eight (38.7%) participants reported ≥1 pregnancy after initiating Option B+; 19 (12.7%) were pregnant at the time of follow-up and 23 (15.3%) were breastfeeding. Long-term retention in care following initiation of Option B+ was 90% (95% CI 84.0%-94.3%), with 135/150 seen in the last 90 days. Assuming those we could not contact had fallen out of care (n=50), retention in care was 67.5% (95% CI 60.5%-73.9%). Among the 150 contacted, 121 (80.7%, 95% CI 73.4%-86.7%) had VS. Assuming those we could not contact had virologic failure, long-term retention in care with VS was 60.5% (95% CI 53.6%-67.3%). Factors associated with VS included disclosure of HIV status to primary partner (OR 5.24, 95% CI 1.21-22.6), no difficulty obtaining ART in the past 3 months (OR 4.16, 95% CI 1.50-11.5), and food security (OR 2.61, 95% CI 1.00-6.88).

**Conclusions:** Following initiation of Option-B+, long-term (2-5 year) retention in care and viral suppression was observed in 90% and 80.7%, respectively. Women who had disclosed their HIV status to their primary partner were 5 times more likely to be virologically suppressed, indicating a significant need for facilitated disclosure interventions to maintain long-term retention in care with viral suppression.

**797 HIV Resistance in Pregnant Women With Detectable HIV-1 RNA at Delivery in Mozambique**

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**Background:** Few data on HIV resistance in pregnant women are available from Mozambique, one of the countries with the highest HIV toll in the world. The HIV resistance implications of reaching delivery with detectable HIV-1 RNA despite prevention of mother to child transmission of HIV (pMTCT) are not fully understood.

**Methods:** We analyzed stored plasma samples from HIV-infected pregnant woman participating in a randomized controlled trial on intermittent preventive treatment of malaria in pregnancy (IPTp) at the Manhiça district hospital (MDH) in a semi-rural area in southern Mozambique. Women attending their 1<sup>st</sup> antenatal (ANC) visit between 2009 and 2013 were followed prospectively through 1 month post-partum. Women with HIV-1 RNA levels >400 c/mL at delivery were included in our HIV resistance analysis. HIV drug resistance mutations (HIVDRM) were determined using MiSeq<sup>®</sup> (limit of detection 1%) at the first ANC visit and at the time of delivery.

**Results:** Overall, 150 plasma samples from 113 pregnant women were analyzed. Ninety and 60 samples were available at the 1<sup>st</sup> ANC and delivery visits, respectively. Women attended the first ANC visit with a mean of 25 years of age and a median gestational age of 22 weeks. Of them, 96% had HIV-1 RNA >400 c/mL, 39% had CD4+ counts <350 c/mm<sup>3</sup> and 20% were on antiretroviral therapy (ART). Thirteen women (14%) had at least 1 HIVDRM at the 1<sup>st</sup> ANC, of whom 2/3 were not on previous ART. The number of women with at least 1 HIVDRM to nucleoside reverse-transcriptase inhibitors (NRTI), non-nucleoside reverse-transcriptase inhibitors (NNRTI) and protease inhibitors (PIs) in the 1<sup>st</sup> ANC visit was 8 (9%), 6 (7%) and 2 (2%), respectively. Eight women (13%) had at least 1 HIVDRM at delivery, 6 (10%), 5 (8.3%) and 0 (0%) to NRTI, NNRTIs and PIs, respectively. Table 1 summarizes the predicted susceptibility to the different antiretrovirals (HIVdb) in both visits. Of the 37 women with longitudinal data available from the two time points, 5 (13.5%) developed at least 1 new HIVDRM during pMTCT; 2 (5.4%) to NNRTI, 2 (5.4%) to NRTI and 1 (3%) to PI.

**Conclusions:** Even with ultrasensitive HIV-1 genotyping, less than 15% of women with detectable viremia at delivery had HIVDRM before initiating pMTCT with 1<sup>st</sup> line ARVs. This suggests that other factors beyond pre-existing resistance, such as lack of adherence or interruptions of the ANC chain, are relevant to explain lack of virological suppression in women receiving pMTCT at the time of delivery.

**798 Maternal Vitamin D Deficiency Is Associated With Preterm Birth in HIV-Infected Women**

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**Background:** Several studies in pregnant women have shown an association between low maternal vitamin D and preterm birth. HIV and antiretrovirals (ARVs) can affect vitamin D levels. Few studies have assessed the relationship between maternal vitamin D and preterm birth in HIV+ pregnant women.

**Methods:** We evaluated data from Latin American HIV+ pregnant women enrolled in the National Institute of Child Health and Human Development (NICHD) International Site Development Initiative (NISDI) cohort from 2002-2009. Preterm birth was defined as delivery at <37 weeks (wks) gestational age (GA). Maternal plasma 25-hydroxyvitamin D (25OHD) levels were measured using the Abbott Architect<sup>®</sup> immunoassay on stored samples collected at 12-34 wks GA. Severe vitamin D deficiency was defined as 25OHD <10 ng/mL, deficiency as 10-20 ng/mL, insufficiency as 21-29 ng/mL, and sufficiency as ≥30 ng/mL. Logistic regression modeling was used to evaluate the effect of maternal vitamin D on preterm birth.

**Results:** Of 715 HIV+ women, 13 (1.8%) were severely vitamin D deficient, 224 (31.3%) deficient, and 233 (32.6%) insufficient. Severely deficient women had lower rates of no ARV use for ≥28 days prior to the date of vitamin D testing compared to deficient, insufficient, and sufficient women (15.4% vs. 55.4%, 49.4%, and 39.2% respectively) and higher rates of non-nucleoside reverse transcriptase inhibitor use (46.2% vs. 17.4%, 17.6%, and 16.7% respectively, p<0.01). Overall, 23.2% (166/715) of pregnancies resulted in preterm birth [median GA of preterm births=36 wks (Interquartile Range: 34-36)]. After adjusting for age, substance use in pregnancy, CD4 count, HIV RNA level, body mass index (BMI), ARV use in pregnancy, pre-eclampsia/eclampsia, and prior preterm birth, severe vitamin D deficiency was associated with preterm birth [adjusted Odds Ratio (aOR)=4.7, 95% Confidence Interval (CI): 1.3-16.8]. In stratified analyses, these results remained the same amongst women with vitamin D testing at <22 wks GA (aOR=2.7, 95%CI: 1.1-6.7) and those with vitamin D testing at >22 wks GA (aOR=7.0, 95%CI: 1.6-30.9). In addition, pre-eclampsia/eclampsia (aOR=5.8, 95%CI: 2.3-14.7), underweight maternal BMI (aOR=1.8, 95%CI: 1.1-3.0), and prior preterm birth (aOR=2.7, 95%CI: 1.6-4.6) were also associated with preterm birth.

**Conclusions:** HIV+ women with severe vitamin D deficiency may be at risk for preterm delivery. Further studies may be warranted to determine if vitamin D supplementation in HIV+ women may impact risk of preterm birth.

Table 2. Logistic Regression Results for Preterm Birth Outcomes

Effect	Adjusted Odds Ratio	95% CI
<b>Maternal Vitamin D Status</b>		
Severe Deficiency (<10 ng/mL)	4.68	1.30 - 16.77
Deficiency (10-20 ng/mL)	1.32	0.84 - 2.08
Insufficiency (21-29 ng/mL)	0.80	0.50 - 1.29
Sufficiency (≥30 ng/mL)	Ref	---
<b>Maternal Age, years</b>		
<20	0.94	0.40 - 2.21
20-29	Ref	---
≥29	1.00	0.68 - 1.49
<b>Maternal BMI (GA-adjusted), kg/m<sup>2</sup></b>		
Underweight (<19.8)	1.81	1.10 - 2.97
Normal (19.8-26.0)	Ref	---
Overweight (26.1-28.9)	1.04	0.58 - 1.88
Obese (≥29)	1.03	0.56 - 1.87
Substance Use in Pregnancy	1.12	0.74 - 1.68
Pre-eclampsia/ Eclampsia	5.78	2.27 - 14.68
Prior Preterm Birth	2.71	1.58 - 4.64
<b>CD4 cell count<sup>a</sup>, cells/mm<sup>3</sup></b>		
<200	0.91	0.50 - 1.69
200 - 499	0.98	0.63 - 1.52
≥500	Ref	---
<b>HIV RNA level<sup>a</sup>, copies/mL</b>		
<1,000	Ref	---
1,000 - 9,999	1.10	0.67 - 1.82
≥ 10,000	1.13	0.67 - 1.93
<b>ARV Regimen<sup>a</sup></b>		
2 NRTIs (dual)/ NRTI (monotherapy)	1.54	0.70 - 3.35
2 NRTIs + 1 NNRTI	1.07	0.60 - 1.90
2 NRTIs + 1 PI	1.18	0.72 - 1.94
Other	0.62	0.13 - 3.00
No ARV	Ref	---

ARV<sup>a</sup>-Antiretroviral; BMI<sup>a</sup>-Body Mass Index; CI<sup>a</sup>-Confidence Interval; GA<sup>a</sup>-Gestational Age; NRTI<sup>a</sup>-Nucleoside Reverse Transcriptase Inhibitor; NNRTI<sup>a</sup>-Non-Nucleoside reverse Transcriptase Inhibitor; PI<sup>a</sup>-Protease Inhibitor  
<sup>a</sup> At enrollment

**799 Timing of ART Initiation in Pregnancy and Birth Outcomes in South African Women**

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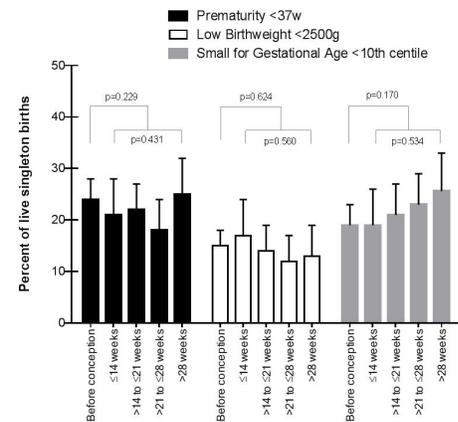
**Background:** Studies have suggested that use of triple-drug antiretroviral therapy (ART) during pregnancy may be associated with prematurity, low birthweight (LBW) and/or small for gestational age (SGA) deliveries. However the nature of any association remains controversial. There are few data from Africa where most ART use in pregnancy occurs; the quality of pregnancy dating in most African studies is poor; and there are limited data on this question involving the most commonly used non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimens.

**Methods:** We recruited a cohort of ART-eligible HIV-infected women (n=1464) making their first antenatal visit at a primary care facility in Cape Town, South Africa, March 2013-June 2014. Pregnancy dating was based on a combination of obstetric ultrasound conducted by a research sonographer on all women, last menstrual period and clinical exam. All women were followed from their first antenatal visit through delivery with outcomes abstracted from clinic records. Analyses compared pregnancy outcomes between women on ART at conception versus those initiating at different gestations.

**Results:** In the cohort (median age, 29y; 17% nulliparous; median CD4 cell count 374 cells/uL), 38% (n=575) were on ART prior to conception (93% NNRTI-based regimens; majority TDF+3TC/FTC+EFV/NVP; 7% PI-based regimens excluded) and the remaining 62% (n=907) initiated the public sector first-line regimen TDF+FTC+EFV. Median gestation at initiation was 21 weeks. Overall, 4% of pregnancies ended in a miscarriage or stillbirth; this did not vary by timing of ART initiation (p=0.86). In 1275 live singleton births (mean birthweight, 3048g; 22% preterm; 14% LBW; 21% SGA), prematurity, LBW and SGA deliveries did not vary systematically between women on ART at conception versus those initiating ART during pregnancy (Figure). The absence of associations between adverse birth outcomes and timing of ART initiation persisted after adjusting for maternal age, parity, height, CD4 and viral load at first antenatal visit; only decreased CD4 was associated with increased prematurity and LBW (p<0.001 for both associations) and only nulliparity was associated with increased SGA (p<0.001).

**Conclusions:** These reassuring findings from a well-characterised routine care cohort demonstrate that timing of initiation of widely used NNRTI-based regimens before or during pregnancy does not appear to be associated with adverse pregnancy outcomes.

Figure. Prevalence of prematurity, low birthweight and small for gestational age deliveries, by timing of ART initiation before or during pregnancy, among 1275 HIV-infected women on ART in Cape Town, South Africa.



**800 Higher Mortality in HIV-Exposed/Uninfected vs HIV-Unexposed Infants, Botswana**

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**Background:** It is unknown whether higher mortality previously observed in HIV-exposed uninfected (HEU) infants (compared with HIV-unexposed [HU] infants) in resource limited settings persists when mothers have access to effective antiretroviral treatment (ART). We compared rates of 2-year infant mortality in HEU vs. HU infants in Botswana to assess outcomes in an era where maternal ART is available.

**Methods:** We enrolled HIV-infected and HIV-uninfected mothers (during pregnancy or 1 week postpartum) and their babies in the prospective observational "Tshipidi" study in 2 sites (1 city and 1 village) in Botswana from May 2010-July 2012. Live born infants and their mothers were followed for 24 months postpartum; data on socio-demographic factors, health, and psychosocial characteristics were collected at baseline and ~6 monthly, and infant health outcomes ascertained. Participants did not receive their primary medical care through the study. Mothers chose infant feeding method with counseling; per Botswana guidelines, HIV-infected mothers choosing replacement feeding could receive free formula.

**Results:** 949 mothers (474 HIV-infected, 475 HIV-uninfected) and 910 live born infants (453 HIV-exposed, 457 HIV-unexposed) were enrolled. HIV-infected women were older (median 29 vs. 25 years old, p<0.001), had median CD4 410 cells/mm<sup>3</sup>, and 32% took ART during pregnancy. Infants born to HIV-infected mothers had significantly higher risk of death compared with HU infants, even after excluding children documented to be HIV-infected (HR 2.9, p = 0.009, 95% CI 1.3-6.6). The 24-month infant mortality rates, stratified by initial feeding method and HIV infection status, are shown in the table.

**Conclusions:** HIV-exposed/uninfected infants were significantly more likely to die before 24 months than HIV-unexposed infants, with most deaths occurring in the first 3 months of life. The small number of breastfed HEU babies does not permit conclusions about the independent effects of HIV-exposure vs. formula-feeding on infant mortality.

Initial Feeding Method	HIV-infected		HIV-Exposed/Uninfected		HIV-Unexposed		Overall	
	# of infants	Number(%) died	# of infants	Number(%) died	# of infants	Number(%) died	Total # of infants	Number(%) died
Breastfed	1	0 (0.00%)	34	1 (2.9%)	451	5 (1.1%)	486	6 (1.2%)
Formula fed	9	4 (44.4%)	395	14 (3.5%)	5	2 (40.0%)	409	20 (4.9%)
Never fed	0	N/A	7	7 (100%)	1	1 (100%)	8	8 (100%)
<b>Total</b>	<b>10</b>	<b>4 (40.0%)</b>	<b>436</b>	<b>22 (5.1%)</b>	<b>457</b>	<b>8 (1.8%)</b>	<b>903</b>	<b>34 (3.8%)</b>

**801LB Cotrimoxazole and Reduced Infectious Morbidity in HIV-Exposed Uninfected Infants**

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**Background:** Diarrhea and respiratory infections are among the leading causes of global morbidity and mortality in young children, with HIV-exposed infants at increased risk. Cotrimoxazole prophylaxis (CPT) may reduce such morbidity in the growing population of HIV-exposed, uninfected infants.

**Methods:** A cohort study was conducted using data from the Breastfeeding, Antiretrovirals and Nutrition (BAN) clinical trial (conducted 2004–2010, Malawi) to assess the association of CPT with respiratory and diarrheal morbidity. All HIV-exposed infants in the BAN trial began receiving CPT (240 mg once daily) in June 2006, in accordance with WHO and Malawi guidelines, from 6–36 weeks of age or until weaning occurred and HIV infection was ruled out. We included all infants who were HIV-uninfected at 8 weeks of age (n=1984). CPT was treated as a time-varying exposure, with infants attending BAN study visits prior to June 2006 considered CPT-unexposed. Outcomes included all documented diarrhea or respiratory infection events occurring from 8–48 weeks of age; events occurring more than two weeks apart were treated as separate events. Conditional gap-time proportional hazards models were used to estimate associations between CPT and diarrheal or respiratory morbidity from 8–48 weeks of age.

**Results:** A total of 1984 infants contributed 1414 person-years (PY) of follow-up (CPT-unexposed: 260 PY, CPT-exposed: 1154 PY). CPT-exposed infants experienced 5.32 respiratory events and 2.87 diarrheal events per 100 person-weeks. The incidence rate of respiratory and diarrheal morbidity among CPT-unexposed infants was 8.09 and 4.39 per 100 person-weeks, respectively. CPT was associated with a 36% relative reduction in respiratory morbidity and a 41% relative reduction in diarrheal morbidity (respiratory HR 0.64, 95%CI 0.60–0.69; diarrheal HR 0.59, 95% CI 0.54–0.65). Adjustment for rainy season (Nov–Mar) and randomization arm resulted in similar findings.

**Conclusions:** CPT was associated with a significant reduction in respiratory and diarrheal morbidity in HIV-exposed, uninfected infants. We have previously shown CPT to also be associated with reduced risk of both clinical and asymptomatic malaria, severe infectious morbidity, and mortality in HIV-exposed, uninfected infants in Malawi. CPT may have an important role to play in reducing the leading global causes of morbidity and mortality in the growing population of HIV-exposed, uninfected infants in malaria-endemic resource-limited settings.

**802 HIV-Exposed Children Account for More Than Half of 24-Month Mortality in Botswana**

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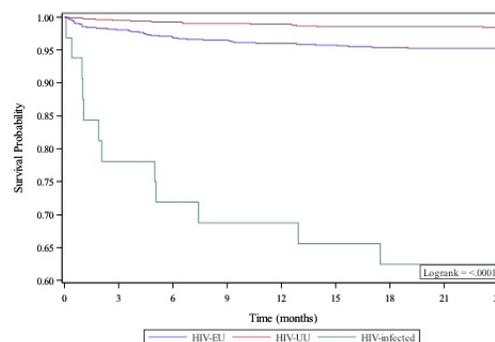
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**Background:** The relative contribution of HIV-exposure and HIV-infection to childhood mortality in a programmatic setting with widespread antiretroviral treatment (ART) use in pregnancy is not well described.

**Methods:** From March 2012–March 2013, women and their children were enrolled within 48 hours of delivery in 5 geographically diverse government-run postpartum wards in Botswana. Maternal obstetric and medical history, demographics and socioeconomic status were collected at enrollment. Women were interviewed every 1–3 months for 24 months by phone (or home visit if not reachable) to assess child HIV status, feeding modality, food security and child and maternal mortality. Mothers and children received all care, including ART, through the government health system. Child feeding was according to Botswana guidelines. Risk factors for 24-month survival were assessed by Cox proportional hazard modeling.

**Results:** A total of 3000 women (1499 HIV-infected) and their 3033 children (1515 HIV-exposed) were enrolled. Among HIV-infected women, 58% were on ART, 23% on zidovudine (ZDV) alone, 11% received no ARVs during pregnancy, and 8% were unknown. Incidence of Mother-to-child HIV transmission (MTCT) was 2.1% through 24 months. Vital status at 24 months was known for 3018 (99.5%) children and there were 106 (3.5%) deaths overall. Mortality differed by HIV exposure status (Figure 1): 12 (38%) among HIV-infected, 70 (4.7%) among HIV-exposed uninfected (HEU), and 24 (1.6%) among HIV-unexposed uninfected (HUU). The only independent risk factors for mortality in adjusted analysis were child HIV-infection (aHR 22.6, 95%CI 10.7, 47.5%), child HIV-exposure (aHR 2.7, 95% CI 1.7, 4.5) and maternal death (aHR 7.7, 95%CI 1.9, 31.9). Although the risk of replacement feeding was significant when modeled separately from HIV-exposure status (aHR 2.3, 95% CI 1.5, 3.6), feeding and HIV-exposure status were co-linear (99.7% of HIV-unexposed but only 13% of HIV-exposed children breastfed) and could not be modeled together. Given 26% HIV prevalence among pregnant women in Botswana, more than half of all 24-month child mortality is accounted for by HEU (46%) plus HIV-infected (8%) children.

**Conclusions:** In a programmatic setting with widespread maternal and child ART availability, HIV-exposed children contribute approximately half the overall deaths at 24 months. Lack of breastfeeding could not be assessed independently of HIV-exposure status, but was a likely contributor to excess mortality among HEUs.

**803 Mortality Risk Associated With Preterm and SGA Stratified by Maternal HIV Status**

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**Background:** Preterm and small-for-gestational-age (SGA), two distinct biological processes leading to low birth weight (LBW), are associated with increased risk of mortality during infancy. The risk of mortality due to these conditions among vulnerable populations such as HIV exposed infants are yet to be estimated. Using the recent intergrowth standard to define SGA, we have evaluated the relative risk of mortality due to preterm and SGA in Tanzania, stratified by maternal HIV status.

**Methods:** Data from five individually randomized trials of multivitamins were pooled. Of the cohorts, two enrolled HIV-positive pregnant women, two enrolled HIV-negative women and one included both HIV-negative and positive women. Preterm birth (gestational age <37 weeks) was defined using date last menstrual period, and SGA (birthweight <10th percentile for gestational age) was defined using the recently published intergrowth standard. We used Cox proportional hazard models to estimate the risk of mortality. Effect modification by maternal HIV status was assessed using the likelihood ratio test.

**Results:** Of the 31,988 infants included in the analyses, 16.6% were preterm, 15.3% were SGA and 7.3% were LBW. Proportion of preterm and SGA births was higher among the HIV infected women. Preterm birth was associated with an increased risk of neonatal and infant mortality, with further increased risk for early preterm. Moderate SGA was associated with an increased risk of mortality, and the risk was even greater for infants born with severe SGA. Compared to term-appropriate-for-gestational-age (AGA) infants, infants born both preterm and SGA had a greater risk of neonatal mortality (HR 5.43, 95% CI: 2.01-14.63) than preterm-AGA infants (HR 2.40 95% CI: 1.89-3.05). Maternal HIV status modified the risk of mortality associated with preterm birth in the post-neonatal and overall infant period ( $p$  for interaction 0.004). Compared to full term infants, early preterm infants born to HIV-positive women were 2.08 times (95% CI: 1.36-3.18) more likely to die during infancy, while early preterm infants born to HIV-negative women were 4.86 times more likely to die during infancy (95% CI: 3.52-6.71). The lower relative risk of death among preterm infants in the HIV infected cohort was driven by the higher mortality among term infants.

**Conclusions:** Preterm and SGA significantly increased the risk of mortality during the neonatal period, and the risk extends well beyond the neonatal period in both HIV exposed and unexposed infants.

#### 804 HBV and HCV Infections in HIV-Infected Pregnant Women: Obstetrical Outcomes

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**Background:** Data on the impact of chronic hepatitis infections on the immunovirological response to antiretroviral therapy (ART) and obstetric outcome in HIV-infected pregnant women are scarce and conflicting.

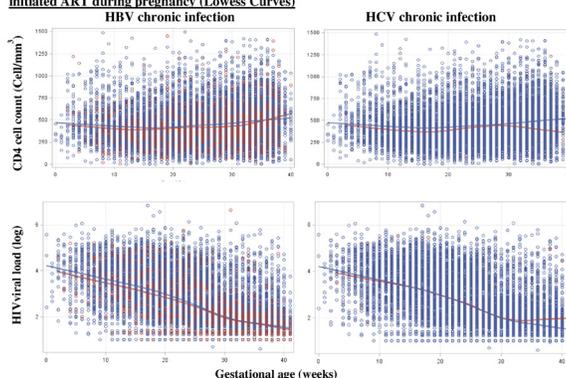
**Methods:** We analyzed data from all HIV-1 infected women included in the national ANRS-C01 French Perinatal Cohort between 2005 and 2013. Prenatal testing for HBV and HCV infections was performed in most cases (95%). HBV/HIV and HCV/HIV co-infected mothers were compared with those infected only with HIV; the rare mothers with all three infections were excluded. Bivariate and multivariate analyses were performed.

**Results:** Among 6548 pregnancies, the overall prevalences of HCV (RNA<sup>+</sup>) and HBV (HBsAg<sup>+</sup>) infections were 3.2% [95%CI: 2.9-3.8] and 6.9% [6.2-7.5], respectively. As expected, HCV infection was strongly associated with a history of drug use, whereas HBV infection was six times more frequent in women originating from Sub-saharan Africa compared with those from mainland France. HIV viral load, CD4 cell count at pregnancy initiation and HIV care were similar in co- and mono-infected HIV mothers except, for ART, with 90% of HBV/HIV co-infected women receiving tenofovir and /or 3TC or FTC, with potential to efficiently decrease HBV viral load. No efficient treatment against HCV was prescribed in the HCV/HIV group.

HCV coinfection was significantly associated with poorer HIV immunovirological status during the third trimester (Fig), and higher risks of gestational diabetes (OR=1.9 [1.0-3.7],  $p=0.05$ ), cholestasis (OR=8.9 [4.9 - 16.3],  $p<0.001$ ) and preterm delivery (OR=3.3 [1.9-5.5], and OR=4.2 [2.3-7.9],  $p<0.001$ ) for moderate and very preterm delivery, respectively:  $p<0.001$ ). The association with prematurity remained significant after adjustment for known risk factors, HIV viral load and antenatal complications ( $aOR=2.3$  [1.1-5.0],  $p=0.03$ ). In HBV/HIV women no association was found with any of these outcomes.

**Conclusions:** In HIV-infected pregnant women, chronic HBV infection, efficiently treated, had no major impact on mother health during pregnancy. In contrast, HCV co-infected mothers, without any efficient treatment against HCV, showed a poorer HIV immunovirological response to ART and higher risk of antenatal complications and prematurity. This suggests that efficient control of HCV activity, before conception, as likely to be obtained in HBV infection, may limit the deleterious impact of co-infection.

**Figure 2 - Trends in CD4 count and HIV viral load according to hepatitis status in women who initiated ART during pregnancy (Lowess Curves)**



#### 805 Maternal ART and Hospitalization or Death Among HIV-Exposed Uninfected Infants

**Scott Dryden-Peterson**<sup>1</sup>; Tatiana Ramos<sup>2</sup>; Roger L. Shapiro<sup>3</sup>; Shahin Lockman<sup>1</sup>

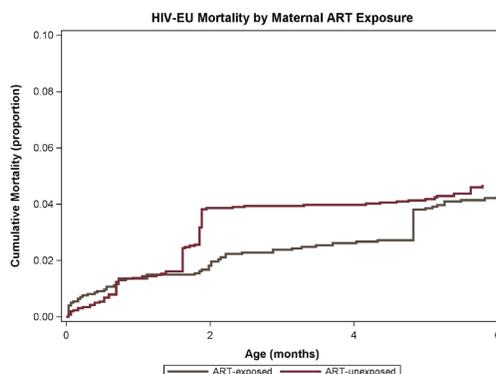
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**Background:** Excess deaths among HIV-exposed uninfected infants (HEU) are a major contributor to under-5 mortality in Africa. Antenatal maternal ART nearly eliminates the risk of mother-to-child transmission (MTCT), but its effect on health outcomes of HIV-uninfected infants is unknown. We examined the effect of maternal ART and risk of HEU infant hospitalization and/or death by 6 months of age.

**Methods:** We pooled data from 2 randomized trials and 2 observational studies that enrolled HIV-infected mothers and their infants from four sites in Botswana from 2001 to 2012. Studies utilized harmonized covariate and endpoint assessments. Analysis excluded HIV-infected infants and stillbirths. Feeding method at maternity discharge defined exposure to formula/mixed or exclusive breastfeeding. Infants born to mothers who continued or initiated ART during pregnancy were considered ART exposed. We developed a Cox marginal structural model to estimate the causal effect of maternal ART. Inverse probability weights were used to adjust for maternal age, antenatal CD4 cell count, socioeconomic status, year of delivery, and participation in a randomized trial or observational study.

**Results:** Following exclusion of 109 HIV-infected infants, 2665 HIV-EU infants were analyzed including 1460 (54.8%) exposed to maternal ART and 1205 (45.2%) exposed to maternal zidovudine or no antenatal antiretrovirals. Most (85.6%) maternal ART was initiated in pregnancy. Median maternal CD4 was lower for ART-exposed infants (354 cells/ $\mu$ L, IQR 219-497) than ART-unexposed (390 cells/ $\mu$ L, IQR 279-538),  $p<0.001$ . The majority (62.5%) of infants were breastfed. A total of 291 (10.9%) infants were hospitalized or died—152 ART-exposed (10.4%) and 139 ART-unexposed (11.5%). Ninety-eight infants died (3.7%)—52 ART-exposed (3.6%) and 46 ART-unexposed (3.8%). In adjusted analyses, no effect of maternal ART was detected on rate of hospitalization or death (HR 1.01, 95%CI 0.65-1.56) or mortality (HR 0.98, 95%CI 0.56-1.71). The effect of maternal ART on HEU mortality appeared to be different between breastfed and formula fed infants, although did not reach significance ( $P=0.075$  for interaction)—breastfeeding was protective among ART-unexposed infants (HR 0.40, 95%CI 0.17-0.93) but not among ART-exposed infants (HR 1.23, 95%CI 0.50-3.00).

**Conclusions:** Expanding ART for prevention of MTCT is not expected to also improve excess mortality among HEU infants. New strategies are urgently needed to mitigate mortality among this large vulnerable group.



### 806 Recombination Elevates HIV-1 Evolution Following Mother-to-Child Transmission

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**Background:** Available data suggest that single HIV-1 variants are transmitted through mother-to-child transmission (MTCT); such analyses have often used samples from a single time point, which may detect only the most replication competent variant at that time point, even when other forms may have been transmitted. Such forms may have a replication advantage later in infection, and may thus be detected in follow-up samples. Because HIV-1 frequently recombines, however, phylogenetic analyses that ignore recombination may miss transmission of multiple forms if they recombine after transmission. The effect of recombination on viral evolution in HIV-1-infected children has not been well defined and warrants analysis of longitudinal samples.

**Methods:** We analyzed full-length *env* sequences after single genome amplification from the plasma of 4 subtype B HIV-1 infected women (11—67 *env* clones from 1 time point within a month prior to delivery) and their non-breastfed *intrapartum* infected children (3–6 longitudinal time points per child starting at the time of HIV-1 diagnosis). To address the potential effects of recombination, we used a recently developed hierarchical recombination detection method based on the pairwise homoplasy index (PHI)-test.

**Results:** Recombination was widespread, occurring in 9—67% of the maternal sequences and in 25—60% of the child sequences. In the child, recombination only occurred between variants that had evolved after transmission; there was no evidence for the recombination of multiple transmitted forms, even in one case where 2 maternal HIV-1 forms were transmitted. We also found evidence for changing effective evolutionary rates of HIV-1 following MTCT, with the highest rates measured in the early years following transmission. Recombination generally inflated the early evolutionary rates, suggesting that it may facilitate adaptation following transmission.

**Conclusions:** Following a transmission bottleneck, the establishment of HIV-1 infection in newborns involves evolutionary dynamics with abundant recombination that elevates the early effective evolutionary rates. Early evolutionary effects of recombination and other selective pressures may facilitate adaptation.

### 807 Infant CD4+ T Cells Have a Distinct Immunophenotype by Single Cell Analysis

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**Background:** The establishment and persistence of HIV reservoirs in memory CD4+ T cell subsets (central memory ( $T_{CM}$ ), transitional memory ( $T_{TM}$ ), effector memory ( $T_{EM}$ ) and stem cell memory ( $T_{SCM}$ )) and to a lesser extent in naïve T-cells ( $T_N$ ) limits the effectiveness of antiretroviral therapy (ART) and the potential for an HIV cure. Compared to adults, infants harbor a greater number of circulating CD4+ T cells with a markedly different T-cell subset distribution. Discrepancies in T-cell activation, differentiation propensity and HIV permissibility have also been described for infant cells. Determining the relative proportion of memory T cell subsets and their transcriptional activity at the single cell level may help uncover potential mechanisms driving HIV pathogenesis.

**Methods:** Memory and naïve CD4+ T-cell subsets ( $T_{CM}$ ,  $T_{TM}$ ,  $T_{EM}$ ,  $T_{SCM}$  and  $T_N$ ) were isolated from single cell suspensions of unstimulated, HIV-unexposed cord blood mononuclear cells (CBMCs) and peripheral blood mononuclear cells (PBMCs), stained with classical activation markers and sorted by flow cytometry. Differential gene expression was compared on flow cytometry sorted naïve CD4+ CD45RA+ CCR7+ CD27+ T cells from CBMCs and PBMCs using single cell RNA sequencing.

**Results:** The distribution of CD4+ T-cell subsets from CBMCs compared to PBMCs was consistent with previous observations, where  $T_N$  dominate CD4+ T cells in CBMCs ( $T_N$  90%,  $T_{CM}$  1.1%) but are decreased ( $T_N$  40%,  $T_{CM}$  22%) in PBMCs. At the single cell level, RNA sequencing on  $T_N$  from CBMCs and PBMCs revealed distinct transcriptional phenotypes. Principal component analysis showed the segregation of  $T_N$  from CBMC versus those from PBMC. 302 genes were differentially expressed (using 'monocle' software package (v1.2.0, R version 3.2.1, p-value cutoff of 0.1), including several genes involved in T cell activation and characteristic of fetal CD4+ T cells (RGS1, CD69, TNFAIP3, JUN, KLF6). Notably, changes in genes that influence the propensity of CD4+ T cells to differentiate into Th17 or regulatory T cells (IL-23R, RGS1, TNFAIP3) and genes that influence HIV pathogenesis (IL-32, PLCG1) were observed.

**Conclusions:** Infant immune cells have a distinct immunophenotype at the transcriptome and cellular level. Understanding the transcriptional and immune mechanisms that support the seeding and proliferation of infected cell types in which viral reservoirs reside, may help determine potential targets and provide insight into both an infant and adult HIV cure.

### 808 Duration of Tenofovir Exposure in Utero and Linear Growth in the First Year of Life

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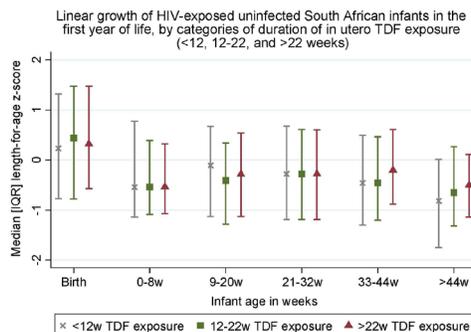
**Background:** Tenofovir disoproxil fumarate (TDF) is widely used as part of first-line antiretroviral therapy (ART) in pregnancy globally, but there are concerns that TDF may impair bone growth in children. Data on the effect of TDF exposure in utero on growth are mixed, with few insights from sub-Saharan Africa. We examined the association between duration of TDF exposure in utero and linear growth in HIV-exposed infants.

**Methods:** We recruited pregnant women initiating TDF (+emtricitabine +efavirenz) at primary care services in Cape Town, South Africa and followed breastfeeding mother-infant pairs through 12 months postpartum. Timing of antenatal ART initiation and birth length were abstracted from clinical records. Infant length was measured at 6, 12, 24, 36 and 48 weeks (wks) of age with a recumbent stadiometer. Analyses used length-for-age z-scores (LAZ) based on Intergrowth-21 and World Health Organization reference

standards for birth and postnatal measures respectively, with correction for prematurity. Mixed effects linear models were used to examine the association between duration of in utero TDF exposure and infant LAZ over time.

**Results:** In 464 mother-infant pairs (median CD4 at ART initiation, 346 cells/ $\mu$ L), the median duration of TDF exposure was 16.7 wks (interquartile range 11-22) with 31%, 44% and 25% of infants exposed to <12, 12-22 and >22 wks of TDF respectively. Longer duration of TDF exposure in utero was associated with higher maternal socioeconomic status (SES) but not with baseline maternal age, CD4 cell count or HIV viral load. Infants with >22 wks of exposure were breastfed for shorter than those with 12-22 and <12 wks exposure (median duration 3, 5 and 6 months respectively). Median LAZ were slightly higher than the expected mean at birth, and slightly below thereafter, but did not vary by duration of TDF exposure at any time point (Figure). In the final model predicting LAZ over time, maternal height was strongly associated with LAZ ( $\beta=0.02$  per cm increment, 95% confidence interval, CI: 0.01,0.04), but there was no association with duration of TDF exposure in utero:  $\beta$  for >22 vs <12 wks, 0.11 (95% CI: -0.25,0.47);  $\beta$  for 12-22 vs <12 wks, -0.03 (95% CI: -0.28,0.22). Results were unchanged after adjustment for breastfeeding duration, maternal CD4 cell count, HIV viral load and SES.

**Conclusions:** These data find no evidence for an association between duration of TDF exposure in utero and linear growth in infancy.



### 809 Severe Respiratory Infections in HIV-Exposed Uninfected Infants: Serologic Analysis

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**Background:** HIV-exposed uninfected (HEU) infants have increased rates of severe lower respiratory tract infection (LRTI), sepsis, hospitalization and death. We examined the incidence of LRTI in HEU according to maternal antibody transfer and infant antibody production.

**Methods:** We enrolled 247 HEU and 88 HIV-unexposed uninfected (HUU) Brazilian infant/mother pairs, including 107 HEU and 16 HUU with LRTI in the first 6 months of life. Antibodies to the following agents were measured by ELISA: respiratory syncytial virus (RSV) and pneumococcus (PNC) 1, 5, 6, 14 in mothers (delivery) and infants (0, 6 months); influenza A (Flu) and parainfluenza viruses (PIV) 1, 2, 3 (infants 0, 6 months); tetanus toxoid (infants 6 months).

**Results:** Compared to HUU, HEU infants had lower antibody levels at birth for all respiratory agents ( $p<0.0001$ ), although maternal antibodies to PNC and RSV did not differ by HIV status. Transplacental transfer of maternal antibodies was lower for RSV in HEU vs. HUU (mean $\pm$ SD ratios=1.3 $\pm$ 3.5 vs. 1.8 $\pm$ 0.8;  $p=0.05$ ). Infant: mother PNC antibody ratios were <1 in both HEU and HUU, but the differences between HEU and HUU were not statistically significant. Compared to mothers of LRTI-, those of LRTI+ HEU had higher antibody levels to PNC 1 and 6 and those of LRTI+ HUU to PNC 5 and 14 ( $p\leq 0.04$ ). Flu, PIV, RSV and PNC antibodies at birth were similar in LRTI+ vs. LRTI- HEU or HUU, except for higher PNC 5 and 14 levels in LRTI+ vs. LRTI- HUU ( $p\leq 0.05$ ). At 6 months, HEU and HUU had similar antibody responses to tetanus vaccine regardless of LRTI status. After controlling for birth levels, HEU had lower RSV ( $p<0.001$ ), higher PIV 1, 2, 3 ( $p\leq 0.001$ ) and similar Flu antibodies ( $p=0.11$ ) compared with HUU at 6 months. At 6 months, LRTI+ HEU had higher anti-RSV antibody levels ( $p=0.08$ ) and rates of seroconversion to  $\geq 1, 2$  or 3 paramyxoviruses ( $p=0.05, 0.02$  and  $0.06$ , respectively) than LRTI- HEU.

**Conclusions:** The incidence of LRTI in HEU infants correlated with the frequency of paramyxovirus infections, but not with low levels of transferred maternal antibodies or with infant failure to make antibodies in response to infections or vaccines. The higher maternal PNC antibody levels in LRTI+ vs. LRTI- infants suggested that mothers of LRTI+ infants had higher rates of PNC infection and/or carriage increasing infant exposure to PNC and possibly contributing to LRTI morbidity. Collectively, our data suggest that environmental factors and innate and/or cell-mediated immune defects predispose HEU to LRTI.

### 810 Similar HIV Protection From ZDV vs NVP Prophylaxis in Formula-Fed Infants in Botswana

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**Background:** The World Health Organization recommends that HIV-exposed formula fed infants receive prophylactic zidovudine (ZDV) or nevirapine (NVP) from birth for 4-6 weeks for prevention of mother-to-child HIV transmission (PMTCT). No studies have evaluated the health consequences of ZDV vs NVP prophylaxis in formula fed HIV-exposed infants.

**Methods:** We analyzed data from the Mpepu study in Botswana, which evaluated infant cotrimoxazole vs. placebo among HIV exposed uninfected (HEU) children. Infant post-exposure prophylaxis for PMTCT changed during the course of the study: single dose of NVP (sdNVP) followed by ZDV was used from May 2011-Jan 2013, and either this regimen or extended NVP (without ZDV) was used after Jan 2013. Mpepu data for formula-fed, full-term ( $\geq 37$  weeks gestation) infants without growth restriction (birthweight  $\geq 2500$  grams) were included to reduce study-specific sources of bias, and analyses were limited to those initiating ZDV or NVP  $\leq 72$  hours from birth with a documented ZDV or NVP 25-35 day course. Incidence of intrapartum mother-to-child HIV transmission (MTCT) and first occurrence of anemia or neutropenia in the first 6 months of life using Division of AIDS grade 3 and 4 criteria were compared using Fisher's exact tests.

**Results:** Of 3164 infants born in the Mpepu Study, 1365 (43%) met inclusion criteria: 687 (50%) received ZDV (86% of ZDV-treated infants were born prior to 2013) and 678 (50%) received NVP. The most common reasons for infant exclusion included AZT or NVP prophylaxis variation, preterm or growth restricted infants, and breastfeeding. Most mothers received 3-drug antiretroviral treatment (ART) in pregnancy (74% in the ZDV group, and 95% in the NVP group). Of 1362 infants with documented negative HIV DNA PCR prior to a 2nd PCR test between 14 to 34 days of life, 4 infants were confirmed HIV-infected on their 2nd HIV DNA PCR test: 2 (0.3%) of ZDV recipients and 2 (0.3%) of NVP recipients ( $p=1.0$ ). Anemia occurred in 19 (2.8%) ZDV recipients vs 13 (1.9%) NVP recipients ( $p=0.37$ ), and neutropenia occurred in 32 (4.7%) ZDV recipients vs 25 (3.7%) NVP recipients ( $p=0.41$ ).

**Conclusions:** Among formula fed infants in the Mpepu Study, an infant prophylactic regimen of either sdNVP/ZDV or NVP were similarly efficacious in achieving low peripartum transmission in the setting of extensive maternal ART use through delivery. Infant ZDV vs. NVP did not result in significant differences in hematologic events in the first 6 months of life.

### 811 Risk of Cancer in Children Exposed to Didanosine in Utero

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**Background:** Antiretroviral treatment during pregnancy has been effective in reducing perinatal HIV transmission. Some nucleos(t)ide reverse transcriptase inhibitors (NRTIs) display genotoxicity *in vitro* so we evaluated the incidence of cancer among children exposed to NRTIs *in utero* between 1990 and 2014 in the ANRS French Perinatal Cohort (EPF).

**Methods:** We updated the evaluation of cancer incidence among children exposed to NRTIs in the cohort by cross checking with the French national cancer registry. Associations between cancer risk and exposure to the various NRTIs were evaluated by univariate survival analysis and Cox proportional hazard models. Standardized incidence ratios (SIR) were used to compare risks with those for the general population.

**Results:** Twenty-one cancers were identified in 15,163 children (median age: 9.9 years [interquartile range (IQR): 5.8-14.2]) exposed to at least one NRTI *in utero* between 1990 and 2014. Five children were exposed to zidovudine monotherapy, and 15 to various combinations, seven including didanosine. Didanosine was included in only 10% of the prescriptions but was associated with one third of the cancers. Compared with the 2281 children exposed to zidovudine monotherapy, the risk of cancer was higher in the 1461 children exposed to combinations including didanosine (adjusted HR = 3.0 [0.9-9.8]), but similar for all other NRTI combinations. The risk was specifically higher in children exposed during the first trimester, than never exposed, to didanosine (HR=5.5 [2.1-14.4]); it was not significantly associated with second or third trimester exposure to didanosine (HR=1.6 [0.2-12.2]).

Overall, the total number of cases was not significantly different from that expected for the general population (SIR = 0.8 [0.47-1.24]), but among those exposed to didanosine it was twice that expected (SIR = 2.5 [1.01-5.19]). Three cases of pineoblastoma, a very rare cancer, were observed, whereas 0.03 were expected; two of these cases were associated with didanosine exposure.

**Conclusions:** There are strong arguments to suggest that didanosine displays transplacental oncogenicity. Although these findings cannot be extrapolated to other NRTIs, they stress the need for comprehensive evaluation of the transplacental genotoxicity of this class of antiretrovirals.

### 812 Elevated Mitochondrial DNA Content in HIV-Exposed Uninfected Children With Autism

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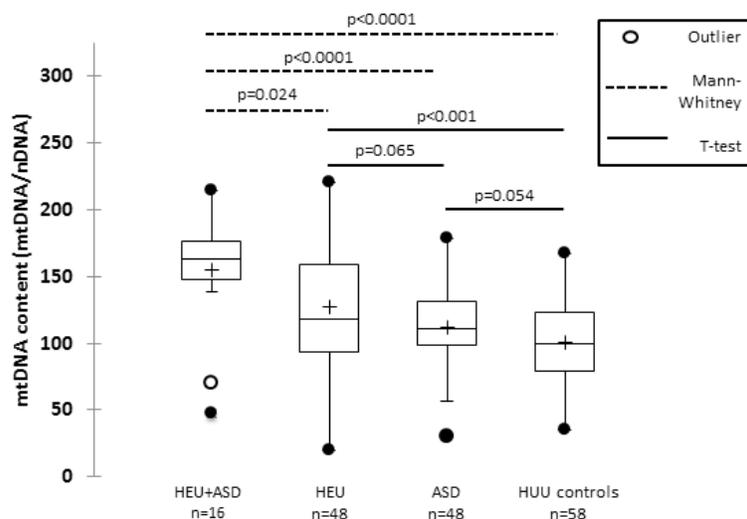
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**Background:** Recently, a Canadian pediatric HIV program reported a high prevalence of autism spectrum disorder (ASD) among HIV-exposed uninfected (HEU) children. Many of these children are enrolled in the prospective Children & Women AntiRetrovirals & Markers of Aging (CARMA) cohort. Of all 296 HEU children in CARMA, 16 have a diagnosis of ASD. Four more are strongly suspected and are undergoing formal assessments. This represents an ASD prevalence (5.4%) >4-fold higher than the North American population estimate of 1.47%. In addition to genetic predisposition, it is postulated that ASD may also be associated with maternal infections in pregnancy and mitochondrial dysfunction. Increased blood mitochondrial DNA (mtDNA) content, which may indicate mitochondrial dysfunction, has been observed in HEU children with perinatal exposure to combination antiretroviral therapy (cART). This study analysed mtDNA content in HEU children with and without ASD, as well as HIV-unexposed uninfected (HUU) children with and without ASD.

**Methods:** CARMA HEU children with confirmed ASD (HEU+ASD, n=16) aged 2-16 years were matched 1:3 on age, sex, and ethnicity with CARMA HEU children without ASD (n=48); and 1:3 on age and sex with HUU children with ASD taking part in the BC Autism Spectrum Interdisciplinary Research (ASPIRE) program (n=48). A fourth group included HUU anonymous controls (n=48) age- and sex-matched 3:1 to the HEU+ASD group, and 10 age- and sex-matched ASPIRE controls (n=58 HUU total). Leukocyte mtDNA content (mtDNA/nDNA ratio) was measured via monochrome multiplex qPCR, and specimens were randomized and blinded to account for inter-assay variability. Comparisons between groups were performed using the Mann-Whitney U test or unpaired t-test.

**Results:** Among the 16 HEU+ASD children, 15 had received approximately 6 weeks of AZT prophylaxis and 13 were exposed to cART *in utero*. Between-group comparisons are shown in Figure 1.

**Conclusions:** HEU children have significantly elevated blood mtDNA levels compared to HUU controls, and HEU+ASD children have higher mtDNA than all other groups. It is unclear if this effect is modulated by exposure to cART or HIV. This finding is consistent with previous studies in younger HEU children, which suggested the increase may be a compensatory mechanism in response to mitochondrial dysfunction. This study suggests an association between mtDNA and both HEU and ASD status which may be cumulative and could help to explain the observed high ASD prevalence among HEU children.



**813 ARV Risk for Speech and Language Impairments in HEU Children at 3 and 5 Years**

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**Background:** Perinatally HIV-exposed but uninfected (PHEU) children have elevated risk of late language emergence at age 1 year, with possible links to *in utero* antiretroviral (ARV) exposure. This study investigated possible risks, including ARV exposure, for speech impairments (SI) and language impairments (LI) in monolingual PHEU children in the important preschool period. This is the first study of SI in PHEU children and the first to examine LI among PHEU children in this age range.

**Methods:** Speech and Language assessments were conducted as part of the PHACS Surveillance Monitoring of ART Toxicities (SMARTT) study at ages 3 (N= 238) and 5 (N = 465, of whom 145 were also tested at age 3) years. Domains of speech, overall language, vocabulary and grammar were assessed. SI/LI was defined as standardized score (SC) below the 15<sup>th</sup> percentile and was subdivided into primary (PSI/PLI) (normal nonverbal IQ ≥85 without hearing loss) versus concomitant (CSI/CLI) (low nonverbal IQ or presence of hearing loss). Mean SCs among PHEU children were compared to population norms by t-test. Logistic regression models were used to estimate adjusted odds of SI and LI for different ARV exposures, controlling for confounding variables.

**Results:** PHEU children had lower SCs compared to population norms (PN) at ages 3 for vocabulary (mean SC = 94.4, 95% CI: 92.4, 96.4; PN = 100) and 5 years for overall language (mSC = 90.4, CI: 89.1, 91.8; PN = 100), vocabulary (mSC = 9.4, CI: 9.2, 9.7; PN = 10) and grammar (mSC=8.4, CI: 8.1, 8.6; PN = 10), but did not underperform on the speech assessment at either age. Risk factors included male sex, black race, and other socioeconomic measures, although these varied somewhat by age group, primary vs. concomitant group, and by the particular speech or language measure. Adjusted logistic regression models revealed possible protective effects as well as increased risk for specific ARVs, including tenofovir at age 3 and both didanosine and zidovudine at age 5 (see table).

**Conclusions:** In the preschool period, risk for LI was higher among older children. There was no indication of overall increased risk for SI. Outcomes with *in utero* ARV exposure depended on the child's age, whether SI/LI was accompanied by other impairments (PSI/PLI vs CSI/CLI), and the dimensions of speech and language outcomes. Both protection and increased risk were seen with specific ARV exposures.

ARV	Age / Group	Impairment	Adjusted OR [95% CI]	P-Value
<b>Protective Effects</b>				
Nelfinavir	5 / CLI	Language	0.52 [0.27, 1.00]	0.05
Tenofovir	5 / CLI	Grammar	0.32 [0.13, 0.77]	0.01
<b>Increased Risk</b>				
Tenofovir	3 / PSI	Speech	5.28 [1.09, 30.39]	0.04
Didanosine	5 / PLI	Vocabulary	3.46 [1.18, 10.21]	0.02
Zidovudine	5 / CLI	Language	2.35 [1.00, 5.50]	0.05

Logistic regression models adjusted for age, sex, race, ethnicity and various socioeconomic factors. For each outcome modeled (CLI, PSI, PLI) the reference group was "no impairment".

CLI = Concomitant Language Impairment; PSI = Primary Speech Impairment; PLI = Primary Language Impairment.

**814 Longitudinal Evaluation of Language Impairment in Perinatally HIV Exposed Adolescents**

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**Background:** We previously documented a significantly higher prevalence (relative to comparable communities) of both primary language impairment (PLI) and language impairment concomitant with hearing/cognitive impairments (CLI) in perinatally HIV-infected (PHIV) and perinatally HIV-exposed uninfected (PHEU) participants (age range: 7-17y) from the Adolescent Master Protocol (AMP), a component of PHACS. This analysis describes longitudinal outcomes of this cohort to determine the persistence of PLI and CLI and associations with antiretroviral (ARV) treatment, disease status, and other risk factors.

**Methods:** The Clinical Evaluation of Language Fundamentals (CELF-4) was repeated on AMP participants 18 months after their baseline assessment. General linear regression models were utilized to identify independent predictors of change in standardized score (SC). The sample was also split by baseline status to LI (SC<85) and no LI (SC≥85), and separate logistic regression analyses were used to identify factors associated with the resolution or development of LI.

**Results:** Complete language data at both time points were available on 319 participants (mean baseline age=12y, 80% black, 9% Hispanic). Among these, 112 (35%) had LI at baseline. Overall, SCs for the study population were highly stable and changes were similar in PHIV (n=212) and PHEU (n=107) participants. Family history of language delays/learning difficulties had a negative association with SC change after controlling for demographic and socioeconomic factors and baseline LI status (coefficient=-3.35, 95% CI: -5.83, -0.88). Among the PHIV group, being on combination antiretroviral treatment (cART) (4.50, CI: 0.31, 8.69) and high CD4 count (≥350 cells/mm<sup>3</sup>) (4.61, CI: 0.48, 8.74) at baseline were associated with a higher mean SC change. Over the follow-up period, initial LI was persistent in the majority of cases (78%) and 21 new cases of LI occurred (10%). No significant risk factors for either developing or resolving LI were found among combined PHIV and PHEU after adjusting for demographics and baseline SC. Among PHIV, not being on cART at baseline was associated with lower odds of LI resolution (adj. Odds Ratio<0.01, CI: <0.01, 0.61) after controlling for demographics and baseline SC

**Conclusions:** Perinatally HIV exposed youth with LI are at risk for persistent LI, irrespective of infection status. Risk factors for declining scores included family history and—for PHIV—not receiving cART and low CD4 count.

**815 Nevirapine (NVP) Concentrations in HIV-Infected Newborns Receiving Therapeutic Dosing**

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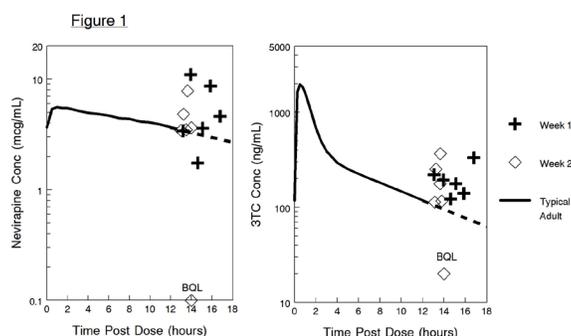
**Background:** Very early antiretroviral treatment (ART) initiation in HIV infected newborns may limit the seeding of viral reservoirs and maintain immune responses, but few antiretroviral agents are approved for use in newborns. Prophylactic NVP dosing is well established, but the appropriate NVP dose for treatment, which requires higher NVP levels than prophylaxis, is still unknown. While NVP is well absorbed in infants, its auto-induction, maturation and polymorphisms in its metabolism all complicate prediction of NVP treatment dosing in newborns. A very early treatment study in Botswana (BHP-074) was recently initiated using a NVP treatment dose of 6mg/kg BID for the first few weeks. Early objectives of this study are to determine NVP safety using 6mg/kg BID and if newborns achieve therapeutic levels with this dose.

**Methods:** HIV-infected infants born at ≥35 weeks gestation and ≥2 kg were enrolled in the early treatment protocol and had pre-dose plasma pharmacokinetic (PK) samples collected at Weeks 1 and 2. Participants received NVP 6mg/kg BID in combination with lamivudine (3TC) 2mg/kg BID and zidovudine (ZDV) 4mg/kg BID until 2 weeks of age (or 40 weeks gestational age equivalent, whichever later). Samples were analyzed for NVP and 3TC by High Performance Liquid Chromatography (HPLC). Drug concentrations were

compared to typical concentrations seen in adults receiving 200 mg NVP BID and 150 mg 3TC BID and the frequency of therapeutic NVP troughs (>3 mcg/mL) determined. Data are presented as mean (range).

**Results:** Six infants born at GA 37 (35-39), starting therapy at age 2.8 (1-5) days and weighing 3.12 (2.30-3.50) kg were evaluated. All infants experienced a reduction in HIV RNA and none had any adverse events considered related to ART during the first month of life. One participant was non-adherent at Week 2 with NVP and 3TC levels that were below the limit of quantitation (BLQ). Excluding the BLQ result, NVP levels averaged 5.10 (1.74-10.95) mcg/mL, and 3TC levels averaged 195 (114-368) ng/mL, obtained 14.2 (13.1-16.8) hrs after the prior dose (Figure 1). At Week 2, all NVP levels in adherent subjects were 3-11 mcg/mL.

**Conclusions:** Therapeutic, but not excessive, NVP levels are achieved in term infants receiving 6mg/kg BID during the first weeks of life. This dose appears to be well tolerated and should be utilized for very early treatment initiation in term and near term infants.



### 816 IMPAACT 1093: Dolutegravir in 6- to 12-Year-Old HIV-Infected Children: 48-Week Results

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**Background:** IMPAACT P1093 is an ongoing Phase I/II multicenter, open-label, pharmacokinetic (PK), safety, dose finding study of dolutegravir (DTG) plus optimized background regimen (OBR) in children and adolescents in age defined cohorts. The pediatric weight band dosing of ~1 mg/kg once a day in adolescents achieved PK exposure comparable to those observed at 50 mg once daily in adults.

**Methods:** Cohort IIA enrolled HIV infected treatment experienced, integrase naive children  $\geq 6$  to <12 years of age with an HIV RNA of  $\geq 1000$  copies/mL (c/mL) into Stage 1 (intensive PK) or Stage 2 (no PK, safety and efficacy). In Stage 1, DTG was added to a stable, failing ARV regimen, with OBR optimization after intensive PK (~Day 5-10); in Stage 2, DTG and OBR at study entry. Safety, tolerability, CD4 cell count and HIV-1 RNA were evaluated at Week 48, a primary objective. Virologic success was defined as achieving an HIV-1 RNA <400 c/mL by Week 48 based on the FDA snapshot algorithm and HIV-1 RNA <50 c/mL as a secondary outcome.

**Results:** Twenty three children (Stage 1, n=11; Stage 2, n=12) were enrolled and 21 (91.3%) completed the 48 week study visit. Demographics were as follows: 70% (16/23) male; 52% (12/23) African American, 17% (4/23) Caucasian; 26% (6/23) were of Hispanic ethnicity. Median age (range) was 10 yrs (6, 11) and median weight (range) was 30.0 kg (18, 54). Median (IQR) baseline CD4+ cell count and % were 645 cells/mm<sup>3</sup> (466, 732) and 24% (14.3%, 28.7%), respectively. Median (IQR) baseline HIV-1 RNA log<sub>10</sub> was 5.0 log<sub>10</sub> c/mL (4.5, 5.5). DTG weight band target dose was 1 mg/kg, (# participants/dose (mg)) distribution as follows: 1 (70); 5 (50); 6 (35); 8 (25). Virologic success (wk 48): HIV RNA < 400 c/mL was achieved in 78.3% (18/23); 95% CI: (56.3% to 92.5%); HIV <50 c/mL achieved in 73.9% (17/23); 95% CI: (51.6% to 89.8%). Median (IQR) gain in CD4 cell count and % at Week 48 was 387 cells/mm<sup>3</sup> (49, 575) and 9% (7, 14), respectively. DTG was well tolerated; none of the four Grade 3 clinical adverse events nor three Grade 3 laboratory events were study drug related. Two subjects went off study: one for virologic failure, and one moved and was lost to follow-up. There were no Grade 4 AEs, SAEs or discontinuations due to AEs.

**Conclusions:** DTG plus OBR was safe, well tolerated and provided virologic efficacy through week 48 in HIV infected children 6-12 years of age.

### 817 Safety and Efficacy of E/C/F/TAF in HIV-1 Infected Treatment-Naïve Adolescents

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**Background:** A subset of Tenofovir DF (TDF) recipients may have renal and bone toxicities. Tenofovir alafenamide (TAF), a novel prodrug of tenofovir shows 91% lower plasma tenofovir levels compared to TDF. In phase 3 adult studies, a single tablet regimen of elvitegravir 150mg, cobicistat 150mg, emtricitabine 200mg and TAF 10mg (E/C/F/TAF) was highly efficacious and well-tolerated, with improved renal and bone safety profiles as compared to TDF-containing regimens. We previously reported favorable pharmacokinetics of E/C/F/TAF in HIV-1 infected treatment-naïve adolescents through Week 24 and now report the safety and efficacy through the preplanned secondary efficacy endpoint at 48 weeks.

**Methods:** Treatment-naïve adolescents (12 to <18 years) with CD4 > 100 cells/mm<sup>3</sup>, weighing  $\geq 35$  kg received open-label E/C/F/TAF for 48 weeks. The primary efficacy endpoint was virologic success (HIV-1 RNA <50 c/mL) at Week 24 using the snapshot algorithm. Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry.

**Results:** 50 adolescents enrolled and two discontinued prior to Week 48. At Week 48, 46/50 (92%) had HIV-1 RNA < 50 copies/mL. The mean (SD) increase in CD4 cell count was 224 (170) cells/ $\mu$ L. No subject developed antiretroviral resistance. The most commonly reported adverse events (AEs) were mild/moderate and unrelated to study treatment. No subjects discontinued study drug due to AE, and none had proximal renal tubulopathy. The median (Q1, Q3) change in serum creatinine (Cr) was +0.07 (0.02, 0.15) mg/dL, consistent with the inhibition of renal tubular Cr secretion by cobicistat. The median (Q1, Q3) percent change from baseline to week 48 in urine protein to Cr ratio, retinol binding protein to Cr ratio, and beta-2-microglobulin to Cr ratio were -27% (-55%, +19%), -22% (-46%, +22%), and -29% (-60%, -4%), respectively. The median (Q1, Q3) change in spine BMD was +3.3% (+0.8%, +7.1%) and that of total body less head (TBLH) BMD was +0.9% (-0.5%, +2.6%). The median (Q1, Q3) change in height-adjusted spine Z-score was -0.03 (-0.16, +0.20) and that of TBLH Z-score was -0.09 (-0.3, +0.07). One subject had a  $\geq 4\%$  decrease in spine BMD from baseline, and none had a TBLH BMD decrease of  $\geq 4\%$ .

**Conclusions:** E/C/F/TAF is an effective first-line therapy and is well-tolerated with favorable renal and bone safety profiles in HIV-1 infected adolescents. These findings support further evaluation of E/C/F/TAF in younger HIV-1 infected pediatric patients.

**818 Pharmacokinetics, Safety, and Efficacy of Maraviroc in Pediatric Patients With R5 HIV**

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**Background:** Maraviroc (MVC) is a CCR5 antagonist approved to treat adults infected with CCR5-tropic (R5) HIV-1. Study A4001031 was conducted to evaluate the pharmacokinetics (PK), safety and efficacy of MVC in treatment-experienced (TE) pediatric patients.

**Methods:** This is an open-label, two-stage (stage 1: dose-finding; stage 2: safety/efficacy), age-stratified, non-comparative, multicenter study to evaluate the PK, safety and efficacy of MVC plus optimized background therapy (OBT) in TE children infected with R5 HIV-1. A total of 103 participants were enrolled into one of four age/formulation cohorts (Table) and dosed twice daily. Initial doses were determined by body surface area (BSA) and OBT, based on interactions with MVC in adults. Dose adjustment and PK re-evaluation occurred if average concentrations ( $C_{avg}$ ) at Week 2 were <100 ng/mL (stage 1).

**Results:** The majority of participants (90/103) included in the Week 48 primary analysis received OBT containing potent CYP3A4 inhibitors. The dosing strategy resulted in 49/50 stage 1 participants rolling over into stage 2 achieving  $C_{avg}$  concentrations  $\geq 100$  ng/mL irrespective of age, BSA or OBT, and an exposure range similar to that seen in adults. MVC was well-tolerated with a safety profile comparable to that of adults. The majority of treatment-emergent adverse events (TEAEs) were of Grade 1 severity. There were no deaths. None of the Grade 3 or 4 TEAEs or serious adverse events was considered to be related to MVC. Fourteen subjects had Grade 3 or 4 laboratory abnormalities with Grade 3 neutropenia the most common (n=8). All cohorts had a median decrease from baseline in HIV-1 RNA of  $>1 \log_{10}$ , while 67/103 subjects (65.0%) achieved HIV-1 RNA <400 copies/mL using the FDA snapshot (MSDF) algorithm, and 49/103 (47.5%) achieved HIV-1 RNA <48 copies/mL. An increase from baseline in median CD4+ cell count and percentage was seen for all age-groups. A total of 23 (22.3%) patients experienced protocol-defined virologic failure with few instances of non-R5 tropism (n=5), MVC resistance (n=1) or clinically relevant resistance-associated mutations (n=3).

**Conclusions:** Participants achieved the target  $C_{avg}$ , with exposure ranges similar to that observed in adults. MVC's safety profile in this population was comparable to that seen in adults with no new safety concerns identified. Virologic efficacy was comparable to what was reported in a similarly treatment-experienced adult population. These data support dosing recommendations for TE patients 2- <18 years old.

	Cohort 1 2- <6 years Liquid MVC (N=16)	Cohort 2 6- <12 years Tablet MVC (N=31)	Cohort 3 12- <18 years Liquid MVC (N=13)	Cohort 4 ≥12- <18 years Tablet MVC (N=43)	Total (N=103)
Race (White:Black:Asian:Other)	1/11/2/2	5/21/3/2	1/12/0/0	9/27/6/1	16/71/11/5
Median baseline log <sub>10</sub> HIV-1 RNA (copies/mL)	4.9	4.3	4.6	4.4	4.4
Median baseline CD4+ count (cells/μL)	977	471	438	406	471
Median treatment duration (days)	958	1093	969	714	914
Week 2 - MVC $C_{avg}$ geometric mean (ng/mL) for stage 1 subjects enrolled in stage 2	237 (N=12)	261 (N=11)	264 (N=10)	240 (N=17)	248 (N=50)
Week 48 - MVC $C_{avg}$ geometric mean (ng/mL)	164 (N=9)	290 (N=8)	169 (N=8)	199 (N=12)	199 (N=37)
Subjects with Serious Adverse Events	2	2	2	6	12
Subjects with Grade 3 or 4 adverse events	2	1	1	2	6
Discontinuations due to Adverse Events	0	0	1	1	2
Median Change from baseline in log <sub>10</sub> HIV-1 RNA at Week 48 (copies/mL)	-2.7	-2.1	-2.3	-1.2	-2.2
Median Change from baseline in CD4+ count at Week 48 (cells/μL)	266	286	143	130	192

**819 Safety of Tenofovir on Bone Mineral Density in HIV-Infected Youths: A 10-Year Study**

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**Background:** HIV-infected adults receiving highly active antiretroviral therapy (HAART) containing tenofovir DF (TDF) have decreased bone mineral density (BMD) measurements. Data in children are conflicting. The aim of this study was to assess the long-term safety of a TDF-containing HAART on BMD in a cohort of pediatric patients.

**Methods:** This was a single-site, longitudinal, controlled, observational study. We enrolled 26 vertically HIV-infected Caucasian youths (13 girls), aged 4.9 to 17.9 years, with undetectable viral load, receiving HAART containing lamivudine, stavudine (d4T) and a protease inhibitor (PI). At enrollment, all subjects replaced d4T with TDF and PI with efavirenz (EFV). We measured BMD at the lumbar spine and in the whole skeleton by dual-energy x-ray absorptiometry yearly for 10 years. BMD measurements were compared to those obtained in 201 healthy youths (90 girls), aged 3 to 25 years. To investigate the natural patterns of the variables over the time, the trend of each variable observed on healthy subjects as function of age was analyzed fitting quantile regression models based on natural spline with knots chosen on quartiles. Analyses were performed by non-linear mixed effect regression models for longitudinal data.

**Results:** During the study period, patients did not differ from healthy subjects in anthropometric measurements, and maintained HIV-RNA < 50 cp/ml and stable CD4+ count: mean values (range) at baseline vs 10-year were 842 (236-1518) and 854 (314-1526) cells/μL, respectively. The BMD regression curves of patients were significantly lower than those built for healthy subjects at the lumbar spine both when data were expressed as absolute values (P=0.017) or as z-scores (P=0.012). Similarly, BMD regression curves of the whole skeleton were significantly lower in patients (P<0.001 both for absolute values and z-scores). The difference between patients and control subjects did not change over time, and the longitudinal analyses did not show differences between patients and control subjects, thus indicating that bone mineral acquisition was not impaired.

**Conclusions:** Our long-term study on BMD measurements demonstrate that switching d4T to TDF and PI to EFV in HIV-infected youths, guarantees an optimal control of infection, does not normalize bone mineral density, but is not associated with a further impairment of bone mass.

**820 HIV Encephalopathy Despite Prolonged Viral Suppression With Slow Spontaneous Recovery**

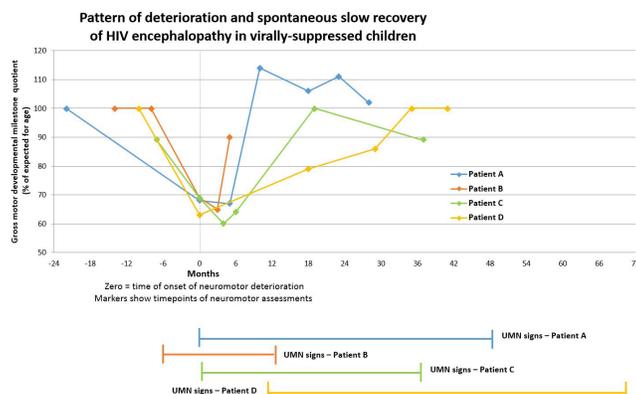
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Stellenbosch Univ and Tygerberg Children's Hosp, Cape Town, South Africa

**Background:** Early antiretroviral therapy (ART) dramatically reduces risk of HIV encephalopathy (HIVE) in children. Neurological deterioration, despite successful viral suppression in blood, has previously been attributed to HIV relapse within the central nervous system, a known sanctuary site. We describe four children with new onset HIVE despite long-standing viral suppression in blood and undetectable HIV DNA and RNA PCR in cerebrospinal fluid (CSF).

**Methods:** Four CHER (Children with HIV Early antiRetroviral therapy) trial participants were followed up clinically 3-monthly for 7 years. Blood HIV viral load and CD4 levels were measured at 6-12 monthly intervals. HIVE diagnosis required two of: (i) Acquired cortical motor deficit manifesting as pathological upper motor neuron (UMN) signs; (ii) Impaired brain growth manifesting as acquired microcephaly or generalized brain atrophy on imaging; (iii) Failure to attain or loss of developmental milestones.

**Results:** The four infants initiated ART at 2, 2, 2 ½ and 15 months of age respectively and attained sustained viral suppression within 6 months. New-onset neuromotor deterioration was seen at 31, 16, 20 and 53 months of age respectively. Two developed simultaneous language delay at 16 and 20 months respectively. Extensive investigations revealed no alternative aetiology, including ultra-sensitive HIV DNA and RNA PCR on CSF tap performed at 38, 42, 34 and 68 months of age respectively. Magnetic Resonance Imaging revealed generalized cerebral atrophy with extensive periventricular and peritrigonal leukoencephalopathy in one child; bilateral mild occipital periventricular hyperintensities in another; and no abnormalities in two children. Slow spontaneous recovery in gross motor and language development was observed over 6 months to 2 years despite no change in ART regimen (see figure). UMN signs resolved far more slowly over 3-5 years.

**Conclusions:** These are the first reported cases of HIVE despite sustained viral suppression in plasma from an early age and undetectable HIV in CSF. Virally-suppressed HIV-infected children remain at risk for HIVE. Slow but complete neuromotor and language recovery is reassuring.



## 821 A Diffusion Tensor Imaging and Neurocognitive Study of HIV-Infected Children

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**Background:** There are no diagnostic criteria for a spectrum of neurocognitive disorders (ND) secondary to HIV infection for children.

**Methods:** A cross-sectional cohort study was initiated in Cape Town, in which 120 participants, including a HIV negative control group for comparison, completed clinical and neurocognitive assessments. HIV infected children were either stable on antiretroviral treatment (ART) for a minimum of 6 months or ART naïve. Neuroimaging was completed on 105 children in the study. We compared 75 children vertically infected with HIV aged 6 to 16 years, including both children on ART and ART-naïve, with 30 matched controls using diffusion tensor imaging (DTI) measures. We then used the detailed neurocognitive battery; an assessment of adaptive functioning and the American Academy of Neurology (AAN) system for diagnosing HIV associated neurocognitive disorder (HAND) in adults, to establish whether this system could detect a spectrum of ND in HIV infected children.

**Results:** When comparing HIV uninfected children to HIV infected children DTI found damaged neuronal microstructure in the HIV infected children. Significant associations were found between failing first line ART regimen, nutritional-hematological status, HIV-relevant clinical variables, cognitive functioning and white matter integrity in children stable on ART. Children with a clinical diagnosis of encephalopathy (HIVE) had greater white matter damage when compared to children without encephalopathy. DTI also found significant myelin loss in ART naïve children when compared with ART treated children. Using the AAN criteria for HAND we found that 45.35% of the HIV infected children had a ND. ART naïve slow progressors, who receive limited attention from health care services, as they are thought to be 'well', were found to have neurocognitive impairment and white matter microstructural damage. HIV infected children were also more likely to have impaired competence in various domains of functioning.

**Conclusions:** Despite the use of ART and improved virological control with immune reconstitution, there were still a significant percentage of children who were found to have ND. Our findings suggest that children on ART remain at risk for developing CNS disease, and that this risk extends to physically well slow progressors. The HAND criteria designed for adults were able to identify children with functional cognitive impairments who don't fit criteria for HIVE and would therefore not have been identified otherwise

## 822 Brain Volumes, HIV Disease Severity, and Substance Use in Perinatally Infected Youth

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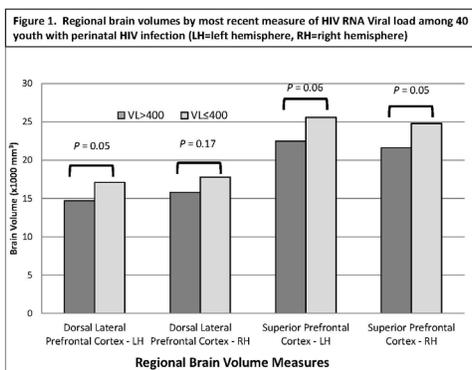
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**Background:** Combination antiretroviral therapy has improved survival in youth with perinatally-acquired HIV (PHIV), but they remain at risk for poor cognitive outcomes. We compared regional brain volumes of PHIV youth to uninfected controls, and among PHIV youth, evaluated associations with HIV disease severity and substance use.

**Methods:** We conducted structural magnetic resonance imaging (MRI) and cognitive testing in 40 PHIV youth recruited from one site of the PHACS Adolescent Master Protocol study. Current and past HIV disease severity measures were obtained from medical charts; self-reported substance use indicators were collected using audio computer-assisted structured interviews. Total gray matter and regional brain volumes were generated via FreeSurfer, and compared to 334 control youth from the PING study, adjusting for age and sex. Among PHIV youth, we evaluated associations of HIV disease severity measures and substance use with 11 primary brain volumes using Spearman correlations and via adjusted linear regression analyses. Associations between regional brain volumes and cognitive functioning measures (working memory and processing speed) were also examined.

**Results:** Regional brain volumes were significantly lower for the 40 PHIV youth (mean age=16.7 years) than the 334 PING youth (mean age=16.1 years), with adjusted decreases of 4-10%. Among the 40 PHIV youth, higher peak plasma viral loads showed significant negative correlations with volumes of left and right hemisphere dorsal lateral prefrontal cortex (Spearman  $r=-0.39$  and  $-0.43$ ) and superior prefrontal cortex ( $r=-0.35$  and  $-0.35$ ), and with total gray matter ( $r=-0.35$ ). Youth with recent unsuppressed viral loads (>400 copies/mL, 15%) had significantly lower volumes for these same brain regions (Figure 1); no association with nadir or current CD4 was observed. In adjusted models, youth reporting alcohol use or marijuana use had significantly lower volumes for postcentral gyrus, superior prefrontal cortex, and total grey matter, with decreases ranging from 9-16%. Significant positive associations of total gray matter and other affected brain regions with working memory and processing speed were observed.

**Conclusions:** PHIV youth had significantly lower total gray matter and regional brain volumes than similarly-aged uninfected youth, with largest decreases among PHIV youth with higher viral loads. Alcohol and marijuana use were also linked to lower brain volumes, suggesting that multiple factors may influence brain development.



**823 Long-term Effects on Basal Ganglia in Youth With Perinatally Acquired HIV Infection**

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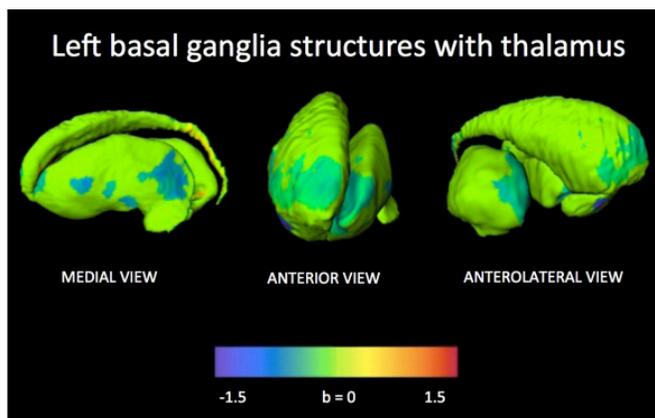
<sup>1</sup>Northwestern Univ, Feinberg Sch of Med, Chicago, IL, USA; <sup>2</sup>Harvard Sch of PH, Boston, MA, USA; <sup>3</sup>Ann and Robert H. Lurie Children's Hosp of Chicago, Chicago, IL, USA; <sup>4</sup>Tulane Univ Sch of Med, New Orleans, LA, USA; <sup>5</sup>Keck Sch of Med at the Univ of Southern California, Los Angeles, CA, USA

**Background:** HIV affects multiple brain regions. Pathological studies have demonstrated prominent and differential distribution patterns of HIV load in the basal ganglia. Neuroimaging studies in adults have also implicated these subcortical structures for HIV prognosis demonstrating a correlation between atrophy and clinical measures of disease severity. Whether these effects are observed in adolescents with perinatally-acquired HIV (PHIV) has not been well-studied.

**Methods:** We conducted structural magnetic resonance imaging (MRI) in 40 youth with PHIV (median age=17.1 years, 48% male) at one PHACS Adolescent Master Protocol study site. Current and past HIV severity measures were obtained from medical charts. Median peak HIV-1 RNA load was 522,000 copies/ml. Median nadir CD4+ T-lymphocyte percentage was 17%. Subcortical surfaces and vertex deformation referenced to a population mean for each region of the basal ganglia (nucleus accumbens, caudate, putamen and globus pallidus) as well as the hippocampus, amygdala and thalamus were obtained via automated FreeSurfer-initiated Large-Deformation Diffeomorphic Metric Mapping (FSLDDMM) pipeline. Principal component analysis (PCA) was performed for each region on the deformation covariance for dimensionality reduction, and components accounting for 75% of the variance were used to quantify subcortical shape. These PCA-based shape measures were evaluated for association with peak HIV-1 RNA viral load and nadir CD4+ T-lymphocyte percentage, adjusting for sex, age, and substance use. Significant associations were then visualized on the specific surfaces using vertex deformation data.

**Results:** Peak viral load showed significant correlations with the shape of the left putamen, globus pallidus, caudate, and thalamus (all p<0.001), but not with right basal ganglia structures. Visualization revealed that higher peak viral load was associated with increased inward deformity (i.e., localized volume loss) in contiguous patterns, primarily in anterior and posterior aspects of these structures (Figure). No association with nadir CD4 lymphocyte percentage was observed.

**Conclusions:** PHIV youth with a history of higher peak viral loads were associated with greater localized volume loss in multiple regions of the basal ganglia. These neuroimaging findings are consistent with histopathologic and clinical studies in adults, and suggest similar patterns of brain dysmorphology in adolescents with life-long HIV given antiretroviral therapy during brain development.



**Figure** Higher peak viral load was associated with increased inward deformity (i.e., localized volume loss) in contiguous patterns, primarily in the anterior and posterior aspects of the left putamen, globus pallidus, caudate, and thalamus. Visualized on the surface were vertex-wise significant (p<0.05, uncorrected) association (parameter estimate b) between vertex deformation and predict peak viral load, account for sex, age, and substance use.

**824 Risk Factors for BCG IRIS in HIV-Infected Infants Starting Antiretroviral Treatment**

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**Background:** Bacille Calmette-Guerin (BCG) vaccine is widely used in Tuberculosis (TB) endemic, resource-limited settings, and is administered soon after birth. In HIV-infected infants, BCG related Immune reconstitution inflammatory syndrome (IRIS) is a complication of ART but its pathogenesis is uncertain.

**Methods:** A multicenter case controlled prospective study (IMPAACTP1073) was conducted to assess incidence of BCG and TB IRIS in infants and children (age 1-72 months) and associated immunopathology. Of 202 enrolled participants, BCG IRIS was diagnosed in 21 children (10.4%) at periods ranging from 2-8 wks after ART initiation. T cell phenotypes (maturation, activation and exhaustion markers), regulatory T cells, monocyte subsets, NK cells and functional studies for intracellular expression of IFN $\gamma$ , TNFa, IL2, IL17, IL22, CD154 and IL10 in CD4 T cells upon stimulation with BCG or gag peptides at study entry (Pre-ART) and at diagnosis of BCG IRIS were investigated. Cases were compared with 21 non-IRIS controls matched by age, nadir CD4 frequency and duration of ART at time of IRIS diagnosis. Groups were compared by Mann-Whitney test.

**Results:** At entry, compared to controls, cases exhibited reduced absolute CD3 (889 vs 1098 cells/ $\mu$ l, p=0.02) and CD4 (182 vs 409 cells/ $\mu$ l, p=0.005) T cells, higher frequencies of CD14<sup>+</sup>CD16<sup>+</sup> inflammatory monocytes (3.5% vs 1.2%, p=0.03) and NK cells (34% vs 14%, p=0.01); lower frequencies of BCG-specific IL22<sup>+</sup>CD4<sup>+</sup> T cells (p=0.004) and gag-specific CD4 T cells expressing IFN $\gamma$  (p=0.004), TNFa (p=0.0005), IL10 (p=0.03) and CD154 (p=0.04). Frequencies of other BCG-specific cytokine-expressing T cells were not different. CD4 T cells increased from entry to the time of IRIS event in cases (p=0.004) and controls (p=0.002), but significant increase in CD4 absolute counts was evident only in cases (p=0.02). A dramatic immune reconstitution of BCG-specific Th1 cells were observed at IRIS diagnosis, compared to control samples (IFN $\gamma$  p=0.005; IL2 p=0.01; TNFa p=0.004). By contrast, reconstitution of gag-specific CD4 T cells was not different across groups. At time of IRIS diagnosis frequencies of NK cells were higher in cases compared to controls (p=0.006).

**Conclusions:** These findings imply that underlying immune deficiency and lack of BCG-specific Th22 cells coupled with inflammation involving monocytes contributes to the pathophysiology of BCG IRIS.

**825 Enhanced Inflammation and Rotavirus Vaccine Responses in Perinatal HIV-1 Infection**

**Priyanka Uprety<sup>1</sup>**; Jane Lindsey<sup>2</sup>; Myron Levin<sup>3</sup>; Kaitlin Rainwater-Lovett<sup>1</sup>; Carrie Ziemniak<sup>1</sup>; Susan Kaplan<sup>4</sup>; Micki Nelson<sup>4</sup>; Amanda Zadzilka<sup>5</sup>; Adriana Weinberg<sup>6</sup>; Deborah Persaud<sup>1</sup>  
<sup>1</sup>Johns Hopkins Univ, Baltimore, MD, USA; <sup>2</sup>Harvard Sch of PH, Boston, MA, USA; <sup>3</sup>Univ of Colorado Anschutz Med Campus, Aurora, CO, USA; <sup>4</sup>Merck Rsr Lab, North Wales, PA, USA; <sup>5</sup>Frontier Sci & Tech Rsr Frnd, Inc, Amherst, NY, USA; <sup>6</sup>Univ of Colorado, Denver, CO, USA

**Background:** Altered gut mucosa from HIV-1 infection leads to microbial translocation, persistent inflammation (IF) and significant morbidity in adults. The effects of IF in perinatal infection are unknown but may modify immune responses to oral live- vaccines in infancy.

**Methods:** Markers of IF (IFN- $\gamma$ , IL-1 $\beta$ , IL-10, IL-2, IL-6, IL-8, TNF- $\alpha$ , IL-4, IL-13 and IL-12p70) and sCD14 were quantified in plasma of HIV-1-infected (HIV+) and HIV-1-exposed uninfected (HEU) African infants enrolled in a double-blind, placebo-controlled clinical trial (IMPAACT P1072) of the safety and immunogenicity of 3 doses of pentavalent rotavirus vaccine (RV5) by age 32 weeks. IF markers and sCD14 were measured at study entry and 21 days post vaccine dose (PD) 1; sCD14 was also measured at 14 and 42 days PD3. Non-parametric tests and correlations were used to compare biomarkers between groups and censored normal regression to explore associations at entry between biomarker levels and IgA and serum neutralizing antibodies (SNA) against RV proteins (G1-G4 and P1) I PD3 in RV5 recipients.

**Results:** This analysis included 68 HIV+ (median age at entry 93 days; 63% breast-fed; 69% received antiretroviral [ARV] prophylaxis for prevention of mother-to-child transmission (pMTCT) and 116 HEU (median age 82 days; 64% breast-fed and 91% received ARV prophylaxis for pMTCT) of 202 infants enrolled. 36/68 HIV+ and 59/116 HEU received RV5, respectively. At entry, the median plasma viral load was 39,827 c/ml; 93% HIV+ were on antiretroviral treatment. HIV+ infants had higher mean concentrations of IFN- $\gamma$ , IL-1 $\beta$ , IL-10, IL-2, IL-6, TNFa, and sCD14 than HEU (ranging from 16% [sCD14] to 204% [IL-6] higher across markers, p $\leq$ 0.05) after adjusting for age, breastfeeding and ARVs for pMTCT. In HIV+, lower concentrations of IFN- $\gamma$  (r= -0.44, p=0.009) and IL-10 (r= -0.39, p=0.022) at entry correlated with higher SNA G1 at PD3 and lower IL-10 (r= -0.37, p=0.029) with higher SNA P1. No correlations were found between entry IF biomarkers and serum IgA titers at PD3. Antibody titers PD3 in RV5 recipients were similar in HIV+ and HEU. There were no significant differences in biomarker changes between placebo and vaccine recipients in either HIV+ or HEU infants.

**Conclusions:** Heightened IF in perinatal HIV-1 infection did not impact humoral responses to RV5. However, whether IF in infancy affects long-term immunity to childhood vaccines requires further study.

**826 Vitamin D Supplementation Decreases Immune Activation and Exhaustion in HIV+ Youth**

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<sup>1</sup>Med Univ of South Carolina, Charleston, SC, USA; <sup>2</sup>Emory Univ Sch of Med, Atlanta, GA, USA; <sup>3</sup>Case Western Reserve Univ, Cleveland, OH, USA

**Background:** Heightened immune activation and exhaustion drive HIV disease progression and co-morbidities. Vitamin D has pleiotropic effects on the immune system, but little is known about the effects of supplementation in HIV infection. Our study investigates these potential effects after 12 months of supplementation in virologically-suppressed HIV+ youth with vitamin D insufficiency.

**Methods:** This is a randomized, active-control, double-blind trial investigating 2 different monthly vitamin D<sub>3</sub> doses [60,000 (medium) or 120,000 (high) IU/month] vs. a control arm of 18,000 IU/month in 8-26 year old HIV+ youth on ART with baseline 25-hydroxyvitamin D (25(OH)D)  $\leq$ 30 ng/mL and HIV-1 RNA <1000 copies/mL. Randomization was stratified by EFV use. Only subjects who maintained an undetectable HIV-1 RNA level for 12 months and had available PBMCs at baseline (BL) and 12 months were included in this analysis. Markers of immune activation and exhaustion were measured by flow cytometry. Comparisons of marker changes from BL to 12 months were made within each of the three dosing groups and between groups (medium vs. control, high vs. control) using appropriate two-sample tests.

**Results:** 50% of enrolled participants (N=51) were included in the analysis: 63% male, 86% black with median (Q1, Q3) age of 20 (15, 22) years and CD4 count of 654 (451, 888) cells/mm<sup>3</sup> (all similar to the rest of the study participants). HIV and ARV duration were 11 (3, 18) and 7 (2, 11) years, respectively. Overall, BL 25(OH)D was 17 (13, 25) ng/mL and not different between arms or to the rest of the study participants. By 12 months, 25(OH)D increased statistically within each dosing group (control: +12 (6, 17); medium: +19 (10, 32); high: +31 (16, 41) ng/mL; all P<0.001) with greater increases in the medium group vs. controls (P=0.04) and high group vs. controls (P=0.008). Overall, all measured markers decreased with CD4 activation (CD4+CD38+HLA-DR+), CD8 activation (CD8+CD38+HLA-DR+), CD4 exhaustion (CD4+CD38+HLA-DR+PD1+), and inflammatory monocytes (CD14+CD16+) reaching statistical significance. These decreases were mostly driven by subjects in the high dose group (Table).

**Conclusions:** Vitamin D supplementation decreased markers of T-cell activation and exhaustion, and monocyte activation regardless of dose in HIV+ youth, but subjects given the highest dose (120,000 IU/month) showed the greatest improvements. These data suggest that high-dose vitamin D may further attenuate immune activation and exhaustion HIV+ youth on ART.

	All Subjects N=51	P <sup>†</sup>	Control Dose N=21	P <sup>†</sup>	Medium Dose N=18	P <sup>†</sup>	High Dose N=12	P <sup>†</sup>	P <sup>‡</sup>	P <sup>‡</sup>
CD4+CD38+HLA-DR <sup>+</sup> a	-0.19 (-0.74, 0.06)	<b>0.005</b>	-0.09 (-0.57, 0.15)	0.31	-0.17 (-0.75, 0.03)	0.07	-0.36 (-0.85, -0.13)	<b>0.04</b>	0.35	0.20
CD8+CD38+HLA-DR <sup>+</sup>	-0.71 (-1.88, 0.20)	<b>0.003</b>	-0.11 (-1.6, 0.43)	0.30	-0.95 (-2.6, 0.16)	0.55	-0.96 (-2.8, -0.07)	<b>0.03</b>	0.54	0.76
CD4+CD38+HLA-DR+PD1 <sup>+</sup> a	-0.15 (-0.36, 0.03)	<b>0.001</b>	-0.15 (-0.36, 0.13)	0.10	-0.06 (-0.32, -0.02)	0.37	-0.26 (-0.42, 0.001)	0.09	0.98	0.27
CD8+CD38+HLA-DR+PD1 <sup>+</sup>	-0.15 (-0.68, 0.20)	0.12	-0.02 (-0.68, 0.29)	0.74	-0.16 (-0.68, 0.16)	1.00	-0.31 (-0.62, -0.01)	0.18	0.68	0.54
CD14+CD16 <sup>+</sup> b	-2.7 (-10.4, 2.6)	<b>0.03</b>	-3.2 (12.2, 5.1)	0.25	0.5 (-2.9, 4.5)	0.70	-8.9 (-18.0, 2.3)	<b>0.004</b>	0.27	0.36
CD14dimCD16 <sup>+</sup> b	-2.6 (-5.8, 4.9)	0.32	-0.16 (-9.3, 7.3)	0.70	-1.9 (-4.3, 3.3)	0.43	-3.5 (-5.7, 3.8)	0.46	0.99	0.26

Data reported as median (Q1, Q3) and represent the % of that cell type expressing the given phenotype; <sup>†</sup>1 subject with no data due to cell viability issues; <sup>‡</sup>3 subjects with no data due to cell viability issues; <sup>†</sup>P value within group; <sup>‡</sup>P value between medium vs. control dosing groups; <sup>‡</sup>P value between high vs. control dosing groups

## 827 Contrasting CTL Impact in Distinct Phenotypes of Nonprogressing Pediatric HIV

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**Background:** ART-naïve non-progressing adult HIV infection is characterized by low viremia, strongly linked with expression of protective HLA I. In contrast, in the natural hosts of SIV infection such as sooty mangabeys non-progressing infection is associated with high viremia and a minimal role of MHC I. Non-progressing pediatric HIV infection is poorly characterized. Here we present two perinatally infected pediatric non-progressors (PNP) who adopt, respectively, an adult elite controller-like and a sooty mangabey-like phenotype in achieving long-term maintenance of normal health and CD4 T-cell counts.

**Methods:** Viral loads, CD4 counts and CTL responses in two South African vertically infected ART-naïve children were monitored over the first decade of life. Full HIV genomes from children and their mothers were ultra-deep sequenced and interrogated for evidence of HLA-mediated CTL selection pressure.

**Results:** ART-naïve child 133C maintained healthy CD4% (mean 32%) and absolute count (1,028c/mm<sup>3</sup>) while gradually suppressing viremia to 520 copies/ml by 9yo. She expresses HLA-A\*74 and B\*81, protective alleles in adult C-clade infection. ART-naïve child 517C maintained normal CD4 counts (43%, 1,232c/mm<sup>3</sup> at 10yo), but in the setting of high viremia (median 35,500copies/ml) and without protective HLA. Both mothers expressed HLA-B\*42:01/B\*58:02. Deep sequencing showed that 133C selected escape mutations in five epitopes: HLA-B\*81-Gag-TL9 (T186M), A\*74-Gag-KR9 (K12N), B\*81-Pol-SL10 (P159S), A\*74-Pol-SR10 (R432K) and B\*81-Nef-RM9 (L76V/I/T). These were mediated by child's HLA-A\*74/81 that were not shared with the mother. Minor variants arose as early as 3m. Variant-specific TL9-T186M responses came to replace wildtype TL9-specific CTL in the child. In contrast, 517C did not show evidence of escape in HLA-restricted epitopes. However, analysis of changes in genome diversity in non-epitopic regions showed more sequence evolution in 517C, particularly between 2.5 to 5yo.

**Conclusions:** These data suggest that distinct immune strategies may be successful in non-progressing pediatric infection: adult elite controller-like CTL-mediated suppression of viremia via protective HLA alleles such as HLA-A\*74 and B\*81 as in child 133C; and a sooty mangabey-like approach in which high viremia is associated with low immune activation and a minimal role for CTL as in child 517C. Further studies are needed to define the mechanisms of low immune activation despite persistent high viremia in non-progressing children such as 517C.

## 828 Impact of HLA-B\*81-Driven Escape Mutation L188F on VRC in Pediatric Slow Progression

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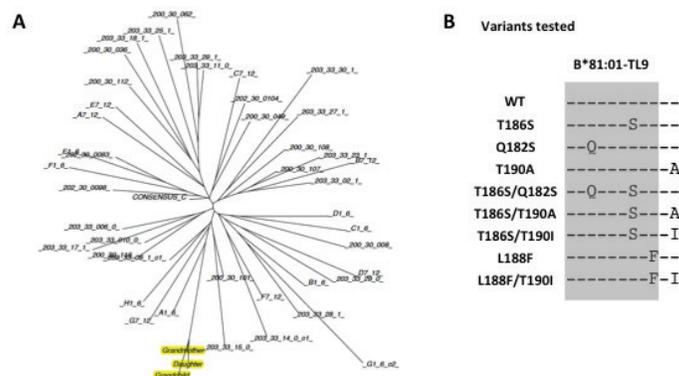
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**Background:** Two ART-naïve HIV-infected children were identified within the same South African family, who were infected by the same donor, via mother-to-child transmission and by grandmother-to-grandchild (breast milk) transmission, respectively (Fig. A). We aimed to study this family trio to define host genetic and virologic factors contributing to slow progression in the two children. All three family members expressed the protective HLA-B\*81:01. In each subject autologous virus encoded the same rare escape mutation L188F within the immunodominant HLA-B\*81:01-restricted TL9 epitope (Gag 180-188).

**Methods:** L188F, the well-described HLA-B\*81:01-associated TL9 escape mutants, most commonly T186S, and selected putative compensatory variants, were introduced by mutagenesis into a modified patient-derived Gag-protease sequence corresponding to Consensus subtype C. These TL9/Gag-Pro variants and the Gag-Pro sequence of the grand-daughter were inserted in the backbone of pNL4-3. These mutant viruses were then generated via electroporation and the viral replication capacity of each determined in comparison with that of NL4-3.

**Results:** L188F or T186S were incapable of replicating at a sufficient level in vitro to measure viral replicative capacity (VRC). Although GFP+ expression in target cells remained negative up to >100 days in culture, viral RNA was successfully extracted from the supernatant and the respective L188F and T186S mutations were confirmed by sequencing. T190I compensated neither L188F nor T186S but Q182S and T190A in combination were able to rescue T186S. The VRC of the grand-daughter's Gag-Pro chimeric virus, however, was similar to that of WT despite the presence of L188F. This indicates that one or more compensatory mutants may be present in the grand-daughter virus to correct for the VRC cost of L188F (Fig. B).

**Conclusions:** HLA-B\*81:01-restricted L188F escape mutation alone substantially reduced VRC to the point where it could not be quantified. However since the VRC cost of L188F was corrected in the grand-daughter, it is likely that this was the virus transmitted and that low VRC did not contribute to slow progression in these two children. This conclusion is consistent with recent data indicating that HLA class I alleles such as HLA-B\*57:01/81:01 that are protective in adult infection contribute little to pediatric slow progression.



## 829 Increasing Adolescent HIV Testing With a Hybrid Mobile Strategy in Uganda and Kenya

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**Background:** Ensuring adequate HIV testing coverage among adolescents in sub-Saharan Africa is crucial given the increased HIV risk faced during transition into adulthood. National survey data estimate that <20% of African adolescents know their HIV status. We sought to increase adolescent HIV testing across rural communities in Uganda and Kenya using a hybrid mobile testing approach, and to identify predictors of undiagnosed HIV.

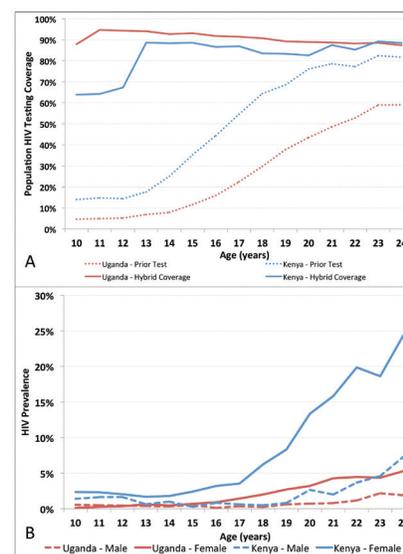
**Methods:** In 2013-14, we enumerated 116,326 adolescent (10-24 years) residents of 32 communities in Uganda (20), and Kenya (12) with a door-to-door census in an HIV "test and treat" trial (SEARCH:NCT01864603). 98,694 adolescents (85%) reported stable residence (living in community ≥6 months). In each community we performed a hybrid testing

strategy of 2-week multi-disease community health campaigns (CHC), that included HIV testing, counseling and linkage to care, followed by home-based testing of CHC non-participants over 1-2 months. We measured stable adolescent HIV testing coverage and prevalence, and determined predictors of self-reported newly diagnosed HIV among HIV+ adolescents, using multivariable logistic regression and accounting for clustering by household.

**Results:** HIV testing was achieved in 86,421 (88%) stable adolescents using a hybrid strategy; testing coverage was 86%, 90%, and 88% in early (10-14 years), mid (15-17) and late (18-24) adolescents, respectively. Self-reported prior HIV testing was 9%, 24%, and 50% in early, mid and late adolescents who tested for HIV, respectively. HIV testing coverage and prevalence by age are shown in Figure. In early, mid and late HIV+ adolescents, 61%, 69% and 56% reported newly diagnosed HIV, respectively. Overall, 51% of newly diagnosed HIV+ adolescents reported ever having a prior HIV test. In multivariate analysis of HIV+ adolescents, predictors of newly diagnosed HIV included male gender (OR=1.97 [95% CI: 1.42-2.73]), Ugandan residence (OR=2.63 [95% CI: 2.08-3.31]), and single marital status (OR=1.62 [95% CI: 1.23-2.14] vs. married).

**Conclusions:** A hybrid mobile HIV testing strategy achieved significant increases in stable adolescent testing coverage to 88% compared to self-reported prior testing. The high proportion of HIV+ adolescents with newly diagnosed HIV (>50%) is likely due to a combination of increasing HIV risk with age and inadequate prior testing. Community-based, mobile testing initiatives to reach 90% of HIV+ adults can and should be leveraged to test adolescents and offer combination HIV prevention services.

**Figure.** Testing Coverage Achieved with the Hybrid Mobile Testing Approach (Panel A solid line), Reported Prior Testing (Panel A, dashed line), and HIV Prevalence (Panel B) among Adolescents in the SEARCH Trial.



**830 Weak HIV Antibody Responses in Perinatally Infected Young Adults**

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**Background:** Young adults who acquired HIV infection perinatally comprise a significant proportion of HIV-infected individuals in some populations. These individuals are often misclassified as recently infected by age-based HIV prevalence models. There is little information characterizing the antibody response to HIV in perinatally-infected adults. Weak antibody responses could impact the performance of serologic assays used for HIV incidence estimation. We compared serologic characteristics in young adults with perinatally-acquired HIV infection (PN) to two groups who were infected as adults who are frequently misclassified as recently infected using cross-sectional HIV incidence assays: elite controllers (EC) and adults exposed to antiretroviral therapy (ART).

**Methods:** Samples were obtained from adults living in the Baltimore-Washington area. (1) PN group: 225 samples from 22 adults perinatally-infected between 1984 and 1996; mean age: 20 years, range: 12-30; 159 samples had viral loads (VL) <400 copies/ml, none had suppressive therapy at birth and were exposed to multiple years of non-suppressive sequential monotherapy. (2) ART group: 480 samples from 379 adults exposed to ART; 290 samples had VL <400 copies/ml, mean minimal duration of infection 13 years, range: 8 to 25 years. (3) EC group: 40 samples from 21 adults. Samples were tested with the Limited Antigen Avidity Assay (LAG-Avidity) and an avidity modified version of the Genetic Systems 1/2 + O EIA (BioRad).

**Results:** The PN group had higher frequency of samples with low LAG-Avidity and low BioRad results than the ART and the EC groups, see table. When comparing the PN to ART group stratified by viral suppression status, PN samples were more likely to have low LAG-Avidity and BioRad values. For VL<400 copies/ml, LAG-Avidity <1.5 OD-n: PN 73/159 vs. ART 25/290, P<0.001 and BioRad AI <80%: PN 46/159 vs. ART 3/290, P<0.001. Among VL>400 copies/ml, LAG-Avidity <1.5 OD-n: PN 3/66 vs. ART 2/190, P=0.11 and BioRad AI <80%: PN 5/66 vs. ART 0/190, P=0.001.

**Conclusions:** Young adults infected perinatally were more likely to be misclassified by incidence assays than their counterparts who were infected as adults. These results could impact the precision of HIV incidence estimates in studies using these assays to analyze populations that include perinatally-infected adults. Further studies are needed to determine reason for the reduced antibody responses in these individuals.

**Table 1. Avidity assay results.**

Group	LAG-Avidity < 1.5 <sup>a</sup> % (# positive / # tested)		P value*	BioRad < 80% <sup>b</sup> % (# positive / # tested)		P value*
	Persons	Samples		Persons	Samples	
<b>PN</b>	50% (11/22)	34% (76/225)	Ref	38% (7/22)	23% (51/225)	Ref.
<b>ART</b>	7% (27/379)	6% (27/480)	<0.001	0.8% (3/379)	0.6% (3/480)	<0.001
<b>EC</b>	20% (4/21)	23% (9/40)	0.06	0% (0/21)	0% (0/40)	0.009

\*Compared to the PN group; <sup>a</sup>Normalized optical density units (OD-n); <sup>b</sup>Avidity index.

**831 Incidence of Virologic Rebound in Perinatally HIV-Infected Adolescents on Stable cART**

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**Background:** With combination antiretroviral therapy (cART), survival of perinatally HIV-infected children has dramatically improved. Yet, maintaining successful treatment during adolescence is challenging. This study aimed to assess the incidence and predictors of virologic rebound among adolescents on cART with previously undetectable virus levels.

**Methods:** Perinatally HIV-infected Asian adolescents (age 10-19 years) followed in the TREAT Asia Pediatric HIV Observational Database who were on cART and had a period of virologic suppression (two consecutive HIV RNA <400 copies/ml ≥6 months apart) before or during adolescence were included in the analysis. Baseline was the date of the first HIV

RNA test <400 copies/ml at  $\geq 10$  years of age, or the 10<sup>th</sup> birthday for those with prior suppression. Cox proportional hazard models were used to identify factors associated with post-suppression virologic rebound (HIV RNA >1,000 copies/ml). Median values are provided with interquartile ranges (IQR).

**Results:** Of 1,379 eligible adolescents, 47% were male. At baseline, 22% were on protease inhibitor (PI)-based regimens, the median CD4 count was 685 (448-937) cells/mm<sup>3</sup>, and 2% had a documented history of pre-adolescent virologic failure before subsequent suppression. The median number of HIV RNA measurements was 1.6 (1.2-2.2) per year. During adolescence, 180 individuals (13%) experienced post-suppression virologic rebound at a rate of 3.4 (95% confidence interval: 2.9-3.9) per 100 person-years (PYs). Median time to rebound was 3.3 (2.1-4.8) years. In multivariate analysis, wasting (weight-for-age Z-score <-2.5), having a grandparent as a primary caregiver, receiving PI-based regimens, starting their first cART regimen after 2005, and having an episode of pre-adolescent virologic failure were significantly associated with post-suppression virologic rebound during adolescence (Table 1). At virologic rebound, the median age was 14.8 (13.2-1.4) years; the most recent CD4 cell count was 507 (325-723) cells/mm<sup>3</sup>. Overall, 16 adolescents were lost to follow-up at a rate of 0.3 events per 100 PYs, and 11 died at a rate of 0.2 deaths per 100 PYs.

**Conclusions:** Post-suppression virologic rebound was not uncommon among adolescents in our cohort on stable cART. Those with wasting, being cared for by grandparents, using PI-based regimens, commencing cART after 2005, or having pre-adolescence virologic failure were at higher risk of rebound, and may benefit from increased monitoring to support long-term treatment success.

**Table 1. Factors associated with post-suppression virologic rebound among perinatally HIV-infected adolescents on stable combination antiretroviral treatment**

Characteristics	Virologic failure, n (%)	Person-years follow-up	Rate per 100 person-years (95%CI)	Univariate analysis		Multivariate analysis	
				HR (95%CI)	P	Adjusted HR (95%CI)	P
<b>Age at initial viral suppression (years)</b>							
• <10	84 (10.7)	2884.1	2.91 (2.35 - 3.61)	Ref			
• 10.0 to 14.9	78 (17.0)	2094.9	3.72 (2.98 - 4.65)	1.25 (0.91 - 1.72)	0.18		
• 15.0 to 19.9	18 (13.4)	381.4	4.72 (2.97 - 7.49)	2.43 (1.41 - 4.18)	0.001		
<b>Sex</b>							
• Male	81 (12.6)	2479.1	3.27 (2.63 - 4.06)	Ref			
• Female	99 (13.5)	2881.3	3.44 (2.82 - 4.18)	1.08 (0.80 - 1.47)	0.61		
<b>Weight-for-age z-score</b>							
• >-1.5	51 (11.0)	1740.7	2.93 (2.23 - 3.86)	Ref		Ref	
• -1.5 to -2.5	49 (11.7)	1682.8	2.91 (2.20 - 3.85)	1.02 (0.68 - 1.53)	0.92	1.06 (0.71 - 1.60)	0.76
• <-2.5	70 (17.0)	1672.9	4.18 (3.31 - 5.29)	1.60 (1.10 - 2.33)	0.01	1.49 (1.01 - 2.18)	0.04
<b>Primary caregiver</b>							
• Parent	31 (7.1)	1314.3	2.36 (1.66 - 3.35)	Ref		Ref	
• Grandparent	56 (17.9)	1230.0	4.55 (3.50 - 5.92)	1.79 (1.12 - 2.84)	0.01	2.05 (1.27 - 3.30)	0.003
• Relative/non-relative/foster	56 (15.6)	1506.8	3.72 (2.86 - 4.83)	1.46 (0.88 - 2.41)	0.14	1.53 (0.92 - 2.55)	0.10
<b>Baseline CD4 cell count (cells/mm<sup>3</sup>)<sup>a</sup></b>							
• >750	61 (10.9)	2041.9	2.99 (2.32 - 3.84)	Ref			
• 500 to 750	36 (10.8)	1378.7	2.61 (1.88 - 3.62)	0.85 (0.56 - 1.28)	0.44		
• <500	73 (18.7)	1693.2	4.31 (3.43 - 5.42)	1.37 (0.96 - 1.94)	0.08		
<b>Baseline cART regimen<sup>b</sup></b>							
• NNRTI-based	119 (11.1)	4443.6	2.68 (2.24 - 3.21)	Ref		Ref	
• PI-based	57 (19.5)	858.3	6.64 (5.12 - 8.61)	2.66 (1.88 - 3.75)	<0.001	2.66 (1.86 - 3.79)	<0.001
<b>Year of the first cART regimen initiation</b>							
• <2005	86 (14.9)	2834.9	3.03 (2.46 - 3.75)	Ref		Ref	
• 2005 to 2008	80 (12.0)	2213.6	3.61 (2.90 - 4.50)	1.75 (1.22 - 2.50)	0.002	1.89 (1.31 - 2.72)	0.001
• >2008	14 (10.4)	311.9	4.49 (2.66 - 7.58)	2.96 (1.59 - 5.48)	0.001	4.15 (2.15 - 7.99)	<0.001
<b>Experienced pre-adolescent virologic failure</b>							
• No	174 (12.8)	5286.0	3.29 (2.84 - 3.82)	Ref		Ref	
• Yes	6 (25.0)	74.4	8.06 (3.62 - 17.95)	2.72 (1.16 - 6.38)	0.02	2.68 (1.11 - 6.48)	0.03

Abbreviation: cART, combination antiretroviral therapy; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; 95%CI, 95% confidence interval; HR, hazard ratio.  
<sup>a</sup>Baseline was defined as the date of the first HIV RNA test <400 copies/ml for children  $\geq 10$  years, or the 10<sup>th</sup> birthday for those with prior suppression.

### 832 Longitudinal Virologic Suppression Among a Cohort of Behaviorally HIV-Infected Youth

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**Background:** Cross-sectional virologic suppression rates among HIV-infected adolescents and young adults (AYA) are significantly lower than those among adults. However, little is known about the longitudinal patterns of virologic suppression among AYA. Recent treatment guidelines suggest reduced monitoring of adolescents and adults with well controlled HIV, however it is unknown if these guidelines relevant for AYA.

**Methods:** This retrospective cohort study included all behaviorally HIV-infected AYA enrolled in care at a U.S. academic medical center from January, 2002-October, 2011. Youth were included if they initiated antiretroviral therapy (ART) during the study period and had a minimum of six months of follow up time after ART start. Subjects were followed until the first episode of virologic failure (defined as HIV plasma RNA >1000 copies/ml once or >200/ml on two consecutive measurements) or censored at transition of care, loss to follow up, death or the end of the study period (December, 2013).

**Results:** Of the 296 eligible AYA, 176 (59.5%) received ART. Of these, 116 (65.9%) initiated during the study period and had a minimum of 6 months follow up time, comprising the study cohort. The cohort was 91% African American and 83% male. Transmission was by male-male sexual contact in 82%. The median age at HIV diagnosis was 18.3 years (IQR 16.9-20). The median age at ART initiation was 20.1 years (IQR 18.6-22) and subjects were followed for a median of 2.6 years (IQR 1.7-4) after ART start. The median time from diagnosis to first regimen was 12.2 months (IQR 5.2-24.8).

Virologic suppression on first ART regimen was achieved by 102 (88%) subjects, 31.3% (n=32) of whom experienced virologic failure within 2 years of ART initiation, with a median time to failure of 8.2 months (IQR 5.2-11.7). There were 45 subjects (44%) who had two years of sustained virologic suppression, and were followed for a median of 3.2 years (IQR 2.6-4.3) after ART initiation. Of these patients, 20% (n=9) subsequently developed virologic failure in their remaining time in the cohort, with 55% of these failing between their second and third years of ART.

AYA remained at high risk of virologic failure throughout the course of treatment, even among those with sustained virologic suppression of  $\geq 2$  years duration. These finding may suggest that recent changes in treatment guidelines recommending decreased frequency of laboratory monitoring among patients with suppression of  $\geq 2$  years duration may not be applicable to AYA.

### 833 Psychological Reactance Is a Novel Risk Factor for Adolescent HIV Treatment Failure

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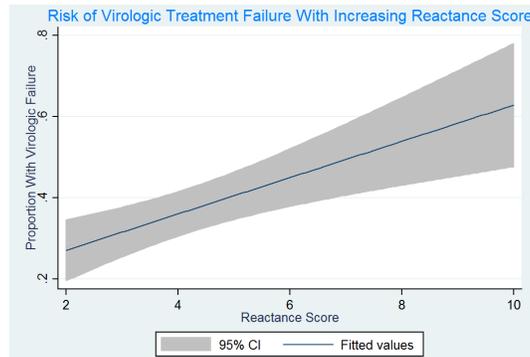
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**Background:** Antiretroviral (ARV) treatment failure rates and death rates among adolescents with HIV remain alarmingly high and adolescent-specific interventional approaches are desperately needed. Adolescents' high failure rates may be partially due to risk factors that are unique to or enhanced by their developmental stage. Psychological reactance is an aversive response to perceived threats to behavioral freedom that may be more common among adolescents. Reactance can be measured and can be increased or decreased through specific parenting or counseling approaches.

**Methods:** In a cohort study of HIV-infected adolescents (age 10-19 years) on ARVs in Botswana, we utilized a 2-question medication-specific reactance tool to assess a) whether having someone tell them to take their ARVs makes adolescents want to avoid taking them and b) whether the adolescents get angry when reminded to take their ARVs. Both questions were scored on a 5-point Likert scale from "definitely false" (1 point) to "definitely true" (5 points). Virologic failure was defined by an HIV viral load >400 copies/mL in the 24 months prior to reactance measurement. Adolescents were classified as "reactant" if their score on the medication-specific reactance tool was >4. Reactance scores were compared between adolescents with and without virologic failure using logistic regression.

**Results:** 289 adolescents were evaluated. 106 (36.7%) had virologic failure during the follow-up period and 89 (30.8%) were classified as reactant. Reactant adolescents had a 2.5-fold (95% CI 1.5-4.2) greater odds of failure than non-reactant adolescents ( $p < 0.001$ ). The risk of failure was 21% greater (95% CI 9%-35%) for each single point elevation in reactance score ( $p < 0.001$ ) (see Figure).

**Conclusions:** Psychological reactance is a novel risk factor for treatment failure among HIV-infected adolescents. Reactance may be a useful interventional target for improving adolescent ARV adherence.



**834 Economic Evaluation of a Novel Adherence Strategy in Perinatally HIV-Infected Youth**

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**Background:** Perinatally HIV-infected youth (PHY) frequently require support to achieve optimum virological control. We identified ART non-adherent patients at risk of AIDS-defining illness in a UK cohort of PHY; explored the cost to the National Health Service of non-adherence in this group and subsequently the potential effect on healthcare costs of providing a financial incentive (FI) and motivational interviewing (MI) based adherence-enhancing intervention to these patients.

**Methods:** 122 patients in receipt of suppressive ART regimens were selected from a service for 141 PHY aged 17-31 years (2006-2015). Patients annually at risk of AIDS-defining illness were defined as having 2 CD4 counts < 350 cells/ml<sup>3</sup> with detectable HIV viral load > 3 months apart during 1 year. Patients annually at risk for > 1 year of their clinic record were designated 'at risk' overall. HIV-related hospitalisations were retrospectively identified, costed from the healthcare provider perspective and compared in 'at risk' and 'not at risk' patients (2010-2015). The published effect of an FI/MI intervention was subsequently applied over a 24-month model to all 'at risk'. Patients newly adherent post-intervention accrued the annual health benefits and healthcare costs of their 'not at risk' peers. Total sample pre/post intervention costs were compared, including: standard outpatient care; HIV-related hospitalisation and intervention provision to 'at risk' patients.

**Results:** 60/122 (49%) patients were designated 'at risk' overall. Median age was significantly higher in these patients ( $D = -0.3, p = 0.003$ ). However, crude rates of social stress, comorbidity, AIDS experience, ART toxicity experience and neurocognitive deficit did not differ significantly between 'at risk' and 'not at risk' groups ( $X^2(1) = 1.9, n = 122, p = 0.2$ ;  $X^2(1) = 0.03, n = 122, p = 0.9$ ;  $X^2(1) = 0.1, n = 122, p = 0.7$ ;  $X^2(1) = 0.2, n = 122, p = 0.7$ ;  $X^2(1) = 0.005, n = 122, p = 0.9$ ). The 5-year rate of hospitalisation was significantly higher in 'at risk' than 'not at risk' patients ( $X^2(1) = 17.6, n = 95, p = 0.00003$ ). Median hospitalisation costs accrued per person-year contributed to the analysis were 113-fold greater in 'at risk' patients. Expansion of the FI/MI intervention to all 'at risk' was cost saving over 24 months, producing a pre/post-intervention total sample healthcare cost ratio of 1:0.98.

**Conclusions:** This novel strategy to enhance ART adherence amongst a vulnerable group of PHY not only conveys enhanced clinical outcomes but could be cost saving.

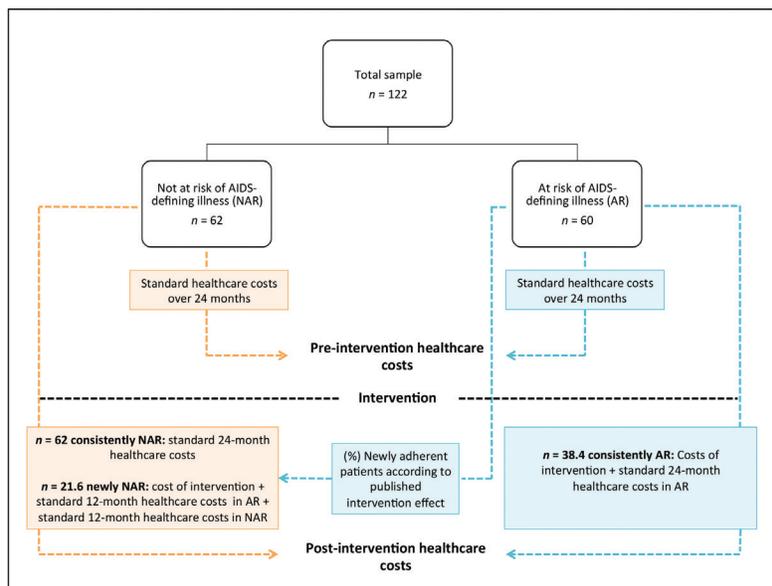


Figure 1: Modelling methodology

## 835 Clinical Outcomes in Adults With Perinatal HIV After Transfer From Pediatric Care

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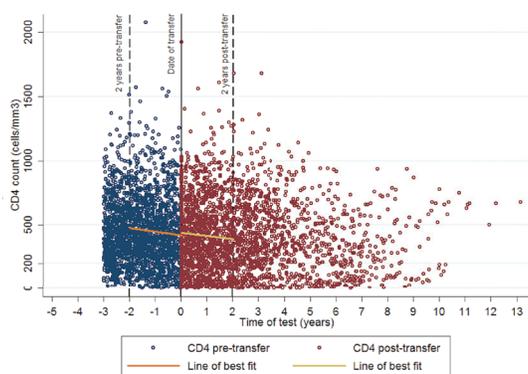
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**Background:** With improved survival, increasing numbers of adolescents with perinatal HIV (PaHIV) are transitioning from pediatric to adult care, but there are few published data on clinical outcomes in adult care. This longitudinal retrospective cohort study investigated retention in care, mortality and clinical outcomes in young PaHIV adults after transfer to 4 large adult HIV clinics in London, UK.

**Methods:** Adult care data for 211 PaHIV adults at the 4 hospitals (collected 2011-2013) were combined with their pediatric data from the Collaborative HIV Paediatric Study (CHIPS). Mean CD4 and the proportion with viral load <50c/mL were compared pre- and post-transfer. Linear and logistic regression models with random effects investigated the effect of clinical, demographic and socioeconomic factors on CD4 trajectories, viral load, and non-attendance (not seen  $\geq 12$  months) and/or death, in the post-transfer period.

**Results:** Median age at last follow-up was 21.9yrs [IQR 19.8,23.6] and median duration of follow-up was 15 years [11,18]. Most (89.6%) were on antiretrovirals. Median age at transfer was 17.6yrs [16.6,18.4] and median follow-up post-transfer was 3yrs [1.4,5.0]. Half (56%) attended  $\geq 1$  pediatric and adult transition clinics during transfer and 43% switched hospitals for adult care. Transfer was associated with increased virological suppression from 43% at 12 months pre-transfer to 44% at transfer and 63% at last visit ( $n=211$ ,  $\chi^2$  trend  $p<0.001$ ). Although mean CD4 decreased from 450c/mm<sup>3</sup> in the 2 years pre-transfer to 420 in the 2 years post-transfer ( $p<0.001$ ), the rate of decline slowed after transfer (from 28.3c/mm<sup>3</sup>/year to 22.8c/mm<sup>3</sup>/year post-transfer,  $p<0.001$ , Figure). Older current age ( $\beta=26.2$ ) and non-progression during childhood ( $\beta=246.5$ ) were multivariate predictors of higher CD4 in adult care, and male sex ( $\beta=-81.0$ ) and virological failure at transfer ( $\beta=-286.2$ ) predictors of lower CD4 (all  $p<0.05$ ). Those who switched hospital for adult care had higher odds of non-attendance (26/211) and/or death (9/211) (aOR=5.3, 95%CI 2.0, 13.7).

**Conclusions:** Viral suppression in PaHIV patients in 4 adult HIV clinics in London improved after transfer from pediatrics. Although mean CD4 decreased after transfer, the decline in immune function was steeper in the 2 years pre-transfer, suggesting a critical time for adherence interventions during transition preparation. Staying at the same hospital had a positive effect on attendance/survival in adult care, suggesting the importance of continuity of care.

836 Strong IFN- $\gamma$  Responses and Limited HIV Reservoirs in Adolescent Virologic Controllers

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**Background:** Insights from the "Mississippi baby" and Visconti cohort have shed light on the benefits of early ART after primary HIV infection to limit reservoir size. We describe two cases of pre- and post-treatment control in perinatally HIV-1-infected youth.

**Methods:** Routine virologic and immunologic parameters were monitored since diagnosis. Study investigations included: HIV reservoir assessments (TILDA and qRT-PCR for total and integrated DNA); cell-mediated immune responses (CMI); and HLA typing. CMI were assessed using pooled clade-matched HIV-1 Gag peptide panels in matrix format and IFN- $\gamma$  ELISpot and expressed as spot-forming units (SFU) per  $10^6$  PBMC.

**Results:** Case 1, diagnosed at age 10 years, had an initial viral load (VL) of <50 c/mL, but was positive for HIV DNA (clade C). Subsequently, she had only 2 detectable VLs of 71 and 57 c/mL at age 12 and 17 years respectively, with the remainder below detection. Now 17 years, her CD4 counts have been normal throughout. Case 2 was diagnosed at age 11 months, at which time VL was 128,400 c/mL (clade D); nadir CD4 count was 1170 cells/mL (18%). She received dual ARV therapy until age 3 years without virologic suppression, followed by 3-drug cART which led to suppression. Treatment was interrupted at age 12 years with ongoing suppression (<50 c/mL) for 5 years. Now 18 years, over the past year VLs have been consistently detectable up to 300 c/mL, with intermittent adherence to cART initiated for a single CD4 count of 326 cells/mL.

TILDA in both cases showed low positivity at 1.5 cells/ $10^6$  CD4+ T cells. Total and integrated HIV DNA measurements were 479 and 102 copies/ $10^6$  CD4+ T cells for Case 1 and 1076 and 564 for Case 2. Both subjects exhibited strong IFN- $\gamma$  responses (Case 1: 97-7032, Case 2: 0-5103 SFU/ $10^6$  PBMC) and breadth of antigen recognition (22/22 and 13/14 peptide pools recognized, respectively). These CMI are in the top quartile compared to chronically infected children and adults. Case 1 possessed no HLA genotypes predictive of elite control; Case 2 possessed one HLA-B\*81 haplotype.

**Conclusions:** In contrast to the recently reported French adolescent post-treatment controller who had weak CMI, these youth (one an elite controller, the other a post-treatment controller) demonstrated HIV-specific CMI that are substantively larger and broader than those observed in pediatric or adult patients with viral suppression on cART. These results underscore the potential impact of enhanced CMI in virologic remission in perinatally infected youth.

## 837 Kinetics of Cell-Associated HIV DNA During Viral Suppression in HIV-Infected Children

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**Background:** Although the success of combined antiretroviral therapy (cART) to control viral replication, HIV eradication has not been yet achieved. One of the major known obstacles is the establishment of viral reservoirs very early after infection. The aim of this study was to determine the decay in the level of cell-associated HIV-DNA (CA-HIV-DNA) in children with sustained virological suppression (VS) for many years on cART.

**Methods:** The research was conducted in an academic pediatric hospital including 37 HIV-infected children by vertical transmission with more than 2 years on VS, defined as plasma viral load below the limit of detection of the available assay. CA-HIV-DNA levels were quantified by a semi-nested real time PCR with Taqman probes targeting LTR-gag region in PBMCs. Samples were tested at pre-cART, at 2 and 4 years ( $\pm 6$  months) on VS. Clinical, virological and immunological data were collected. Mann-Whitney test and with Pearson's or Spearman correlation coefficient were used for data analysis.

**Results:** Of the 37 children, 14 started cART during the first year of infection (3-12 mo) and 23 children between 20-131 mo. Three time points (pre cART, 2 and 4 years on VS) were able to study in 23 children while in 14 only pre cART and at 4 years on VS could be performed. The median CA-HIV-DNA levels pre cART was 2.67 (IQR: 1.72- 3.07)  $\log_{10}/10^6$  PBMCs and was significantly lower when compared to 2 and 4 years on VS with median levels of 1.96 (IQR:1.32-2.56)  $\log_{10}$  ( $p<0.05$ ) and 1.91 (IQR: 0.78-2.36)  $\log_{10}/10^6$  PBMCs ( $p<0.005$ ), respectively. However, no changes in CA-HIV-DNA levels between 2 and 4 years on VS was found. Moreover, initiation of therapy before or after 12 months of age did not modify CA-HIV-DNA levels. No correlation between clinical stage or CD4 counts and CA-HIV-DNA levels were found.

**Conclusions:** CA-HIV-DNA levels is markedly reduced by therapy after 2 years of VS. However, once CA-HIV-DNA levels reaches a set point, it remains stable despite prolonged therapy and they could not be reduced by cART initiation between 3 -12 months of age. Collectively, these findings suggest that the strongest effect of cART on viral reservoirs is restricted to a very short period after infection, and extended ART prophylaxis may play a central role to limit the set point of CA-HIV-DNA levels.

### 838 HIV-1 DNA Dynamics Over a Decade or More of Viral Suppression in Perinatal Infection

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**Background:** In perinatal HIV-1 infection, whether age at and duration of virologic suppression (VS) effects long-term decay of HIV-1-infected cells following  $\geq 10$  years of combination antiretroviral therapy (cART) is unknown.

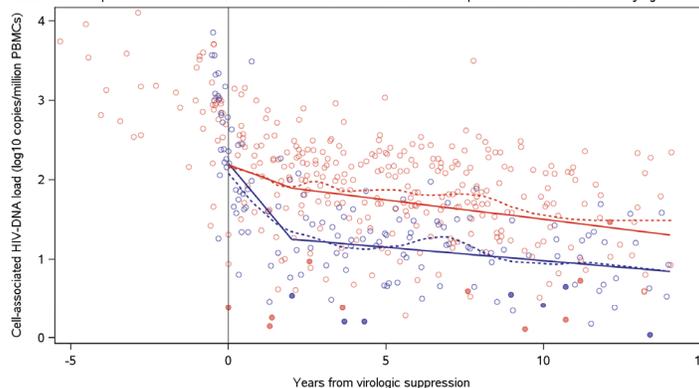
**Methods:** Total HIV-1 DNA was quantified using droplet digital PCR methods in peripheral blood mononuclear cells (PBMCs) from perinatally-HIV-infected youth enrolled in the US-based Pediatric HIV/AIDS Cohort Study who achieved VS (two consecutive plasma viral load [pVL]  $<400$  copies/mL) after cART initiation and maintained suppression during follow-up. Piecewise linear mixed effects regression models estimated HIV-1 DNA trajectories following VS for all study participants (N=61 youths; n=402 PBMC samples during VS), and after stratification into youth with VS before one year of age (Group 1; N=13; n=118), and youth with VS between one and five years of age (Group 2; N=48; n=284).

**Results:** Groups 1 and 2 initiated cART at a median age of 2.1 months and 1.7 years and achieved VS within a median of 5.9 and 10.2 months of cART, respectively. The median duration of VS was 11.9 and 9.5 years for Groups 1 and 2, respectively, with a median of 9 and 5.5 PBMC samples tested per study participant during VS. The median ages at last study visit were 12.6 years for both groups. PVLs and CD4% at cART initiation or at last study visit did not differ between the two groups.

Estimated mean HIV-1 DNA concentrations at the time of VS were 2.21 and 2.19  $\log_{10}$  copies/million PBMCs in Groups 1 and 2, respectively. In the two years following VS, HIV-1 DNA decreased by -0.48 (95% CI: -0.77, -0.18) and -0.15 (95% CI: -0.23, -0.07)  $\log_{10}$  copies/million PBMCs per year in Groups 1 and 2, respectively (Figure). Between two and 14 years after VS, HIV-1 DNA decreased by -0.03 (95% CI: -0.07, -0.00) and -0.05 (95% CI: -0.07, -0.03)  $\log_{10}$  copies/million PBMCs per year, respectively. Following 10 years of VS, estimated HIV-1 DNA concentrations averaged 0.98 and 1.50  $\log_{10}$  copies/million PBMCs in Groups 1 and 2, respectively.

**Conclusions:** HIV-1 infected cells continually decreased during a decade or more of cART in perinatal infection, irrespective of age at VS. A more rapid decrease in the first two years of VS was observed among those with VS before 1 year of age. The basis of decreasing concentrations of circulating HIV-1-infected cells in perinatal infection requires further study.

Figure: Piecewise linear mixed effects model results of HIV DNA decay stratified by age at virologic suppression (VS). Blue circles represent youth who achieved VS by  $<1$  year of age. Red circles represent youth who achieved VS by 1-5 years of age. Closed circles represent HIV-DNA levels below the limit of detection. Dashed lines represent fitted LOESS curves by age at VS.



### 839 Value of Ultrasensitive HIV Assay in Infants With Negative Routine Laboratory Results

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**Background:** Early cART after vertical infection can prevent the development of HIV specific antibodies and has the potential to restrict the size of the HIV reservoir. Ever since the Mississippi baby (who temporarily retained HIV control after early initiated therapy was discontinued) parents question if therapy should be continued in HIV infected children who initiated cART in an early phase after birth and are seronegative.

**Methods:** Case-series of vertically infected children in whom the presence of an ongoing HIV infection was questioned. Clinical data and standard diagnostic data were collected. DNA was extracted from PBMCs or dried blood spots. Detection and quantification of HIV DNA was performed by different ultrasensitive assays (1cp/E6 cells). HIV specific T-cell activity was investigated by ELISPOT

**Results:** 7 children born in Africa who presented at Dutch hospitals after adoption (median age 12 months) were included. At presentation all children had negative HIV serology. Child 1 was treated for 8 weeks with monotherapy and had no written documentation of HIV infection. This infant repeatedly tested negative for HIV-RNA during 21 months off therapy, HIV antibody and ultrasensitive HIV-DNA tests were also negative. All other children had documented records of positive HIV status in the country of origin and had locally initiated cART. HIV RNA could only be detected in infant 2. In this child a slow viral decay was seen before full suppression was reached at 13 months on cART. Despite the prolonged viremia no HIV antibodies or HIV-specific T-cell activity were observed. In child 3 cART was discontinued resulting in a viral rebound ( $5.64 \times 10^6$  cp/mL) and HIV seroconversion. Retrospective analysis of PBMC obtained before therapy interruption showed positive HIV DNA results. The four other children continued cART after ultrasensitive DNA assays confirmed the HIV infection.

**Conclusions:** Standard diagnostic tests can provide false hope of a cure and erroneous discontinuation of cART with risk of viral rebound. By using ultrasensitive tools developed to investigate the viral reservoir, HIV infection could be confirmed in children with negative HIV serology and undetectable viral loads. In one child, without any proper documentation of HIV status, the negative ultrasensitive DNA tests reinforced the decision to stop ART and led to the conclusion that it is unlikely this child was infected with HIV. Remarkably no HIV specific T-cell immunity was mounted in one child despite prolonged viremia.

**840 HIV Antibodies and Reservoir Size in Perinatally Infected Children on HAART**Josephine Brice<sup>1</sup>; Fatoumata Tiguem Telly<sup>2</sup>; Maxime Grudé<sup>3</sup>; Anne Derache<sup>4</sup>; Deenan Pillay<sup>4</sup>; Francis Barin<sup>5</sup>; Vincent Calvez<sup>3</sup>; Maryam Sylla<sup>6</sup>; Almoustapha Maiga<sup>7</sup>; Anne-Geneviève Marcelin<sup>3</sup><sup>1</sup>UMR-S 1136 Pierre Louis Inst of Epi and PH, Paris, France; <sup>2</sup>SEREFU, Univ of Scis Techniques and Technologies of Bamako, Bamako, Mali; <sup>3</sup>Sorbonne Univs, Paris, France; <sup>4</sup>Africa Cntr for Hlth and Pop Studies, Mtubatuba, South Africa; <sup>5</sup>François-Rabelais Univ, Tours, France, Tours, France; <sup>6</sup>CHU Gabriel Toure, Bamako, Mali; <sup>7</sup>SEREFU, Univ of Scis Techniques and Technologies of Bamako, Bamako, Mali**Background:** Absence of detectable viremia after treatment cessation in perinatally HIV-infected (PHIV) children suggests that early initiation of highly active combination of antiretroviral therapy (HAART) may lead to functional cure. By stopping the viral replication, the early virostatic treatment may prevent the development of the HIV-1-specific antibody responses and limit the establishment of the viral reservoir. Here we describe the factors associated with the anti-gp41 antibodies activity and the viral reservoir size in PHIV HAART-treated children. Our second objective was to identify global HIV seroreversions.**Methods:** This transversal prospective study involved 97 PHIV HAART-treated children with virological suppression (HIV-1 RNA plasma  $\leq$  50 copies/mL). It took place in Gabriel Touré hospital, in Bamako, Mali, between August 2013 and April 2014. We measured the anti-gp41 antibodies activity (binding to the immunodominant epitope), determined by an enzyme-immunoassay (ELISA), and the quantification of antibodies to HIV by the Architect ELISA (Abbott). The size of viral reservoir was determined by measuring HIV blood cell associated total DNA.**Results:** The PHIV children studied had a median of 9.8 years of age (IQR = 7.0 - 13.1) at time of inclusion. In median, they had started HAART at 3.3 years of age (IQR = 1.9 - 7) and were on HAART for the past 5.4 years (IQR = 3.5 - 7). The median level of total HIV DNA was 445 copies/10<sup>6</sup> cells (IQR = 187 - 914), the median anti-gp41 antibodies activity was 0.29 UA (IQR = 0.18 - 0.75). A low activity of anti-gp41 antibodies was associated with a younger age at treatment initiation ( $p = 0.01$ ). No association was found between anti-gp41 antibodies and HIV DNA ( $p = 0.17$ ). The 9 children having an HIV DNA under the threshold ( $< 66$  copies/10<sup>6</sup> cells) tended to have a lower anti-gp41 antibodies activity *versus* children with an HIV DNA  $> 66$  copies/10<sup>6</sup> cells ( $p = 0.11$ ). Overall, eight seroreversions were identified (negative Architect ELISA) in which 2 children had an HIV DNA under the threshold (1 detectable and 1 undetectable) and a low anti-gp41 antibodies activity.**Conclusions:** This study may be helpful to identify candidates with low viral reservoir and low antibodies level for future strategies aiming at reduce the burden of antiretroviral therapy or control the HIV reservoir in children.**841 Hybrid HIV Testing Strategy Achieves High Coverage of Rural East African Children**James Ayieko<sup>1</sup>; Gabriel Chamie<sup>2</sup>; Craig R. Cohen<sup>2</sup>; Tamara Clark<sup>2</sup>; Edwin Charlebois<sup>2</sup>; Maya Petersen<sup>3</sup>; Moses R. Kamya<sup>4</sup>; Diane V. Havlir<sup>2</sup>; Theodore D. Ruel<sup>2</sup><sup>1</sup>Kenya Med Rsr Inst, Kisumu, Kenya; <sup>2</sup>Univ of California San Francisco, San Francisco, CA, USA; <sup>3</sup>Univ of California Berkeley, Berkeley, CA, USA; <sup>4</sup>Makerere Univ Coll of Hlth Scis, Kampala, Uganda**Background:** Despite scale-up of early infant diagnosis programs, many HIV+ children remain undiagnosed and suffer high mortality. Efficient ways to screen children  $\geq$  2 years old in the context of universal test-and-treat policies are needed. We evaluated the HIV-testing coverage of children in a hybrid mobile multi-disease screening campaign that achieved 89% HIV testing of adults in Uganda and Kenya (SEARCH, NCT:01864603).**Methods:** From 2013-14, children in 32 rural Ugandan and Kenyan communities were enumerated by door-to-door census. Children were tested for HIV using a "hybrid" mobile testing strategy that included 2-week multi-disease community health campaigns (CHCs) offering malaria testing, deworming and vitamin A, followed by targeted home-based testing (HBT) for children "at-risk" for HIV, with mothers' serostatus positive (HIV+) or unknown (HIVunk). Children aged 2-9 years old living in the study community for  $\geq$  6 months of the prior year ("stable") were evaluated for HIV testing coverage, seroprevalence and newly diagnosed infection.**Results:** Of 87,700 stable children (mean age 5.4 years), maternal serostatus was negative in 57,655 (66%), positive in 7,055 (8.0%), and unknown in 22,990 (26%). Both parents were alive for 92%, 1.3% were maternal orphans, 6.0% were paternal orphans, and 0.9% had lost both parents (unknown in 0.3%). The CHC was attended by 69,906 (80%) children; overall hybrid model testing coverage of at-risk children was 81% (see Table). Among children of HIV+ mothers, older children (8-9 vs 2-3 years aOR 2.5, 95%CI:2.0-3.1) and Kenyan residence (aOR 3.4, 95%CI:2.4-4.8) were predictive of not being tested in generalized estimating equation modelling, but low wealth (lowest tertile aOR 1.0, 95%CI:0.8-1.3) and maternal CD4 count  $< 350$  (aOR 1.1, 95%CI:0.9-1.4) were not. Among 819 HIV+ diagnoses, 444 (54%) had no prior positive testing reported. The prevalence of HIV was 0.4% in Southwest Uganda, 0.6% in East Uganda and 2.6% in Kenya, among children tested.**Conclusions:** The integration of HIV-testing of children into a CHC, with subsequent targeted HBT for at-risk children, resulted in 81% testing coverage of at-risk children and doubled the number of children recognized to be HIV+. In Ugandan communities, coverage of children born to HIV+ women reached 95%. Similar approaches, integrating pediatric screening into mobile population-level health campaigns, could serve as efficient tools for identifying HIV+ children as countries seek to achieve universal testing and treatment.

Testing coverage of at-risk children in SEARCH communities

Region	n=	Community Health Campaign Testing	Targeted Home-based Testing	Total Coverage
<i>Southwest Uganda</i>				
Mother HIV+	1,115	855 (77%)	209 (19%)	95%
Mother HIV unk	6,157	4,485 (73%)	1,009 (16%)	89%
<i>Eastern Uganda</i>				
Mother HIV+	816	701 (86%)	77 (9%)	95%
Mother HIV unk	7,816	5,791 (74%)	1,014 (13%)	87%
<i>Kenya</i>				
Mother HIV+	5,124	3,583 (70%)	797 (16%)	85%
Mother HIV unk	9,017	5,154 (57%)	726 (8%)	65%
<b>All at-risk children</b>	<b>30,045</b>	<b>20,569 (68%)</b>	<b>3,832 (13%)</b>	<b>81%</b>

**842 Outcomes of the Test and Treat Policy for HIV-Infected Children and Adolescents**

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**Background:** In 2014, Uganda was the first high-burden country to adopt the Test and Treat model for all children and adolescents living with HIV under the age of 15 years. We compare baseline and 12 months outcomes of children who initiated ART under the Test and Treat model and WHO 2010 guidelines at an HIV clinical center of excellence in Uganda.**Methods:** All children and adolescents under 15 years were started on ART. For this analysis, participants were eligible if they were aged 5-15 years and had initiated first line ART between 1<sup>st</sup> April 2014 and 31<sup>st</sup> March 2015. Participants were further categorized into 2 groups namely those eligible for ART based on WHO 2010 guidelines and those only eligible based on the new Test and Treat policy. Data was retrospectively reviewed and comparisons made between the 2 groups for CD4 levels, nutritional status, drug adverse events, viral load and retention in care. Data was analyzed using STATA SE/13.0

**Results:** We studied 500 children; the majority 381/500 (76.2%) initiated ART due to the new Test and Treat policy while 119/500 (23.8%) required ART based on the 2010 WHO guidelines. Children initiated based on the new policy had a median baseline CD4 count of 745 [551-1089] compared to 313 [77-558] for those initiated based on the 2010 WHO guidelines. Children initiated based on the new policy were less likely to develop malnutrition within the first 6 months of treatment OR 0.13 [95%CI 0.05; 0.30], were more likely to be adherent to ART OR 1.85 [95% CI 1.02; 3.29] and were also more likely to have viral suppression at 6 months OR 4.42 [95% CI 2.29; 8.53]. The rates of retention at 12 months were 99% for the Test and Treat group versus 85% for children initiated based on 2010 WHO guidelines. There were no other statistically significant differences between the two groups.

**Conclusions:** The Test and Treat model of treatment is viable for resource limited settings with demonstrated favorable treatment outcomes.

**843 48-Week Outcomes of African Children Starting ART at CD4 >500 With Streamlined Care**

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**Background:** With global guidelines shifting to universal antiretroviral therapy (ART) for HIV+ children, strategies are needed to scale-up ART efficiently while ensuring good clinical outcomes. Data on children initiating ART at high CD4 counts are particularly limited. We evaluated retention in care, viral suppression, and safety in asymptomatic HIV+ children starting ART at CD4 >500 cells/µl in rural African clinics using a streamlined ART delivery system.

**Methods:** In 16 rural Ugandan and Kenyan clinics, HIV+ adults and children were offered ART (SEARCH Study, NCT:01864603) in 2013-15. We studied children 2-14 years old who initiated ART with CD4 >500 cells/µl and were ineligible for ART by country guidelines. Streamlined care included: (1) nurse-driven triage and visits focused on symptom-based ART toxicity screening, (2) on-site nurse referral of complex cases to a physician, (3) a patient-centered care system, fostering a welcoming/ supportive environment, (4) viral load (VL) measurement and structured VL counseling, (5) provision of 3 months' ART refills, and (6) appointment reminders and patient tracking. Patients had visits at baseline, 4, 12, then every 12 weeks. VL and basic safety laboratory tests were assessed at baseline, 24 and 48 weeks.

**Results:** Overall, 77 HIV+ children initiated ART. Median age was 8 years (IQR 6-11), 56% were female, and 34% were orphans from mother/father/both. No prior + HIV test was reported by 43/77(56%). At baseline, children had a median VL of 14,851 copies/ml (IQR 1,651-66,479) and a median CD4 count of 847 cells/µl (IQR 659-1,103). Overall, 74/77(96%) of children were retained at week 48. Two children withdrew consent when they moved out of the study area and one because the parent declined ART. One child was last seen at enrollment, another at 12 weeks. There were no deaths. Overall, 3/77 (4%) patients had grade III or IV clinical adverse events, including thrombocytopenia(III), and neutropenia (III) and rash(IV). One patient each switched from nevirapine and abacavir due to rash. Viral suppression was achieved in 62/68 (91%) children tested at 48 weeks (see table).

**Conclusions:** HIV+ children 2-14 years old with CD4 >500 cells/µl receiving ART in rural African clinics employing streamlined ART delivery had 94% retention in care at 48 weeks with 91% viral suppression among those tested. This suggests that as nations adopt universal ART, streamlined nurse-driven care systems can safely and effectively deliver ART to HIV+ children.

Outcomes at the 48 week visit after ART initiation	
<i>Retention:</i>	
Attended	72 (94%)
Did not attend	2 (3%)
Withdrew consent prior	3 (4%)
<i>HIV RNA Levels*</i>	
< 500 c/ml	62 (91%)
500-10,000 c/ml	4 (6%)
> 10,000 c/ml	2 (3%)

\*Of 68 tested; 4 attended but lacked test results.

**844 Family Clinic Day RCT in Uganda: Child Antiretroviral Therapy Retention and Adherence**

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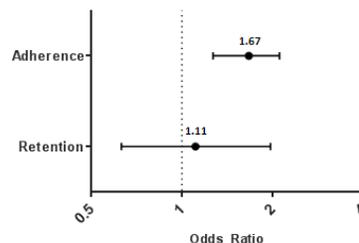
**Background:** Uganda recently adopted a test-and-treat policy for HIV patients 15 years or younger. But despite increased eligibility, low retention rates among pediatric and adolescent antiretroviral therapy (ART) initiates could severely limit the impact of this new policy. This evaluation tested the impact of the Family Clinic Day (FCD) program, a facility based family-centered appointment scheduling and health education intervention.

**Methods:** We conducted a clustered randomized controlled trial for 6 months, from October 2014 to March 2015. Forty-six facilities were stratified by implementing partner and facility type and randomly assigned to the control arm receiving standard practice of care or the intervention are receiving FCD. Retrospective data collection of ART registers occurred after study completion in May 2015. Outcome indicators for paediatric and adolescent patients included the proportion retained in care and the proportion adherent to their appointment schedule. Six focus group discussions (FGD) were conducted with FCD participants and 17 interviews with health workers were conducted to understand perspectives on FCD successes and challenges.

**Results:** A total of 4,715 pediatric and adolescent patient records were collected, of which 2,679 (n=1,319 from control facilities and 1,360 from intervention facilities) were eligible for inclusion. The FCD did not improve retention (adjusted odds ratio [aOR] 1.11; 90% CI 0.63 – 1.97, p=0.75), but as shown in figure 1, was associated with improved adherence to appointment schedule (aOR 1.64; 90% CI 1.27 – 2.11, p<0.01). Qualitative findings suggested that FCD patients benefited from the health education and increased psychosocial support. Health workers identified few challenges with FCD implementation, but broader challenges in ART care delivery may be impacting the ability of health facilities to implement FCD.

**Conclusions:** FCD scale-up in Uganda would likely produce improvements to adherence following an increase in knowledge based on health education, peer support and adherence to appointments. Broad challenges facing ART clinics, such as under-staffing and poor filing systems, should be addressed in order to maximize the impact of programs such as FCD.

Figure 1. Pediatric and adolescent adjusted odds of retention and adherence



Weighted generalized estimation equation regression analysis of the odds of pediatric and adolescent patient retention and adherence among FCD intervention compared to control, adjusting for initiation category, age, region, gender, appointment representation, and facility level.

## 845 12-Month Treatment Outcomes Amongst HIV-Positive Orphans and Nonorphans

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**Background:** The AIDS epidemic has resulted in a large population of orphans in South Africa – without parents, these children may be more at risk of delayed healthcare and poorer outcomes. Little research has investigated treatment outcomes for HIV-positive orphans versus non-orphans; the research that has been done shows mixed results. We sought to evaluate the association between orphan status and antiretroviral treatment (ART) treatment outcomes among HIV-positive infants, children and adolescents initiating ART at 2 large public-sector HIV clinics in Johannesburg, South Africa.

**Methods:** Retrospective cohort study among HIV-positive infants, children and adolescents aged one month to 18 years initiating on standard first-line ART between June 2004–May 2013. We used modified Poisson regression to evaluate the association of orphan status with all-cause mortality, loss to follow-up (LTF;  $\geq 3$  months late for a scheduled visit) and having a detectable viral load ( $\geq 400$  copies/ml) at 12 months on ART.

**Results:** We included 244 (27.1%) patients classified as orphans (either maternal, paternal or both) and 658 (72.9%) as non-orphans at ART initiation in our analysis. Median ages were 8.5 years (IQR:5.2–11.6) and 3.0 years (IQR:1.0–7.4) for orphans and non-orphans, respectively. At ART initiation about 36% were classified as WHO stage III/IV and 17% had TB. A total of 1 (0.4%) orphan and 16 (2.4%) non-orphans died in the first 12 months following ART initiation while 7.8% and 17.8% of orphans and non-orphans were LTF. A total of 37 (18.7%) orphans and 133 (29.4%) non-orphans had a detectable viral load after 12 months on ART. Adjusted modified Poisson regression (Table 1) showed that being an orphan has a protective effect on the risk of death (RR 0.26; 95%CI:0.20–0.35), risk of LTF (0.60; 95%CI:0.44–0.82) and risk of failure to achieve viral suppression (0.77; 95%CI:0.70–0.84) when compared to non-orphans.

**Conclusions:** Results show that orphans were less likely to die, be lost to follow-up and fail to achieve viral suppression. This surprising result needs to be analysed further, but may arise from orphans being more integrated into care due to orphan-specific programming or foster care. Understanding the impact of orphan status on short- and long-term ART outcomes could improve targeted strategies, and subsequent treatment and developmental outcomes, for HIV-positive infants, children and adolescents. Additional research investigating age-specific outcomes will be important to further elucidate these effects.

**Table 1. Adjusted predictors of mortality, loss to follow up and viral suppression among infants, children and adolescents 12 months after initiating ART**

	Adjusted risk ratio and 95% confidence interval (CI)		
	Mortality (n=17)	Loss to follow-Up (n=136)	Failure to achieve viral suppression (n=170)
<b>Orphan status at ART initiation</b>			
Non-orphan	Reference	Reference	Reference
Orphan	0.26 (0.20-0.35)	0.60 (0.44-0.82)	0.77 (0.70-0.84)
<b>Sex</b>			
Female	Reference	Reference	Reference
Male	0.95 (0.47-1.92)	0.94 (0.81-1.09)	1.08 (1.00-1.16)
<b>Age at ART initiation (years)</b>			
< 1 year	0.94 (0.40-2.22)	1.06 (0.83-1.36)	1.32 (1.08-1.62)
1 to 4.9	Reference	Reference	Reference
5 to 9.9	0.47 (0.40-0.55)	0.51 (0.30-0.85)	0.65 (0.27-1.56)
$\geq 10$ years	0.60 (0.26-1.33)	0.40 (0.18-0.86)	0.99 (0.88-1.12)
<b>CD4 classification at ART initiation*</b>			
Very low	4.30 (3.19-5.81)	1.78 (1.69-1.89)	0.91 (0.58-1.42)
Low	0.43 (0.29-0.63)	0.91 (0.56-1.49)	0.96 (0.94-0.98)
Moderate	0.47 (0.11-1.93)	0.95 (0.84-1.07)	0.94 (0.80-1.10)
High	Reference	Reference	Reference
<b>Hb at ART initiation (ug/dL)</b>			
< 10	Reference	Reference	Reference
$\geq 10$	0.64 (0.59-0.69)	0.99 (0.71-1.36)	0.96 (0.78-1.19)
<b>WHO clinical stage at ART initiation</b>			
I or II	Reference	Reference	Reference
III or IV	0.97 (0.63-1.48)	0.82 (0.73-0.92)	1.21 (1.10-1.32)
<b>NRTI</b>			
Stavudine (d4T) or Zidovudine (AZT)	Reference	Reference	Reference
Abacavir (ABC) or Tenofovir (TDF)	0.41 (0.21-0.79)	1.18 (1.11-1.24)	2.56 (1.88-3.48)
<b>PI**</b>			
No	Reference	Reference	Reference
Yes	1.89 (0.90-3.99)	0.69 (0.68-0.70)	0.97 (0.96-0.99)

\*CD4 classification as follows: Very low CD4: $<5\%$  for  $<6$  and  $<25$  cells/mm<sup>3</sup> for  $\geq 6$ ; Low CD4:5–15% for  $<6$  and 25–50 cells/mm<sup>3</sup> for  $\geq 6$ ; Moderate CD4:15–25% for  $<6$  and 50–200 cells/mm<sup>3</sup> for  $\geq 6$ ; High CD4: $>25\%$  for  $<6$  and  $>200$  cells/mm<sup>3</sup> for  $\geq 6$

\*\*PI is either Lopinavir or Ritonavir

## 846 An Audit of Weight-Based Antiretroviral Therapy Dosing in Children

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**Background:** Paediatric antiretroviral therapy (ART) dosing is weight dependent and guidelines recommend ART dose adjustment according to a child's weight. We conducted an audit to assess ART dosing in a public sector paediatric HIV outpatient clinic in Harare, Zimbabwe. At this clinic children are routinely reviewed by a clinician every three months.

**Methods:** Children and guardians consecutively attending the HIV clinic from May to July 2015 at Harare Hospital were enrolled with consent. Data was extracted from patient-held and clinic records including date of ART initiation, current CD4 count, ART regimen, ART dosage, weight and changes to ART regimen and/or dose at the last clinic (doctor) visit. Correct weight-based dosing was defined according to the National ART Guidelines. The proportion of children with incorrect dosing (either over-dosed or under-dosed) at the time of the clinic visit was determined for each drug.

**Results:** A total 458 children attended the clinic; 244 (53%) were male; 22 (5%) 0-3 years, 192 (42%) 4-10 years and 244 (53%) 11-17 years. Median CD4 count was 713.5 cells/mm<sup>3</sup> (IQR 440-1088) and median time on ART 16 months (IQR 8-30). Changes to ART regimens were documented in 241/458 children (53%). ART regimen combinations were prescribed outside of recommended guidelines in 2/22 (9%) aged 0-3, 41/192 (21%) aged 4-10 and 19/100 (19%) aged 11-17. The majority (n=13, 59%) of infants were over-dosed on one drug of the ART regimen (Table 1). Among the young (4-10) and older children (11-17) 82 (46%) and 41 (18%) were either under- and/or over-dosed on one drug. Drugs commonly over-dosed in the older age groups were efavirenz and boosted lopinavir. Zidovudine was more frequently under-dosed and nevirapine was both frequently under- and over-dosed. 311/448 (69%) children were prescribed a fixed-dose combination (FDC). 77% (160) on FDC had all three drugs correctly dosed compared to those not on a FDC 62% (151) were correctly dosed.

**Conclusions:** This study shows that ART regimens in children are often prescribed outside of national guidelines and at doses not adjusted to their weight. Suboptimal ART dosing might result in unwanted side effects, development of virological failure and potentially drug resistance. Training of health care workers should address appropriate dosing and simple aide-memoires may support accurate dosing.

**Table 1. Summary of incorrect ART dosing**

	0-3 years		4-10 years		11-17 years	
	13/22 incorrectly dosed		82/177 incorrectly dosed		41/229 incorrectly dosed	
	Over-dosed	Under-dosed	Over-dosed	Under-dosed	Over-dosed	Under-dosed
EFZ	2		Ataz/R	5	Ataz/R	1
Lop/R	10		EFZ	15	EFZ	2
NVP	1		Lop/R	11	Lop/R	2
			NVP	21	NVP	16
					EFZ	18
					AZT	22
					Lop/R	2
					NVP	2

EFZ=Efavirenz, Lop/R=Lopinavir/ritonavir,  
NVP=Nevirapine, Ataz/R=Atazanvir/ritonavir

#### 847 Persistence of HIV Drug Resistance in Children Exposed to Nevirapine Prophylaxis

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**Background:** Nevirapine (NVP) is administered to breastfeeding infants as prophylaxis for prevention of mother-to-child-transmission of HIV. Since drug-resistant viruses can compromise future antiretroviral therapy (ART) when prophylaxis fails, we studied HIV-infected, NVP-exposed children to determine the persistence of NVP resistance and correlate the detection of resistance with duration of exposure to NVP.

**Methods:** HIV-infected, NVP-exposed children initiating ART were enrolled in a prospective observational study in Pretoria, South Africa. Dried blood spots were collected at enrollment and during monthly follow-up visits. Amplifiable HIV templates were quantified by real time PCR of *gag*, so that a target of 300 templates could be submitted to nested HIV *pol* PCR. NVP-resistance was assessed by an oligonucleotide ligation assay optimized for HIV-1 subtype C with the capability to quantify  $\geq 2\%$  mutant. The proportion of virus population comprised of mutant and wild-type viruses were quantified at four codons encoding high-level resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs).

**Results:** A total of 88 HIV-infected children enrolled in the study from 2010-2013 at a median of 8 months (mo) of age (IQR:4-15). Children received single-dose (sd)-NVP at birth (n=40) or extended NVP prophylaxis (n=48) for a median of 6 weeks (IQR:4-9). At study enrollment, NVP-resistance was detected in 52% of children and among them, mutations were detected at Y181C (63%), K103N (43%), G190A (22%) and V106M (22%). Children with resistance had viral populations with high proportions of mutant variants (median 96% (IQR:82-100)). These children were also younger, with more recent NVP-exposure (median of 4 (IQR:2-7) vs. 14 (IQR:9-27) mo prior to enrollment,  $P < 0.0001$ ), and a greater proportion had received prolonged NVP-prophylaxis vs. sdNVP (60% vs. 43%;  $P = 0.133$ ). Of children with resistance at enrollment, 59% were followed longitudinally for a median of 13 mo (IQR:12-16) and resistant viruses persisted at a median concentration of 98% (IQR:77-100) to a median age of 21 mo (IQR:17-32).

**Conclusions:** HIV-infected children who fail NVP-prophylaxis often have HIV populations consisting of NVP-resistant viruses, suggesting maternal transmission of resistant variants or NVP-selection at the time the HIV reservoir was established. The persistence of high mutant concentrations supports drug resistance testing in NVP-exposed children prior to NNRTI-ART in resource-limited communities.

#### 848 Independent lineages of HIV-1 Multidrug Resistance in Children Failing Early ART

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**Background:** Early protease inhibitor (PI) based antiretroviral therapy (ART) is recommended in vertically infected children, bringing immediate clinical benefit and limiting the reservoir size, which may impact on future chances of cure. We previously reported linked multi-class drug resistance (MDR) in children that received nevirapine prophylaxis and failed early PI based ART (Lange et al, JAIDS 2015). Here we report evolutionary histories of drug resistant lineages in these children.

**Methods:** Single genome sequencing was used to characterise genetically linked MDR in HIV-1 protease and reverse transcriptase from longitudinal plasma samples. Sequences were obtained from the pre-ART populations, during suppressive therapy, treatment failure, treatment interruption and re-started ART. The mean pairwise genetic distances (MPDs) between these populations were calculated. The evolution and population diversity of MDR lineages were analysed by Maximum Likelihood (ML) phylogenetic reconstruction.

**Results:** 442 sequences were obtained from 2/10 children: n=249 from 7 time points in the first child; n=193 from 5 time points in the second child. Sampling depth was estimated as  $\geq 5-10\%$  of the viral population. ML trees showed multiple emergences of MDR on distinct intra-patient lineages. One child had a clonally expanded MDR lineage persisting for 85 weeks of ART. Accordingly, MPD at weeks 12, 40, 72 and 96 of ART were significantly lower than at baseline (0.6%, 0.5%, 0.2% and 0.3% vs 0.7% respectively;  $p < 0.0001$ ). In stark contrast the ML tree of the second child indicated temporal stepwise accumulation of resistance mutations. In this scenario the MPD at weeks 40 and 96 of ART and at week 60 of re-started ART were significantly higher than at baseline (0.3%, 0.4% and 0.3% vs 0.1% respectively;  $p < 0.0001$ ). MDR populations with PI resistance most frequently evolved from RT M184V mutated viral populations in both children.

**Conclusions:** We report distinct patterns of multiple class HIV-1 drug resistance acquisition over time in children treated with early PI based ART: firstly clonal expansion from an MDR reservoir, and secondly through stepwise accumulation of major resistance mutations.

**849 Transmitted Drug Resistance and First-Line ART Treatment Outcomes in Ugandan Children**

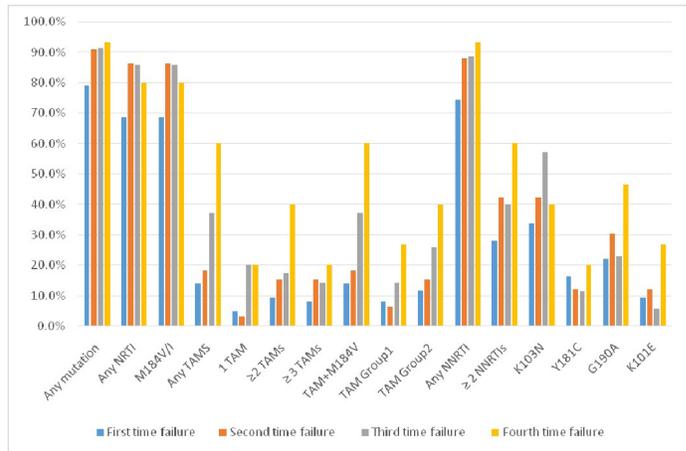
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**Background:** Transmission of drug-resistant (TDR) HIV-1 can impair virologic response to antiretroviral therapy (ART) especially in children. This study evaluated the effect of TDR on virologic and acquired drug resistance (ADR) outcomes among children initiating first-line ART.

**Methods:** Children ≤12 years initiating first-line ART were enrolled at 3 sites in Uganda between 2010 and 2011. Blood was taken at baseline and 6-monthly during 24 months of ART for later determination of viral load (VL) and pol genotypic testing if VL>1,000 copies/ml at JCRC Kampala laboratories. The 2014 IAS-USA mutation list and Stanford algorithm were used to score drug resistance mutations (DRMs) and susceptibility. Patients were classified into two groups: fully active (no TDR or TDR without reduced susceptibility) and patients with TDR and partially active ART. Virological failure (VF) was defined as 2 consecutive VLs >1000 copies/ml, at least 6 months from ART initiation. Factors associated with VF and acquired drug resistance (ADR) were assessed in multivariate logistic regression analysis and evolution of resistance was described.

**Results:** 317 children median age 4.9 years were enrolled on mainly NNRTI based regimens (91.2%). TDR mutations were detected in 47(16.9%) participants of whom 22(46.8%) initiated a partially active ART. 256(80.8%) participants were still on first line ART and in care at 24 months of follow-up of whom 32%(92/287) had VF. Children with TDR and partially active ART had significantly higher risk of VF (aOR:15.25, 95%-CI:3.77-61.7, p<0.001) and ADR (aOR:3.47, 95%-CI:1.31-9.22, p<0.012). A single VL >1000 copies/ml at 6 months on treatment was strongly associated with VF (OR:22.09, 95%-CI:9.68-50.42, p<0.001) and ADR (OR:9.89, 95%-CI:5.16-18.94; p<0.001). Other factors associated with VF in the adjusted model were higher baseline VL (aOR:2.28, p<0.001) and WHO stage 2 compared to Stage 1 (aOR:10.3, p=0.022). Among the 66 children with prolonged viraemia, there was a high rate of acquisition of DRMs.

**Conclusions:** In this pediatric cohort, TDR was found to be high and strongly associated with VF and ADR. Accumulation of DRMs was high and may jeopardize future therapeutic options. Efforts are needed to incorporate affordable drug resistance testing in developing countries to prevent the use of suboptimal ART. In the context of universal access of VL monitoring, programmatic use of a 6 month VL on treatment would detect majority of future VFs early and allow for treatment adjustment to prevent ADR.



First time failure (N=86), Second time failure (N=66), Third time failure (N=35), Fourth time failure (N=15)

**850 Drug Resistance Compromises Second-Line ART in Mozambican Children Failing First-Line**

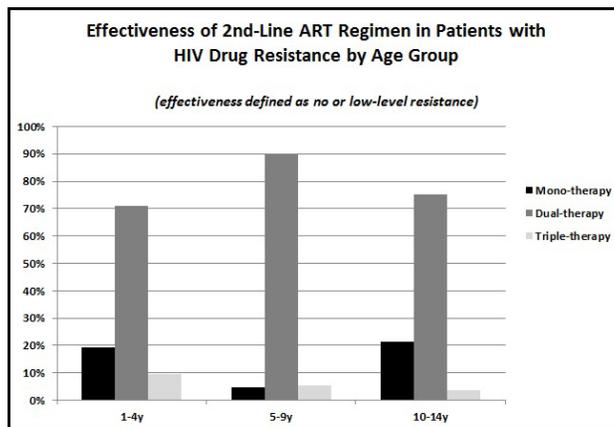
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**Background:** Mozambique's pediatric antiretroviral treatment (ART) program grew from 9,393 patients in 2007 to 41,400 in 2013. This rapid and ongoing expansion has made assurance of quality care a challenge and has occurred without viral load monitoring. In this context, we assessed the prevalence of virologic failure (VF) and HIV drug resistance mutations (DRM) in a cohort of ART-experienced children to understand implications for the current pediatric second-line regimen of LPV-r/3TC/ABC or TDF.

**Methods:** A cross-sectional study was conducted at six clinics providing pediatric ART in Maputo. Children aged 1-14 years and active on ART for at least 12 months were enrolled from August 2013 to March 2014. Clinical and demographic information were collected and viral load was performed. Dried blood spots were prepared from samples with VF (>1000 copies/mL) for genotyping. DRM interpretation and drug susceptibility were assessed using HIVdb (Stanford v7.0). Statistical analysis was done with SAS (v9.2).

**Results:** In total 713 children were enrolled; mean age of 102.9 months (95%CI: 81.0-124.7), mean time on ART of 60.3 months (95%CI: 38.8-81.2), 73.2% (95%CI: 43.5-90.6) were on d4T/3TC/NVP, 85.8% (95%CI: 75.2-92.3) had no immune suppression and 20.2% (95%CI: 15.0-26.6) had PMTCT exposure. VF was observed in 256 patients (35.9%) (95%CI: 26.9-46.2%), and 96.9% (n=248) were successfully genotyped, with DRM found in 94.8% (n=235). High levels of NRTI and NNRTI mutations were observed with M184V (90.7%) and Y181C (49.6%) most common in each class. K65R and major PI DRMs occurred in 2.4% and 1.6% of sequences, respectively. Thymidine analog mutations (TAMs) were observed in 33.5% (n=83), all in children ≥5 years. TAM-2 was the main pathway observed (n=69; 83.1%) with a mixture of both pathways in 12 patients. Second-line ART had three, two, and one-drug efficacy in 5.4%, 82.4%, and 12.2% of patients with DR, respectively, age-stratified results in Fig. 1.

**Conclusions:** VF was common (35.9%) in this large, treatment-experienced, older pediatric ART cohort, and was strongly associated with DRM (94.8%). Many of these children did not have immunologic failure and had likely been on failing regimens for some time, accumulating DRMs. These findings support the ongoing roll-out of viral load testing in Mozambique and have implications for pediatric second-line outcomes. Additional analysis of these data will look at differences in VF and DR by age group and associated risk factors.



### 851 Dysregulated Epigenome in Perinatally HIV-Infected Children on ART

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**Background:** Perinatal acquisition of HIV and early initiation of antiretroviral therapy (ART) takes place in a critical developmental period during which the epigenome may be influenced. To investigate the cumulative impact of perinatal HIV infection and ART on the epigenetic profile of school-aged children and identify affected biological pathways, we conducted an epigenome-wide association study.

**Methods:** Genome-wide DNA methylation profiling was used to identify differentially methylated regions (DMRs) associated with HIV in 120 HIV-infected and 60 frequency age-matched HIV-uninfected children aged 4-9 years participating in an observational study at two sites (Empilweni Services and Research Unit and Perinatal HIV Research Unit) in Johannesburg, South Africa. HIV-infected children were initiated on ART  $\leq 24$  months of age, consistently on treatment (did not interrupt), and currently suppressed (HIV RNA  $< 400$  copies/mL) on a LPV/r-based regimen. DNA methylation was assayed using the Illumina Infinium HumanMethylation450 BeadChip array. Pre-processing, including filtering and beta-mixture quantile normalization, and analysis was performed with the R/Bioconductor RnBeads package. We identified significant DMRs at defined promoter regions and genes with an adjusted FDR p-value  $< 0.05$  using the limma package. Gene functional classification was explored using the Database for Annotation, Visualization, and Integrated Discovery (DAVID). The analysis was repeated stratified by sex.

**Results:** After pre-processing and normalization a total of 424343 sites and 179 samples were suitable for analysis. Mean age was similar between HIV-infected and uninfected children ( $6.4 \pm 1.4$  vs.  $6.4 \pm 1.4$ , NS), as was the proportion of males ( $46.2$  vs  $50.0\%$ , NS). 17067 significant DMRs at genes and 12700 at promoters were identified. Functional analysis of the top 1000 DMRs at genes identified functional clusters related to transmembrane receptors including chemokine ligands and G protein-coupled receptors, defensins, and zinc finger proteins. Among boys, 671 significant DMRs at genes and 1263 at promoters between HIV-infected and uninfected children were identified, involving similar genes. Among girls, only 1 significant DMR at a gene and 12 DMRs at promoters were identified.

**Conclusions:** Our novel study provides evidence that perinatally acquired HIV infection may dysregulate the epigenome in school-aged children on ART. We further note that HIV may influence the epigenome differentially in boys and girls.

### 852 Timing of Pubertal Onset in Perinatally Infected South African Adolescents on ART

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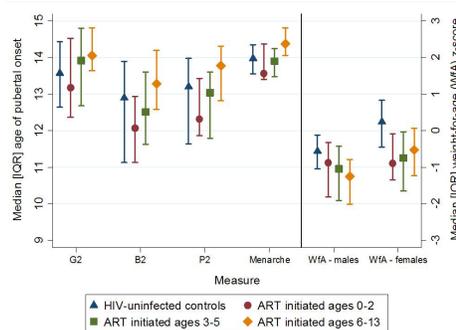
**Background:** Paediatric HIV infection is associated with delayed puberty but there are few comparative data including HIV-negative controls from southern Africa. We examined the onset of puberty in perinatally-infected adolescents in the Cape Town Adolescent Antiretroviral Cohort (CTAAC).

**Methods:** We recruited 515 perinatally HIV-infected adolescents ages 9-14 years on ART for at least 6 months. At enrolment, individuals underwent a structured clinical interview and examination including Tanner staging and anthropometry; medical history was abstracted from routine clinical records. A comparator group of 110 age- and sex-matched HIV-negative controls underwent parallel assessments. Analyses used semi-parametric models to compare the onset of puberty (Tanner stage II or higher on genital development in males [G2] and breast and pubic hair development in females [B2 & P2, respectively]) and menarche according to age, age at ART initiation, current ART regimen, and between HIV+ and HIV- adolescents.

**Results:** Overall 49% of HIV+ participants were female (mean age, 12.0 years; median age at ART initiation, 4.3 years; median CD4 cell count, 712 cells/uL; 76% with viral load  $< 50$  copies/mL). Comparing HIV+ participants to HIV- controls, the median weight-for-age z-score for boys was -1.14 versus -0.56 and for girls -0.71 versus 0.24. Comparing all HIV+ children to age- and sex-matched controls, the median age of pubertal onset was delayed by approximately 0.3 years in males (G2: 13.9 versus 13.6,  $p=0.039$ ) and was similar among females (B2: 12.8 versus 12.9,  $p=0.524$ ; P2: 13.1 versus 13.2,  $p=0.993$ ). The median age of menarche was 14.2 in HIV+ girls versus 14.0 in HIV- girls ( $p=0.127$ ). Among HIV+ adolescents, pubertal onset was persistently associated with age at ART initiation: across all measures for boys and girls, older age at ART initiation was strongly associated with later age of pubertal onset ( $p < 0.05$  for all measures), and children who initiated ART earlier ages had timing of pubertal onset that was comparable to that of HIV- controls (Figure). Later age of pubertal onset was also associated with lower current CD4 cell count ( $p < 0.05$  for all measures) but not with viral load or current PI vs NNRTI use.

**Conclusions:** Differences in the timing of pubertal onset between HIV-infected adolescents and local HIV- controls may be less marked in this setting than has been observed in North America, and appear to be heavily modified by age at ART initiation.

Figure. Median age of pubertal onset and weight-for-age (WFA) z-score by HIV status and age at ART initiation.



### 853 Left Heart Abnormalities in HIV-Infected Children in Harare, Zimbabwe

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**Background:** Due to the remarkable scale-up of antiretroviral therapy (ART), HIV-infected children are expected to survive into adolescence and beyond. However, chronic complications such as cardiac abnormalities have become new challenges for this age-group. The main aim of this study was to characterise left sided cardiac abnormalities in a cohort of HIV-infected older children and adolescents taking ART.

**Methods:** HIV-infected children aged 6-16 taking ART for at least 6 months were enrolled from the Paediatric HIV clinic at Harare Central Hospital. Assessment included clinical history, New York Heart Association (NYHA) scoring, incremental shuttle walk testing, viral load, CD4 count and transthoracic echocardiography using 2-Dimensional, M-mode, pulsed wave, continuous wave and tissue Doppler imaging. Cardiac abnormalities were assessed using European reference ranges and a z-score  $>+2$  was considered abnormal. **Results:** 201 children, median age 11 (IQR 9-12), 48% females and median age at HIV diagnosis of 5 (IQR 3-7) years were enrolled. The median CD4 count 726.5 (IQR 473-935) and 78.2% had a viral load of  $<400$  copies/ml. Most of the children were stunted and wasted, 76.1% and 78.1% respectively. Chest pain on exertion was reported in 10.9%, tachypnea 10.8%, palpitations in 4.5% and 14.4% (N=29) were in New York Heart functional score II and 0.5% (N=1) in class IV. The most common cardiac abnormality was left ventricular hypertrophy (LVH) (39.5%; N=70) which was either interventricular septal (63.8%) or posterior wall (7.2%) or both (29%), LV diastolic dysfunction (23.2%; N= 41) and LV systolic dysfunction (15.8%; N=28). There was no association between symptoms and cardiac abnormalities.

**Conclusions:** The findings show a high burden of echocardiographic abnormalities in HIV-infected older children and adolescents the majority of whom were optimally controlled on ART. While more than a third of the HIV-infected children had evidence of left heart abnormalities, only a few were symptomatic. Further studies are needed to investigate the progression and cause of these abnormalities, in particular the strikingly high prevalence of left ventricular hypertrophy.

#### 854 Cardiovascular Disease Biomarkers in Perinatally HIV-Infected Adolescents on ART

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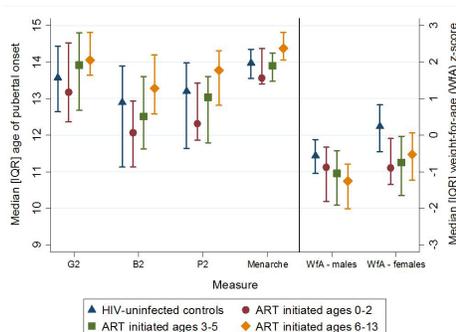
**Background:** HIV infection and antiretroviral therapy (ART) are independently associated with increases in cardiovascular disease (CVD) biomarkers in children. However little is known about CVD biomarkers in perinatally-infected adolescents in southern Africa.

**Methods:** We enrolled perinatally HIV-infected (HIV+) adolescents ages 9-14 years on ART for at least 6 months and age- and sex-matched HIV-uninfected (HIV-) controls. Individuals underwent phlebotomy after fasting for total cholesterol (TC), low-density lipoprotein (LDL-C), high-density lipoprotein (HDL), triglycerides (TG) and highly-sensitive C-reactive protein (hs-CRP). Analyses compared biomarker levels according to current age, sex, age at ART initiation, current ART regimen, and between HIV+ and HIV- adolescents; abnormal lipid values were based on US National Heart, Lung and Blood Institute cutoffs, and hs-CRP measures excluded children with a recent history ( $<1$  week) of or current acute illness or antibiotic use.

**Results:** Lipid measures for 487 and 72, and hs-CRP measures for 431 and 104, HIV+ and HIV- adolescents, respectively, were included. The mean age of HIV+ adolescents was 12.0 years (median CD4 cell count, 711 cells/uL; median duration of ART use, 7.0 years; median BMI z-score, -0.24 [IQR, -0.93-0.38]). 36% were on PI-based regimens (predominantly lopinavir/ritonavir) and 10% on a regimen containing stavudine (d4T). Overall 15%, 11%, 7% and 5% of HIV+ children had abnormal TC, LDL-C, HDL and TG, respectively. The prevalence of lipid abnormalities was higher among female participants and decreased with increasing age in children on PIs (Figure). When compared to HIV- controls and adjusting for age and gender, HIV+ adolescents on PIs had elevated TC, LDL-C and TG (OR, 8.2; 95% CI, 1.9-35.5; OR, 2.7; 95% CI, 0.9-8.1; OR, 8.8; 95% CI, 1.2-67.4). Although adolescents on NNRTIs had a markedly higher level of TC abnormalities compared to controls (OR, 5.3; 95% CI: 1.3-22.8), TG and LDL-C abnormalities were similar. Current use of d4T was not associated with lipid abnormalities. The median hs-CRP was 1.1 mg/L (16%  $>3.0$  mg/L) in HIV+ children and did not vary with age or PI use, versus 0.5 mg/L in controls ( $p<0.001$ ).

**Conclusions:** Elevated CVD biomarkers are relatively common among perinatally-infected HIV+ adolescents in this setting. The long-term implications of these abnormalities require ongoing investigation.

Figure. Median age of pubertal onset and weight-for-age (WFA) z-score by HIV status and age at ART initiation.



#### 855 Lipoatrophy/Lipohypertrophy Outcomes After ART Switch in Children in the UK/Ireland

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**Background:** Following extensive use of thymidine analogue antiretroviral therapy (ART) over the past three decades, up to a third of children may have lipoatrophy (LA) and/or lipohypertrophy (LH). Following phasing-out of stavudine, incidence of newly-diagnosed LA and LH has declined dramatically. However, the natural history of existing cases is uncertain. Previous longitudinal studies found variable (although generally poor) rates of resolution.

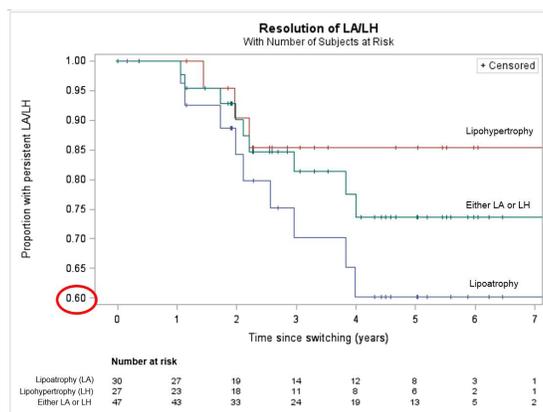
**Methods:** The Collaborative HIV Paediatric Study (CHIPS) is a multicentre cohort study of virtually all HIV-infected children in care in the UK and Ireland. All with an LA/LH assessment recorded in 2003-2011 were included. Using the 0-3 grading system, unequivocal case definition was defined as grade 2 or 3. Resolution was defined as return to grade 1 or 0 in all body regions. LA and LH were assessed and analysed separately. Kaplan-Meier analysis investigated time to resolution of LA and/or LH following diagnosis (which typically occurred at the same visit as switching the drug thought to cause the LA/LH). Multivariable logistic regression identified factors predicting recovery.

**Results:** LA/LH assessments were available on 1345 children followed for a median (IQR) of 5.5 (2.9, 8.2) years after ART initiation. Thirty developed LA and 27 developed LH. (10 had both LA and LH).

Recovery was delayed until 1 year after ART switching (see figure), with a steady incidence of recovery between 1 and 2 years (for LH) or between 1 and 4 years (for LA). Cases persisting beyond 2 years (for LH) or beyond 4 years (for LA) did not recover, although only 6 (25%) and 8 (40%) of unresolved cases respectively remained in follow-up at 5 years. Thus, eventual resolution occurred in only 10 (33%) of LA cases, with a median time to resolution of 2.3 (1.8, 3.6) years, and 3 (11%) of LH cases, with a median time to resolution of 2.0 (1.7, 2.1) years.

Children with LA were more likely to resolve if older at diagnosis of LA (adjusted odds ratio [aOR] 1.51/additional year of age [95%CI 1.01, 2.26], p=0.05) or had greater duration of exposure to LPV prior to diagnosis of LA (aOR 1.65/additional year of exposure [95%CI 1.00, 2.74], p=0.05), and less likely to resolve if they were girls (aOR 0.06 [95%CI 0.00, 0.87], p=0.04). No factors were associated with increased likelihood of resolution of LH.

**Conclusions:** Established LA and LH in children are largely persistent, requiring on-going follow-up to mitigate stigmatizing psychosocial effects and any other morbidity.



**856 Patterns of Systemic Hypertension Among Adults With Perinatally Acquired HIV**

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**Background:** Patients with perinatally acquired HIV may be at risk for the development of age-related non-AIDS diseases. The primary aim of this study was to describe patterns of systemic hypertension among a cohort of adults (≥18 years) with perinatally-acquired HIV.

**Methods:** This retrospective study was conducted among patients with perinatally-acquired HIV infection who received care in the University of Maryland Medical System. Participants were included if they had reached at least 18 years of age as of September 30, 2013. The objectives were to characterize the incidence, prevalence, and patterns of systemic hypertension. Incidence rates of systemic hypertension were calculated and rate ratios were generated based upon age at hypertension diagnosis within one of two age groups, ≥18 and <18 years. Systemic hypertension was defined as one or both of the following: 1) Provider documentation of systemic hypertension diagnosis, 2) Receipt of antihypertensive medications for blood pressure management. Descriptive statistics, including mean, median, standard deviation and quartiles for continuous measures were used to characterize the study population. Multivariable logistic regression was used to evaluate independent predictors of hypertension.

**Results:** The mean age of the overall cohort (N=109) was 22.8 years (range 18.5-30.2 years). The majority (93%) was African American, and 55% were female. The overall prevalence of hypertension in the cohort was 26.6%, and the incidence rate of hypertension was significantly higher among those aged ≥18 years (4.59 per 100 person-years) compared to those who had reached ages <18 years (0.46 per 100 person-years) at time of diagnosis (RR 10.0, CI 7.29-13.71). By multivariable analysis, only coexisting renal disease was associated with an increased risk of hypertension diagnosis (OR 4.93, 95% CI 1.79-13.62), though this association appeared to be strongest among those with a hypertension diagnosis at <18 years.

**Conclusions:** The prevalence of systemic hypertension among adults with perinatally-acquired HIV in this cohort greatly exceeded normative values for the general population (0.3% at 25 years) and incidence rates appeared to increase during early adulthood (≥18 years). Coexisting renal disease may be a risk factor for systemic hypertension though this relationship may not be as strong in adulthood. Adult providers may need to closely monitor for the development of hypertension in perinatally-infected patients as they age into adulthood.

**Table 1: Demographic and clinical characteristics of hypertension, non-hypertension cohorts**

	Hypertension Cohort	Non Hypertension Cohort
<b>N</b>	29	80
<b>Gender</b>	Male: 13 (45%) <sup>†</sup> Female: 16 (55%)	Male: 36 (45%) <sup>†</sup> Female: 44 (55%)
<b>Ethnicity</b>	AA: 27 (93%) <sup>†</sup> W: 1 (4%) NR: 1 (4%)	AA: 76 (95%) <sup>†</sup> W: 4 (5%) NR: 0
<b>Age as of 9/30/13 (years)</b>	Mean: 23.2 <sup>†</sup> Median: 22.6	Mean: 22.6 <sup>†</sup> Median: 22.0
<b>Past Durable HIV RNA Suppression ≥12 mo, (%)</b>	15 (51.7%) <sup>†</sup>	55 (61.1%) <sup>†</sup>
<b>Past Durable HIV RNA Suppression ≥3 y, (%)</b>	9 (31%) <sup>†</sup>	35 (38.9%) <sup>†</sup>
<b>Past Durable HIV RNA Suppression ≥5 y, (%)</b>	6 (20.7%) <sup>†</sup>	24 (26.7%) <sup>†</sup>
<b>Past Durable HIV RNA Suppression ≥10 y, (%)</b>	2 (6.9%) <sup>†</sup>	7 (7.8%) <sup>†</sup>
<b>HIV RNA Suppression Most Recent Visit, (%)</b>	13 (44.8%) <sup>†</sup>	36 (40%) <sup>†</sup>
<b>Nadir CD4 Count (cells/mL, median) (%)</b>	176 <sup>†</sup>	179 <sup>†</sup>
<b>Nadir CD4/CD8 Ratio</b>	0.2 <sup>†</sup>	0.3 <sup>†</sup>
<b>Mean Rate of NRTI Use (per 100 PY)</b>	13.96 <sup>†</sup>	15.70 <sup>†</sup>
<b>Mean Rate of NNRTI Use (per 100 PY)</b>	2.81 <sup>†</sup>	2.69 <sup>†</sup>
<b>Mean Rate of PI Use (per 100 PY)</b>	4.42 <sup>†</sup>	4.04 <sup>†</sup>

Note: <sup>†</sup>p<NS; Abbreviations: AA: African American; W: White; NR: not recorded; NRTI: nucleos(t)ide reverse transcriptase inhibitor; NNRTI: non-nucleoside reverse transcriptase inhibitor; PI: protease inhibitor; coples/mL: copies/mL; PY: person-years

**857 Prevalence of Persistent Renal Dysfunction in Perinatal Thai HIV Adolescents**

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**Background:** With access to antiretroviral therapy (ART), perinatally HIV-infected children can live longer but chronic diseases including renal complications play more roles. There are limited data of persistent renal dysfunction (PRD) in this population. Here, we report prevalence and incidence of PRD and associated factors.

**Methods:** Data of perinatally HIV-infected Thai adolescents from a pediatric HIV cohort with their current aged ≥10 years old and had documented serum creatinine (Cr) ≥2 times after ART initiation were included for analysis. Children with history of renal diseases before ART initiation were excluded. Cr and urine examination were performed every 6

months since 2006. Persistent renal dysfunction was defined by having  $\geq 2$  records of any of 1) estimated glomerular filtration rate (eGFR)  $< 60$  mL/min/1.73 m<sup>2</sup> for age and height adjusted, 2) increased Cr for age; Cr  $\geq 0.7$  mg/dl for aged 10-12 years and Cr  $\geq 1.0$  mg/dl for aged  $\geq 13$  years, or 3) proteinuria by a dipstick method  $\geq 1+$ . Prevalence and incidence of PRD were analyzed. Predictors of persistent RD were assessed by logistic regression.

**Results:** Data of 255 adolescents were included. In their last visit, median (IQR) of age and duration of ART were 16.7 (14.5-18.8) and 10.3 (7.1-12.4) years, respectively. 56% used boosted protease inhibitors (PI)-based regimen and 64% used tenofovir disoproxil fumarate (TDF) with median (IQR) duration of 3.0 (1.4-4.0) years. Median (IQR) of current CD4 was 678 (478-915) cells/mm<sup>3</sup> and 74% had HIV-RNA  $< 50$  copies/mL.

The overall prevalence of PRD was 14.1% (95% CI 9.8-18.4), owing to low eGFR and/or increased serum Cr 9% and proteinuria 6.3%. The prevalence of PRD in TDF vs non-TDF group was 18.3% vs 6.7% ( $p=0.02$ ). Three adolescents (10%) who took TDF and had PRD needed to discontinue TDF. By multivariate analysis, predictors of PRD were age  $< 15$  years (aOR 5.7; 95%CI; 2.5-12.8), male (aOR 3.1; 1.4-7.0), exposed cotrimoxazole  $> 3$  years (aOR 5.3; 1.7-16.2).

In overall, incidence of PRD was 4.4 (95% CI 3.2-6.1) per 100 person-years. Table 1 shows the incidence of PRD by duration of TDF exposed and by type of regimens.

**Conclusions:** Renal dysfunction is not uncommon in the perinatally HIV-infected adolescents. Creatinine and urine analysis monitoring should be emphasized on young adolescents using TDF with protease inhibitors especially in the first 3 years of TDF initiation.

Table 1. Incidence rate of persistent renal dysfunction

	Person-years	Number of adolescents developed persistent renal dysfunction	Incidence rate per 100 person-years (95%CI)	Hazard ratio (95%CI)	P-value
<b>Overall incidence</b>	820	36	4.4 (3.2-6.1)	-	-
<b>Incidence by duration of tenofovir</b>					
• Never used	254	6	2.4 (1.1-5.3)	1	
• Exposed 6 months to 3 years	180	22	12.2 (8.0-18.6)	4.6 (1.9-11.3)	0.001
• Exposed $\geq 3$ years	386	8	2.1 (1.0-4.1)	0.9 (0.3-2.7)	
<b>Incidence by type of regimens</b>					
• Non TDF	254	6	2.4 (1.1-5.3)	1	
• TDF+NNRTI	172	5	2.9 (1.2-7.0)	1.2 (0.4-3.9)	0.02
• TDF+PI	393	25	6.4 (4.3-9.4)	2.8 (1.1-6.8)	

TDF: tenofovir disoproxil fumarate, NNRTI: non-nucleoside reverse-transcriptase inhibitors, PI: protease inhibitors. 95%CI: 95% confidence interval  
Hazard ratio were calculated by cox-proportion hazards model

**858 Early Weight and Height Changes in Asian Children Using Cotrimoxazole With ART**

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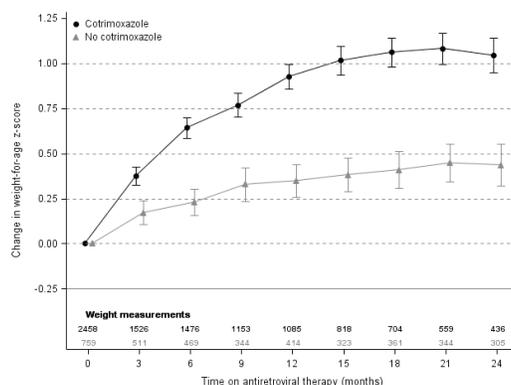
**Background:** Cotrimoxazole (CTX) prophylaxis is indicated for most HIV-infected children starting antiretroviral therapy (ART) in resource-limited settings. In addition to protecting against a range of opportunistic infections, CTX also slows the loss of weight- (WFA) and height-for-age (HFA) when ART is not available. However, it is uncertain if CTX enhances growth during the early stages of ART use.

**Methods:** Children enrolled in the TREAT Asia pediatric HIV Observational Database who initiated ART after 31/Dec/2002 aged 1 month - 14 years and had both weight and height documented at ART initiation (baseline) were included. Follow-up was censored at the time of CTX cessation for children using CTX at baseline; the time of CTX initiation for those not using CTX at baseline; or the last recorded clinic visit. Generalized estimating equations adjusted for country income status and including an interaction between CTX use and time were used to identify factors associated with change in WFA and HFA from baseline during the first 24 months of ART.

**Results:** Overall, 3217 children were eligible for analysis. At ART initiation, 76.4% were using CTX prophylaxis, median age was 5.5 years, 50.9% were male, median WFA z-score was -2.5, and median HFA z-score was -2.4. Median baseline CD4 was 27% in both CTX users and non-users. Amongst CTX users, the median duration of CTX use after starting ART was 1.2 years. Smaller increases in WFA z-score were associated with older age at baseline, higher baseline WFA, anemia, and lower CD4%. Using a broad spectrum antibiotic (other than CTX) was associated with greater increases in WFA z-score. The adjusted mean increase in WFA z-score became significantly higher in CTX users compared with non-users after 6 months of ART (0.64 [95%CI 0.59-0.70] vs 0.23 [0.16-0.30],  $p<0.01$ ) and remained significantly higher thereafter (Figure 1). Older age at baseline, higher baseline HFA, double orphanhood, anemia, and low CD4% were associated with smaller increases in HFA z-score. The adjusted mean increase in HFA z-score became significantly higher in CTX users compared with non-users after 15 months of ART (0.41 [95%CI 0.36-0.46] vs 0.24 [0.17-0.31],  $p=0.02$ ) and remained significantly higher at 24 months.

**Conclusions:** CTX prophylaxis was associated with greater increases in WFA and HFA during the first 24 months of ART in this Asian cohort of HIV-positive children. Wider use of CTX may compliment ART and nutritional intervention programs for children entering HIV care in the region.

Figure 1. Adjusted mean  $\pm$  95%CI change in weight-for-age z-score by cotrimoxazole exposure amongst Asian children using antiretroviral therapy



**859 Effects of Vitamin D Supplementation on BMD and Bone Markers in HIV+ Youth**

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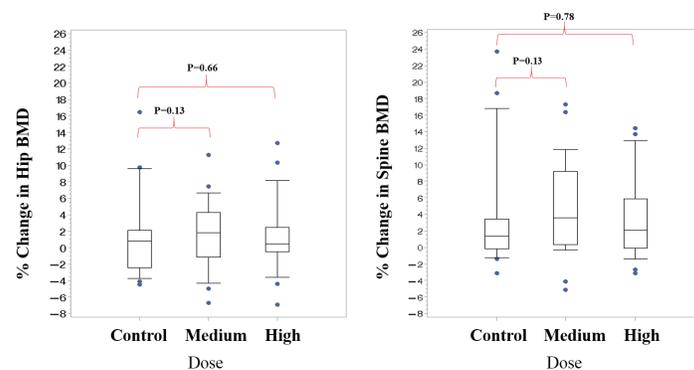
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**Background:** Low bone mineral density (BMD) is a significant co-morbidity in HIV. Vitamin D supplementation in the general population improves BMD in those with vitamin D deficiency. In HIV, studies are limited and show conflicting data. Our study investigates changes in BMD and bone turnover markers after 12 months of supplementation in HIV+ youth with vitamin D insufficiency.

**Methods:** This is a randomized, active-control, double-blind trial investigating 2 different monthly vitamin D<sub>3</sub> doses [60,000 (medium) or 120,000 (high) IU/month] vs. a control arm of 18,000 IU/month in 8-26 year old HIV+ youth on ART with baseline 25-hydroxyvitamin D (25(OH)D)  $\leq$ 30 ng/mL and HIV-1 RNA <1000 copies/mL. Randomization was stratified by EFV use. Spine and total hip bone BMD by DXA and plasma bone turnover markers were measured at baseline (BL) and at 12 months. Comparisons of BMD and bone marker changes from BL to 12 months were made within each group, and between the HIV+ control dose vs. combined supplementation (medium+high dose) using appropriate two-sample tests.

**Results:** Overall, 102 participants enrolled: 64% male, 89% black, median (Q1, Q3) age of 20 (17, 23) years. HIV & ARV duration were 8 (2, 17) and 3 (1, 10) years, respectively with a CD4 count of 652 (450, 871) cells/mm<sup>3</sup>. Only 26% were on EFV. BL 25(OH)D was similar between groups (control group: 17 (11, 21) vs. medium+high group: 18 (14, 22) ng/mL; P=0.49) and increased to 31 (22, 37) and 42 (33, 53) ng/mL in the control and supplementation (medium+high dose) groups at 12 months, respectively (within and between groups P<0.001). Over 12 months, HIV+ subjects in medium+high dose group had significant % increases in BMD at the spine (+2.8%, P=0.001) and hip (+0.9%, P=0.03). In the control dose group, increases were seen in % BMD for hip (+0.6%, P=0.002), but not spine (P=0.87). Osteocalcin,  $\beta$ -crosslaps, and procollagen type 1 N-terminal propeptide trended down in the medium+high group (P=0.046, 0.049, 0.053, respectively). None of the endpoints were significantly different between supplementation group (medium+high) and controls (P $\geq$ 0.15). Changes in BMD seen in the medium+high dose group appeared to be driven by subjects in the medium dose group.

**Conclusions:** BMD increased and bone turnover markers decreased in HIV+ youth with vitamin D insufficiency after 12 months of vitamin D supplementation. A dose of 60,000 IU/month appeared to have the greatest impact on BMD. Vitamin D repletion with this dose should be studied further in deficient HIV+ youth.

**860 Seroprevalence of Hepatitis B Among HIV-Infected Children and Adolescents in Asia**

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**Background:** Hepatitis B (HBV)-HIV coinfection is associated with liver inflammation which can progress to liver fibrosis/cirrhosis. We determined HBV seroprevalence in children and adolescents participating in the TREAT Asia Pediatric HIV Observational Database.

**Methods:** A multi-site cross-sectional study was done among HIV-infected patients currently aged <25 years receiving antiretroviral treatment (ART) who had HBV surface antigen (HBsAg), HBV surface antibody (anti-HBs), and/or HBV core antibody (anti-HBc) tested during 2012-2013. HBV coinfection was defined as having positive HBsAg. Seroprotection was defined as having anti-HBs  $\geq$ 10 mIU/mL. Isolated anti-HBc was defined as a positive anti-HBc test with negative HBsAg and anti-HBs tests.

**Results:** A total of 3,380 patients from 6 countries (Vietnam, Thailand, Cambodia, Malaysia, Indonesia, and India) were enrolled; 96% had perinatal HIV-infection. The current median (interquartile ranges, IQR) age was 11.2 (7.8-15.1) years, and duration on ART was 5.9 (3.4-8.0) years. History of HBV vaccination was documented in 39% (1323/3380). Of the 2,754 patients with HBsAg testing (81.5%), 130 were positive, representing a prevalence of HBV coinfection of 4.7% (95% confidence interval [CI] 3.9-5.5). For patients with HBV-HIV coinfection, 50% were male, the median (IQR) age was 11.6 (8.1-14.7) years, the median (IQR) CD4 count was 806 (558-1172) cells/mm<sup>3</sup>, and 84% (49/58) had HIV RNA <400 copies/mL. Among 73% (95/130) with serum alanine transferase (ALT) available, 28 (29%) had mild to moderate elevated ALT to 1.25-5 times the upper limit of normal (xULN), while 3 (3%) had ALT > 5xULN.

Of 1,093 patients with anti-HBs testing, 257 had positive tests. The prevalence of seroprotection was 23.5% (CI 21.0-26.0); with 30% (180/599) among those with documented history of HBV vaccination before ART, and 45% (120/267) among those revaccinated after immune recovery. Of the 1,036 patients with all three tests, 13 (1.3%) had isolated anti-HBc.

**Conclusions:** The estimated prevalence of HBV coinfection in this cohort of Asian HIV-infected children and adolescents on ART was 4.7%. The majority of those with a history of early childhood or revaccination had insufficient levels of HBV protective antibody. HBV screening of HIV-infected children can guide use of ART with anti-HBV activity, and facilitate revaccination of those without seroprotection.

**861 Correlates of Injectable Contraceptive Discontinuation Following HIV-1 Seroconversion**

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**Background:** The risk of vertical HIV transmission during acute and recent HIV infection is elevated, highlighting the importance of effective contraception in addition to barrier methods during this period. While progestin-only injectable contraception (IC) is the most common highly effective contraceptive method currently used in sub-Saharan

Africa, its use has not been well described in HIV-infected women in the post-seroconversion period. We aim to examine factors associated with discontinuation of IC following seroconversion in the VOICE trial.

**Methods:** Following seroconversion during an HIV prevention trial (VOICE), 255 African women enrolled in a longitudinal observational study (MTN-015). Family planning method was assessed by self-report via face-to-face interview at MTN-015 entry and at 3, 12, and 24 months (m) post-seroconversion. Women reporting IC use at baseline were evaluated longitudinally for continued use from time of seroconversion. Correlates of IC discontinuation were examined by Cox proportional hazard modeling.

**Results:** The majority of women were from South Africa (93%), young (median age 23 years (y), IQR 21-26y) and unmarried (93%). Median follow-up was 24m (IQR 13-26m) post-seroconversion. IC use was reported at baseline by 198 (78%) women, of whom 182 (91%) had follow-up family planning data available (Figure 1). During follow-up 34% (61/182) of women discontinued use of IC completely without a non-barrier substitution. Baseline factors associated with lower rates of IC discontinuation were having children (HR 0.39, CI 0.20-0.82,  $p=0.01$ ) and earning one's own income (HR 0.51, CI 0.30-0.87,  $p=0.02$ ). Initiation of antiretroviral therapy (ART) (HR 0.51, CI 0.26-0.95,  $p=0.03$ ) during follow-up was also associated with a lower rate of IC discontinuation. Other baseline demographic factors, partnership status and characteristics, depression, and disclosure of HIV status were not significantly associated with IC discontinuation in time-dependent analyses.

**Conclusions:** Discontinuation of effective contraception was common post-seroconversion in HIV-infected women despite onsite provision of family planning services. Many women with recently acquired HIV face complex decision-making regarding family planning. Linkages between HIV testing, HIV/ART care, and family planning services is essential.

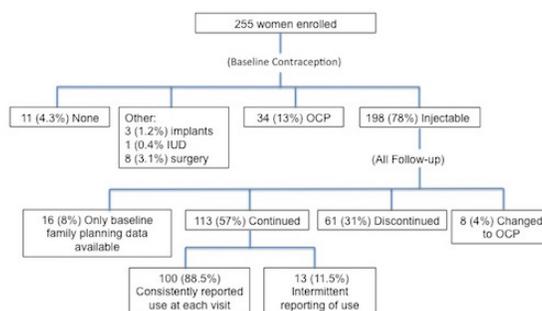


Figure 1. Schematic of contraceptive use in women enrolled in the MTN-015 VOICE cohort. (None= participants not reporting use of any other non-barrier form; OCP= oral contraceptive pill; IUD= intrauterine device.)

## 862 Correlation Between Cotherapy of Efavirenz-Based ART and Pregnancy Among Women

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**Background:** Uncertainties exist around potential interactions between hormonal contraceptive implants (HCI) and antiretroviral therapy (ART). In Swaziland, 12.4% of women taking Efavirenz (EFV)-based regimens while using the Jadelle implant became pregnant and EFV co-therapy was the only variable that significantly correlated with pregnancy outcomes. We examined the association between EFV and non-EFV based ART regimens and pregnancy outcomes among women who were on HCI in a peri-urban HIV clinic in Tororo, Uganda.

**Methods:** Using a retrospective review, we extracted routinely collected data from the TASO Tororo HIV clinic family planning register. All women >18 years of age and on ART who received HCI between January, 2012 and June, 2014 were included in the study. Our primary outcome of interest was confirmed recorded pregnancy. The association between ART regimen and pregnancy outcome was assessed using fisher's exact statistics

**Results:** A total of 148 HCI users were identified, 62 (41.9%) on an EFV- based regimen and 86 (58.1%) on a non EFV-based regimen. The median age of the women was 33.7 years (IQR 22-48); mean weight was 57.76kg (IQR 28-95) and median duration on ART was 22.7 months (IQR 17-37). For women that conceived, the median duration between HCI placement and confirmed pregnancy was 22.6 months (IQR 16-29). Of the 148 women identified during the review period, 9 (6.1%) conceived. All women who conceived were on an EFV-based regimen, while none of the women on non-EFV based regimens conceived ( $p=0.0003$ ).

**Conclusions:** We observed a significant association ( $p=0.0003$ ) between HIV-positive HCI users on EFV-based ART regimens as compared to HIV-positive HCI users on non-EFV based ART regimens. This association is similar to findings in other studies, and strengthens the evidence that women on EFV-based regimens desiring contraception may need to be cautioned about a potential increased risk of pregnancy with use of HCI. A more systematic national surveillance of pregnancy among women on both ART and hormonal contraception may help to confirm a correlation and inform potential guidance on hormonal contraceptive use among women on ART

## 863 Is Menopause Associated With Unprotected Sex in High-Risk HIV-Positive Kenyan Women?

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**Background:** Many HIV-positive women now live well beyond menopause. Postmenopausal women are no longer at risk for pregnancy, and some studies suggest that they may use condoms less often than premenopausal women. This study tested the hypothesis that unprotected sex is more common at postmenopausal visits than at premenopausal visits among HIV-positive women who reported trading sex for cash or in-kind payment in Mombasa, Kenya.

**Methods:** Women in this prospective cohort study were HIV-positive,  $\geq 18$  years old, and reported transactional sex. At enrollment and monthly follow-up visits, participants completed a standardized interview and received comprehensive risk reduction counseling. Study clinicians collected genital samples at enrollment and quarterly visits.

Menopause was assessed using a clinical decision tool. The primary outcome of unprotected sex was determined by the presence of prostate specific antigen (PSA) in vaginal secretions. Log-binomial generalized estimating equation (GEE) models, with working independence correlation structure and robust standard errors, were used to estimate relative risks (RRs) and 95% confidence intervals (CI). Models were adjusted for age.

**Results:** We followed 403 HIV-positive women who contributed 2753 quarterly examination visits. Detection of PSA was less frequent at postmenopausal visits compared to premenopausal visits (55/540, 10.2% versus 397/2210, 18.0%; RR 0.57, 95%CI 0.38-0.86) (Table 1). After adjusting for age, this association was no longer statistically significant (adjusted RR 0.70, 95%CI 0.45-1.11). Women were more likely to report no sex in the past week at postmenopausal visits (RR 1.67, 95%CI 1.44-1.95). When sexually active, women reported the same rate of condom use at postmenopausal and premenopausal visits (RR 0.97, 95%CI 0.84-1.12).

**Conclusions:** In this population of high-risk HIV-positive Kenyan women, postmenopausal status was not associated with an increased risk of unprotected sex. The relationship between menopause and unprotected sex is likely to be context-specific and may differ with varying risk groups, regions, and levels of exposure to sexual health education.

Table 1: Associations between menopause and sexual risk behavior

Outcomes	Postmenopausal	Premenopausal	RR (95% CI)	aRR <sup>1</sup> (95% CI)
	Positive visits/ Total visits (%)	Positive visits/ Total visits (%)		
Positive PSA	55/540 (10.2)	397/2210 (18.0)	0.57 (0.38-0.86)	0.70 (0.45-1.11)
Sperm detected	24/513 (4.7)	183/2181 (8.4)	0.56 (0.32-0.98)	0.73 (0.36-1.45)
Any STI <sup>2</sup>	33/534 (6.2)	187/2201 (8.5)	0.73 (0.38-1.38)	1.66 (0.80-3.46)
Self-reported behaviors in the past week				
Abstinent	919/1266 (72.6)	2095/4834 (43.3)	1.67 (1.44-1.95)	1.16 (0.96-1.40)
Unprotected sex	70/1265 (5.5)	493/4836 (10.2)	0.54 (0.29-1.02)	0.76 (0.39-1.46)
100% condom use <sup>3</sup>	276/346 (79.8)	2246/2739 (82.0)	0.97 (0.85-1.12)	0.97 (0.84-1.12)
# vaginal sex acts (>2) <sup>3</sup>	65/347 (18.7)	953/2739 (34.8)	0.54 (0.32-0.92)	0.85 (0.47-1.52)
# sex partners (>1) <sup>3</sup>	90/347 (25.9)	1157/2742 (42.2)	0.61 (0.34-1.13)	0.94 (0.51-1.76)

<sup>1</sup> Adjusted for age at visit.  
<sup>2</sup> STIs included: Neisseria gonorrhoeae, Chlamydia trachomatis, and Trichomonas vaginalis.  
<sup>3</sup> Restricted to visits where the participant was not abstinent in the past week.

**864 Fertility Desire, Unprotected Sex, and Viral Load in HIV-Positive FSWs in Kenya**

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**Background:** In HIV-positive female sex workers (FSWs), fertility desire may increase secondary HIV transmission risk if it is associated with unprotected sex, and with poor ART adherence, leading to detectable viral load (VL). We conducted a prospective cohort study of HIV-positive FSWs in Mombasa, Kenya to examine fertility desire as a risk factor for semen detection by prostate specific antigen test (PSA) and detectable plasma VL. We also explored fertility desire as a risk factor for HIV transmission potential (TP), the co-occurrence of unprotected sex and detectable VL.

**Methods:** Participants were HIV-positive FSWs taking ART, ≥18 years old, pre-menopausal, and not pregnant. Fertility desire (*wants children*) was assessed quarterly. Primary outcomes were semen detection in vaginal secretions by PSA (quarterly) and detectable VL (≥180 copies/ml, semi-annual). HIV TP was defined as visits with both positive PSA and detectable VL. We evaluated effect modification by contraceptive use (modern method vs. none/condoms only). We used log-binomial generalized estimating equations with independent working correlation structure and robust standard errors to obtain relative risks (RR) and 95% confidence intervals (CI). Final models were adjusted for age and other covariates identified through model building.

**Results:** Overall, 211 women contributed 1,581 visits to the analysis. Fertility desire was common (72 women, 22.9% visits). Semen detection by PSA was similar at visits with and without fertility desire (67/360, 18.6% vs. 201/1,207, 16.7%, Table 1). The association between fertility desire and PSA detection differed in women not using contraceptives compared to those using a modern method besides condoms (p-value for interaction=0.06). Fertility desire was associated with significantly higher risk of PSA detection at visits with no contraceptive use (adjusted RR [aRR] 1.52, 95%CI 1.14-2.03), though not at visits with contraception use (aRR 0.63, 95%CI 0.28-1.40). Fertility desire was not associated with significantly higher risk of detectable VL (aRR 0.83, 95%CI 0.52-1.34) or HIV TP events (aRR 1.22, 95%CI 0.47-3.11).

**Conclusions:** Fertility desire was common in this sample of HIV-positive FSWs, and was associated with higher risk of unprotected sex in women not using contraception. However, given the high level of viral suppression, there was no substantial increase in events when HIV transmission would likely occur. Combination HIV prevention should better address fertility desire in this key population.

Table 1. Unadjusted and adjusted analyses of the associations between fertility desire and semen detection by PSA test, detectable viral load, and HIV transmission potential in HIV-positive FSWs

Outcome	% E	% NE	RR, 95% CI	aRR, 95% CI
<b>Association between fertility desire and semen detection by PSA, stratified by modern contraceptive use<sup>1</sup></b>				
Semen detection by PSA, in visits with none/condoms only	61/307 (19.9)	125/617 (15.3)	1.30, 0.98-1.71	1.52, 1.14-2.03 <sup>2</sup>
Semen detection by PSA, in visits with any modern method	6/53 (11.3)	76/390 (19.5)	0.58, 0.29-1.25	0.63, 0.28-1.40 <sup>2</sup>
<b>Association between fertility desire and viral load ≥180 c/ml</b>				
Viral load ≥180 c/ml	18/145 (12.4)	78/513 (15.2)	0.82, 0.51-1.51	0.83, 0.52-1.34 <sup>3</sup>
<b>Association between fertility desire and HIV transmission potential (visit with PSA &amp; viral load ≥180 c/ml)</b>				
HIV transmission potential	6/145 (4.1)	13/512 (2.5)	1.63, 0.63-4.21	1.22, 0.47-3.11 <sup>4</sup>

<sup>1</sup> Contraceptive use categories are visits where women reported no method or condoms only versus any modern method, which includes DMPA, OCP, Norplant, Tubal ligation, or IUD (hysterectomy and 'other method' excluded). 14/1,581 visits had missing contraceptive data.  
<sup>2</sup> This model adjusted for age (continuous) and number of live births at enrollment (continuous). The model with non-contracepting women included 165 women and 1,033 visits. The model including visits when women were using modern contraception included 72 women and 428 visits.  
<sup>3</sup> Model adjusted for included age (continuous) and education level (binary), and included 181 women and 658 visits.  
<sup>4</sup> Model adjusted for included age (continuous) and male partner controlling behaviors (binary, experienced at least one of 7 acts), and included 181 women and 657 visits.

**865 Inaccurate Reporting of Condom Use Among Women Using Injectable Contraception**

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**Background:** Observational analyses of HIV acquisition by women using injectable contraception versus no hormonal contraception are potentially biased by differential accuracy in self-reported condom use, a key confounding factor. Laboratory markers of semen exposure can be used to objectively determine whether over-reporting is occurring with different frequency among women using injectable contraception and no contraception.

**Methods:** Using stored vaginal swab specimens from a random sample of HIV-uninfected women participating in a randomized clinical trial of pre-exposure prophylaxis (the Partners PrEP Study), we measured the presence of Y chromosomal (Yc) DNA among those who reported 100% condom use with all partners during the past month and sex within the past 7 days. Half of the samples tested were from women reporting injectable contraceptive use at the visit when the swab was collected and half were from women using no contraception aside from condoms. Multivariate logistic regression was used to estimate the association between injectable contraceptive use and Yc DNA detection.

**Results:** The median age of women was 34 (interquartile range [IQR] 29-39), most (97.0%) had at least one child, and the median number of sex acts during the month prior to vaginal swab collection was 3 (IQR 2-5). Among 428 specimens tested (213 from injectable contraceptive users and 215 from women using no contraception), 32.0% had Yc DNA detected with a mean of 193 copies/10,000 human cells (range 0.1-8201). The frequency of detection did not differ by contraceptive use: 34.2% of DMPA users versus 29.8% of women using no contraception, with an odds ratio [OR] of 1.3 (95% confidence interval [CI] 0.9-2.0) after adjustment for participant age, number of children, and sexual frequency.

**Conclusions:** One third of women in this study potentially over-reported condom use, but the frequency of over-reporting did not differ among women using injectable contraception compared to women using no contraception. These results refute the hypothesis that residual behavioral confounding may entirely explain the increased risk of HIV

acquisition among injectable contraceptive users in some observational studies. For studies of HIV prevention among women, Yc DNA detection is a feasible biomarker to obtain and complements self-reported sexual behavior data.

### 866 Higher Cumulative TFV/FTC Levels in PrEP Associated With Decline in Renal Function

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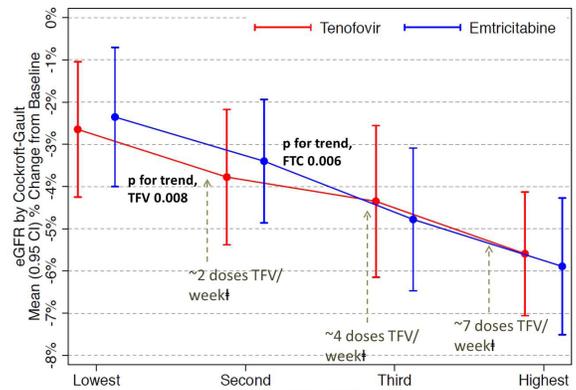
**Background:** PrEP is proven to reduce the risk of HIV acquisition. Drug levels (as markers of adherence) have been critical to interpreting disparate outcomes in PrEP trials, but can also be assessed (as markers of exposure) in relationship to adverse effects. Concentrations of tenofovir (TFV) and emtricitabine (FTC) in hair represent cumulative exposure and may be associated with toxicities in HIV-uninfected persons. We report for the first time, in a large PrEP demonstration study, the relationship between TFV/FTC levels in hair and renal function over time.

**Methods:** The iPrEx Open Label Extension (OLE) study enrolled HIV-negative MSM and transwomen and all were on PrEP. Hair samples were collected every 12 weeks and levels of TFV/FTC measured via liquid chromatography/tandem mass spectrometry. Serum creatinine (Cr) was measured every 12 weeks and glomerular renal function (eGFR) estimated by Cockcroft-Gault (CG) or the MDRD equation. The association between change in eGFR over time (adjusted for baseline eGFR) and TFV/FTC levels (categorized into quartiles) was analyzed by generalized estimating equations.

**Results:** Hair data and creatinine measures were available for 1144 person-visits in 202 participants followed for a median of 16.8 months. Median age 29 years (19-70); 91% MSM; 22% White, 11% Black, 6% Asian, 60% Latino/mixed. Baseline mean Cr level was 0.89mg/dL with a median baseline eGFR of 112ml/min (99-128). The eGFR for all participants on TFV/FTC decreased over 18 months, but there was a monotonic relationship between % decrease in eGFR with increasing quartile of hair level for TFV (p 0.008) and FTC (p 0.006) (Figure). For instance, mean % change in eGFR from baseline was -2.6ml/min (SE 0.8) in person-visits with TFV levels in the 1st quartile, but -5.6 (SE 0.7) when hair levels were in the 4<sup>th</sup> quartile. The odds of eGFR falling below 70 ml/min (6.1% of sample) increased with increasing quartile of TFV/FTC concentration (OR 4.4 (1.1-17.4) for 4<sup>th</sup> TFV hair quartile, p trend 0.045; OR 4.0 (0.9-17.2) for 4<sup>th</sup> FTC quartile, p trend 0.027).

**Conclusions:** We show for the first time that greater long-term exposure to TFV or FTC in patients on PrEP is associated with declining renal function over time. Hair levels of TFV/FTC were associated with decreases in eGFR and a higher likelihood of eGFR falling to <70ml/min in a monotonic fashion in iPrEx OLE. Establishing thresholds of TFV/FTC exposure that protect from HIV, but minimize the risk of toxicity, is essential to the real-world roll-out of PrEP.

Figure: Relationship between % change in eGFR from baseline (calculated by Cockcroft Gault\*) and hair concentration\*\* of either TFV (red) or FTC (blue)



\*Similar findings were seen when creatinine clearance or GFR was estimated either by the Cockcroft Gault or Modification of Diet in Renal Disease (MDRD) equation.  
\*\*Hair assays of TFV and FTC in UCSF lab peer-reviewed and approved by the DAIDS Clinical Pharmacology and Quality Assurance (CPQA) program  
†Median hair levels for 2, 4 and 7 doses/week of tenofovir estimated from the STRAND study (Liu A et al. PLOS ONE 2014. PMID 25296098)

### 867 Changes in Renal Function Associated With TDF/FTC PrEP Use in the US Demo Project

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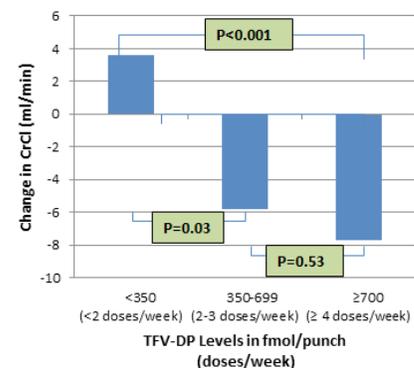
**Background:** Several trials have demonstrated the safety and efficacy of TDF/FTC pre-exposure prophylaxis (PrEP). Renal toxicity was uncommon in randomized trials of healthy individuals, but has not been assessed in clinical settings. We evaluated changes in renal function among participants enrolled in the open-label US PrEP Demonstration Project.

**Methods:** The Demo Project enrolled HIV-negative MSM and transwomen (TGW) in STI clinics and a community health center. Eligible participants [creatinine clearance (CrCl)  $\geq$  60 ml/min] were offered 48 weeks of TDF/FTC PrEP. Creatinine (Cr) was measured every 12 weeks and CrCl estimated by the Cockcroft Gault equation. Tenofovir diphosphate (TFV-DP) levels in dried blood spots (DBS) were measured in a subset of subjects. The associations of time-dependent factors with visit-to-visit changes in CrCl were assessed using linear mixed models.

**Results:** From October 2012 to January 2014, 557 MSM and TGW enrolled. Median age was 35 (range 18-65); 48% were White, 35% Latino, 7% Black, 5% Asian, and 6% other; 98% were MSM. Baseline median Cr was 0.92 (0.59-1.55), with a median CrCl of 124 ml/min (71-309). DBS were tested at 1,067 person-visits among 294 participants, with TFV-DP levels consistent with  $\geq$  4 doses/week in 82% of person-visits. Median CrCl declined 6 ml/min (5%) from baseline to week 12 and remained stable through week 48 (p=0.96), with no differences by race/ethnicity, weight, or NSAID use. However, 30% had >10% decline in CrCl at week 12. TFV-DP levels  $\geq$  2 vs. <2 doses/week were associated with a greater decline in CrCl at week 12 (-7.6 ml/min vs. +3.6 ml/min, p=0.001) (figure). In a multivariable model, age <25 (7.7 ml/min greater decline), use of hypertension or diabetes medications (6.1 ml/min greater decline), and TFV-DP levels  $\geq$  2 doses/week (12.8 ml/min greater decline) were independently associated with greater CrCl loss. The age effect was not explained by alcohol or recreational drug use. No subjects had CrCl <60 ml/min during follow-up. TDF/FTC was held in 3 subjects due to elevated creatinine, however these were not confirmed on repeat testing, and PrEP was restarted in all cases with no further interruptions.

**Conclusions:** MSM initiating PrEP in the Demo Project had an overall modest, non-progressive decline in renal function, with a threshold effect for TFV-DP levels >2 doses/week. Younger PrEP users and those taking medications for hypertension or diabetes had greater decreases in CrCl and may warrant additional monitoring during PrEP use.

Figure: Mean Change in CrCl from baseline to week 12, by TFV-DP concentrations



**868 Rare Incidence of Proximal Tubular Dysfunction With Tenofovir-Based Chemoprophylaxis**

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**Background:** Tenofovir disoproxil fumarate (TDF) is infrequently associated with proximal tubular dysfunction in HIV-infected persons when used as part of combination antiretroviral therapy, but limited data are available for HIV-uninfected persons on TDF for pre-exposure prophylaxis (PrEP).

**Methods:** Data are from the Partners PrEP study, a randomized trial of daily oral TDF and emtricitabine (FTC)-TDF PrEP among African HIV-uninfected men and women (ClinicalTrials.gov:NCT00557245). We conducted: 1) a cohort analysis to determine whether FTC-TDF PrEP causes proximal tubular dysfunction among HIV-uninfected persons randomized to FTC-TDF versus placebo, and 2) a nested case-control analysis of persons on TDF or FTC-TDF to determine whether tubular dysfunction predicts subsequent clinically relevant decline in estimated glomerular filtration rate (eGFR). The primary outcome was subclinical proximal tubulopathy (PT), pre-defined as any two of the following markers of tubular dysfunction: tubular proteinuria, euglycemic glycosuria, increased urinary phosphate excretion, or increased urinary uric acid excretion. PT was assessed in concurrently obtained urine and serum samples at the 24-month visit or last on-treatment visit. For the nested case-control analysis, cases were persons on TDF or FTC-TDF with confirmed  $\geq 25\%$  eGFR decline from baseline and controls were persons with similar drug exposure without the  $\geq 25\%$  eGFR decline.

**Results:** Of 1549 persons included in the cohort (776 on FTC-TDF, and 773 on placebo), 64% were male. Median age was 37 years (range 18-64) and median duration of study drug exposure was 24 months (range 3-27). The frequency of PT was 1.7% in FTC-TDF versus 1.3% in the placebo arm [odds ratio 95% confidence interval: 1.30 (0.52, 3.33);  $p=0.68$ ]. PT occurred in 2 of 52 (3.8%) persons who experienced  $\geq 25\%$  eGFR decline versus 3 of 208 (1.4%) controls (adjusted odds ratio, 95% confidence interval: 1.40 (0.10, 14.1);  $p > 0.99$ ). One person on FTC-TDF and potentially nephrotoxic co-medications developed features indicative of Fanconi syndrome.

**Conclusions:** In this large placebo-controlled study, proximal tubular dysfunction was rare and was not significantly associated with daily oral FTC-TDF PrEP over up to 24 months of observation, nor did it predict a subsequent clinically relevant decline in eGFR. These findings support the safety of TDF-based PrEP as a component of HIV prevention in healthy HIV-uninfected individuals.

**869 STI Data From Community-Based PrEP Implementation Suggest Changes to CDC Guidelines**

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**Background:** Current CDC Clinical Practice Guidelines for the provision of pre-exposure prophylaxis (PrEP) recommend screening for sexually transmitted infections (STI) only every 6 months, unless patients report symptoms. CDC's 2015 STD Treatment Guidelines recommend STI screening for MSM every 3-6 months, but emphasize testing those with past STI history.

**Methods:** SPARK is a community-based PrEP demonstration project conducted at the largest LGBT health center in New York City. Patients prescribed PrEP are screened for urethral/rectal gonorrhea and chlamydia as well as syphilis every 3 months, and also visit the clinic for STI testing and treatment between study visits if they experience symptoms. STI data for patients in the 6-months prior to starting PrEP and in their first 12-months taking PrEP were abstracted from electronic medical records (EMR). We examined: a) the number and type of STIs that were diagnosed at each time point; b) diagnosis due to symptomatic patient presentation versus routine screening; and c) whether or not a positive STI at a previous time point would have triggered asymptomatic screening (absent the SPARK study protocol).

**Results:** Among the 280 patients who began PrEP, 21% ( $n = 58$ ) had an STI in the 6-months prior to starting PrEP (including 11% who tested positive for an STI at their PrEP prescription visit). At 3-month follow-up, 13% of patients were diagnosed with STIs, with 77% of these cases (10% of the total sample) resulting from routine screening, rather than symptomatic presentation. In addition, only 33% of patients with STIs at 3-month follow-up had a prior history of STI that would have triggered screening. At 9-month follow-up, 15% of patients were diagnosed with STIs, with 68% (10% of the total sample) diagnoses as a result of routine rather than symptomatic screening. Even though the percentage of patients with repeat STI diagnoses increased over time, basing STI screening on prior diagnosis at the 9-month visit would have missed 16% of STI cases. Overall, STI screening according to current CDC guidelines would have delayed diagnosis and treatment for 24% of PrEP patients, including 40 cases of rectal STI and 3 cases of syphilis.

**Conclusions:** Current CDC guidelines may miss a significant number of asymptomatic STI among PrEP users. STI screening may be particularly important at the first 3-month follow-up visit. Routine STI testing at each PrEP prescription visit appears warranted, with particular attention to those with past STI history.

**Table 1. STI diagnoses of PrEP patients by time-point, routine screening, STI history, and diagnosis site.**

	Patients with STI Diagnoses						Type of STI Diagnosis <sup>1</sup>						
	Total		Diagnosis due to Routine Screening		STI at Any Previous Visit		Rectal		Urethral		Syphilis		
	n	%	n	% of STI	% of sample	n	% of STI	n	% of STI	n	% of STI		
6M Pre-PrEP (n = 280)	35	13%	N/A	--	--	N/A	--	25	71%	15	43%	2	6%
Prescription Visit (n = 280)	31	11%	31	100%	11%	8	26%	25	81%	5	16%	3	10%
3M Follow-up (n = 225)	30	13%	23	77%	10%	10	33%	23	77%	8	27%	3	10%
6M Follow-up (n = 196)	41	21%	34	83%	17%	20	48%	29	71%	13	32%	4	10%
9M Follow-up (n = 169)	25	15%	17	68%	10%	21	84%	25	100.0%	6	24%	0	0%
12M Follow-up (n = 128)	17	13%	13	77%	10%	13	77%	13	77%	5	29%	2	12%

<sup>1</sup>Diagnoses do not sum to patient N per time period, because some patients were diagnosed with multiple STIs.

**870 Quarterly STI Screening Optimizes STI Detection Among PrEP Users in the Demo Project**

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**Background:** Pre-exposure prophylaxis (PrEP) is a highly effective HIV prevention tool but does not protect against sexually transmitted infections (STIs). The US CDC recommends that men who have sex with men (MSM) on PrEP be screened for STIs every 6 months. Among a cohort of participants in a PrEP demonstration project, we assessed: 1) the number and percent of gonorrhea (GC) and chlamydia (CT) infections that would have been missed if extra-genital screening had not been conducted and 2) The number and percent of participants infected with GC, CT or syphilis for whom treatment would have been delayed without quarterly screening.

**Methods:** MSM and transgender women participating in an open-label PrEP demonstration project were tested for syphilis and urethral (U), pharyngeal (P) and rectal (R) GC and CT at screening and at weeks 12, 24, 36 and 48, and treated promptly if positive. Participants were considered asymptomatic if they denied STI symptoms on a structured review of symptoms and did not have any signs on physical examination. To determine the number of infections that would have been missed without extra-genital screening, we calculated the number and proportion of R and P GC and CT infections in which there was not a concurrent U GC or CT infection. To determine the number of participants with GC, CT or syphilis

for whom treatment would have been delayed without quarterly screening, we tabulated the number of participants diagnosed with GC, CT or syphilis at weeks 12 and 36 who were asymptomatic at all potential sites of infection.

**Results:** Between October 2012 and January 2014, 557 participants were enrolled. Overall, 50.9% of participants were diagnosed with  $\geq 1$  STI during follow-up. The number of GC, CT and early syphilis infections by visit week, anatomic site (for GC and CT) and symptom status are shown in the Table. A total of 150 (82.9%) GC infections and 159 (75.7%) CT infections would have been missed if extra-genital screening had not been conducted. If screening had been conducted only semi-annually or based on symptoms, 62 (34.3%) of participants with GC, 86 (41.0%) of participants with CT and 11 (20.4%) of participants with syphilis would have been missed, extending the period of infectivity by up to 3 months/case.

**Conclusions:** Quarterly STI screening, including testing at extra-genital sites, significantly increases detection of GC, CT and syphilis, providing opportunities for prompt treatment, partner therapy, and prevention of STI related morbidity.

**Table. Gonorrhea, Chlamydia and Syphilis infections by visit week, anatomic site, and symptom status**

Type of STI, by site	Visit Week								Total
	12		24		36		48		
	# Infxn; n/N	# Asx; N (%)	# Infxn; n/N	# Asx; N (%)	# Infxn; n/N	# Asx; N (%)	# Infxn; n/N	# Asx; N (%)	
<b>Gonorrhea</b>									
Any site	45/481	32 (71.1)	29/464	19 (65.5)	45/434	30 (66.7)	62/417	46 (74.2)	181
Rectal	21/478	17 (81)	14/456	11 (78.6)	24/426	19 (79.2)	31/406	28 (90.3)	90
Pharyngeal	33/481	29 (87.9)	17/463	14 (82.4)	28/432	24 (85.7)	38/415	33 (86.8)	116
Urethral	6/481	1 (16.7)	5/464	1 (20.4)	9/434	2 (22.2)	11/416	1 (9.1)	31
<b>Chlamydia</b>									
Any site	62/481	50 (80.7)	49/464	42 (85.7)	41/434	36 (87.8)	58/417	46 (79.3)	210
Rectal	51/480	47 (92.2)	34/457	31 (91.2)	33/427	30 (90.9)	49/406	43 (87.8)	167
Pharyngeal	7/481	6 (85.7)	7/463	6 (85.7)	9/432	8 (88.9)	7/415	5 (71.4)	30
Urethral	13/481	6 (46.2)	12/464	8 (66.7)	13/433	12 (92.3)	13/416	8 (61.5)	51
<b>Syphilis</b>	15/480	7 (46.7)	14/463	7 (50.0)	10/432	4 (40.0)	15/418	9 (60.0)	54

Asx = Asymptomatic  
Infxn = Infections

**871 Phase I Trial to Assess Safety, PK, and PD of Film and Gel Formulations of Tenofovir**

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**Background:** Fast dissolving vaginal film formulations of topical microbicides may provide more efficient vaginal drug delivery than gels because films dissolve directly into vaginal fluid. In this first in human Phase 1 study of tenofovir vaginal films, the safety, PK, and PD of gel and two doses of film formulations of tenofovir were compared to matched placebo.

**Methods:** 75 healthy HIV negative women (mean age 27.9, 73% white) were randomized to 1 of 5 arms: 1) 4 mL of daily tenofovir 1% gel containing 40mg of tenofovir, 2) 4 mL of placebo gel, 3) 10 mg tenofovir film, 4) 40 mg tenofovir film or 5) placebo film used daily for 7 days. All films were 2 X 2 inches. Adverse event (AE) data were collected via questionnaire and physical exam at follow-up visits on day 7 and 30. Grade 2 and higher AEs deemed related to study product were compared across arms using Fisher's exact test. Plasma tenofovir was measured before and 2 hours after the last product use. Two hours after final product use, two sets of cervical biopsies were obtained, of which one set was assessed for tissue tenofovir-diphosphate levels and the other exposed to HIV-1 in an *ex-vivo* challenge assay. Tissue HIV infection was monitored by p24 levels in culture supernatant.

**Results:** There was 1 Grade 2 related AE for vaginal pain in the tenofovir gel group and no difference in the rate of urogenital complaints between groups. After 6 daily doses, women in the 40 mg film group had 4-fold higher concentrations of tenofovir in plasma compared to either the 10 mg film or 40 mg gel group (1.9, 0.4, and 0.5 ng/mL respectively), suggesting that women using the 40 mg film had higher plasma trough levels. Two hours after the 7th dose, median concentrations in plasma were comparable in the 40 mg film and gel groups (2.6 and 2.3 ng/mL) while the 10 mg film group was lower (1.0 ng/mL). Cervical tissue biopsies obtained from women after 7 days of tenofovir film or gel use had significantly lower HIV infectivity than placebo treated women. As shown in Figure 1, there was a significant PK/PD correlation in cervical tissue with higher tenofovir-diphosphate concentrations correlated with lower HIV replication (Figure 1).

**Conclusions:** This study demonstrates that vaginal films can efficiently and safely deliver tenofovir to the cervicovaginal tissues, achieving plasma levels higher than those of gels containing identical doses of drug. All active products were protective against HIV-1 infection in the *ex-vivo* challenge model using cervical tissue.

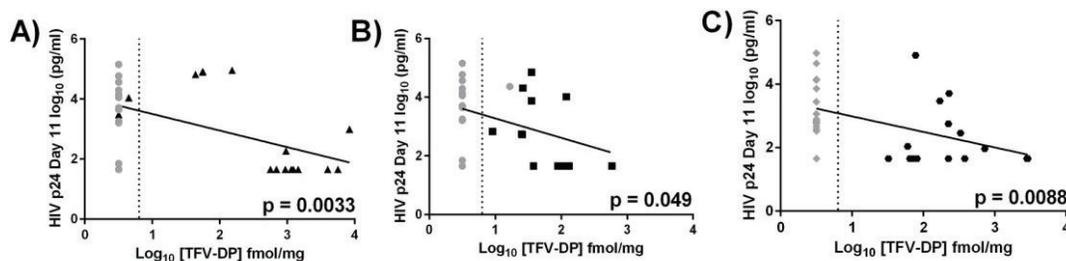


Figure 1. Higher tenofovir-diphosphate (TFV-DP) concentrations in cervical tissue correlate with significantly lower HIV-1 replication. A) 40 mg TFV film (grey circle; placebo film, black triangle; 40 mg TFV film), B) 10 mg TFV film (grey circle; placebo film, black square; 10 mg TFV film), and C) TFV gel (grey diamond; placebo gel, black circle; TFVgel).

**872 Safety and Pharmacokinetics of Dapivirine Vaginal Rings in Postmenopausal US Women**

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**Background:** Microbicide studies have not been conducted among postmenopausal women although this age group may be at higher risk for HIV than reproductive-age women due to vaginal atrophy and reduced innate antiviral activity. This study evaluated the safety and pharmacokinetics of a vaginal ring (VR) containing dapivirine (DPV), an NNRTI, compared to placebo in postmenopausal women.

**Methods:** We enrolled 96 HIV-negative postmenopausal U.S. women in a Phase 2a multi-site, double-blind, randomized (3:1) trial to evaluate monthly VRs containing 25 mg DPV or placebo used continuously for 12 weeks. Menopause was defined as amenorrhea for  $\geq 12$  months, or  $\geq 6$  months status post-bilateral oophorectomy, and a follicle-stimulating hormone level  $\geq 40$  mIU/mL. Safety was assessed by adverse events (AE). DPV was quantified in plasma for all women and in vaginal fluid (VF) for 47 women. Cervical biopsies were obtained in 10 women after DPV VR removal at week 12. Steady-state DPV concentrations at 4, 8, and 12 weeks were analyzed using repeated measures ANOVA. Used rings were analyzed for residual DPV levels.

**Results:** Mean age was 56.8 years (range 46-65); 66% were white, 31% were black, and 3% were of other race. Retention was 97%. There was no difference in the number of women with related Grade 2 or higher reproductive system AEs in the DPV vs placebo arms (6/72 (8%) vs 3/24 (13%),  $p=.68$ ), and no difference in Grade 3 or higher AEs in the DPV vs placebo arms (4/72 (6%) vs 0/24 (0%),  $p=.57$ ). One grade 3 AE, vaginal pain, was deemed related to study product. There were 6 protocol-required product holds for 5 women, all due to AEs which resolved; 2 women in the DPV arm declined to restart product. Median DPV concentrations in plasma and VF showed no change over 12 weeks. DPV was detectable in cervical tissue in only 5/10 women though median biopsy weights were 36% lower in women with undetectable levels. The median residual drug level for returned VRs across all visits was 21.1 mg, consistent with adherence to VR use.

**Conclusions:** DPV VRs were safe and well tolerated in postmenopausal women; only 2/96 women chose not to continue VR use due to AEs. Plasma and VF DPV concentrations remained constant over 12 weeks of use. Compared to published data on DPV VR use in reproductive-age women that found mean plasma DPV levels of 217.5 pg/mL, plasma DPV levels were not lower in postmenopausal women. Further studies are needed to assess biological differences in the postmenopausal genital tract.

Plasma DPV (pg/mL)		
	Median (IQR)	Mean (95% CI)
Week 4 (n=69)	268.00 (213.00, 325.00)	273.50 (249.91, 297.09)
Week 8 (n=70)	287.50 (217.50, 323.80)	289.00 (259.49, 318.50)
Week 12 (n=69)	262.00 (227.00, 351.00)	298.20 (263.85, 332.64)
Vaginal fluid DPV (ng/mg)		
	Median (IQR)	Mean (95% CI)
Week 4 (n=33)	33.69 (26.46, 60.80)	64.30 (40.94, 87.66)
Week 8 (n=34)	45.17 (32.41, 75.95)	78.51 (46.35, 110.67)
Week 12 (n=33)	40.58 (21.90, 81.49)	72.13 (43.75, 100.51)

**873 Adherence and Acceptability of a Dapivirine Vaginal Ring in Postmenopausal US Women**

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**Background:** Microbicide vaginal rings (VR) provide sustained release of the NNRTI dapivirine (DPV). In a Phase 2a trial, we evaluated the adherence and acceptability of DPV VRs among postmenopausal U.S. women, a population with high biological, behavioral and social risks, in which 12% of new HIV infections occur.

**Methods:** We enrolled 96 HIV-uninfected postmenopausal women in MTN-024/IPM 03, a 2-arm, double-blinded, multi-site, randomized trial (3:1) of a monthly silicone VR containing 25 mg DPV or placebo, used continuously for 12 weeks. Adherence was assessed by case reports and computer-assisted self-interviewing (CASI) at monthly follow ups; and acceptability by CASI at the final visit, and by in-depth-interviews (IDIs) in a random subset (n=24). Analysis was blinded and behavioral data were combined across Study Groups.

**Results:** Mean age was 56.8 years (range 46-65); 61% had a main partner, and 66% were currently sexually active. Study retention was 97%; 73% reportedly had the ring in place during the entire 12 weeks of use; 91% never had the ring out for more than 12 hours. Ever reporting the VR out decreased from 17% (week 4) to 5% (week 12). Six women reported full expulsions and 26 partial slippage, primarily due to bowel movements; 18 reported removals due to physical discomfort, worries, or to clean the VR. Most (99%) said the VR was very easy/easy to use; 96% indicated it never interfered with daily activities, 91% very much liked/liked the VR, 83% were never worried about it, and 65% preferred VR to condoms while 24% liked both equally. Thirty six percent reported vaginal changes with the VR, including wetness (21%) or dryness (10%). Of those sexually active, 49% did not feel the VR during sex, 82% said it did not change her sexual pleasure and 10% said her pleasure increased. Only 2 disliked wearing the VR during sex because their partners had sexual dysfunctions. During IDIs, women typically said the ring was empowering, "super-easy" to use, and preferred over condoms, as VR do not break, impact male performance, or interrupt sex. Side effects like vaginal wetness were perceived as beneficial and none had complaints about the VR interfering with other postmenopausal bodily changes. A few women had challenges with VR insertions and removals and needed staff assistance, for example due to obesity.

**Conclusions:** Participants reported high adherence, found VRs acceptable and preferred it to condoms. VR are a promising microbicide approach for HIV prevention in postmenopausal women.

**874 Distinct Pharmacodynamic Activity of Rilpivirine in Mucosal Explant Tissue**

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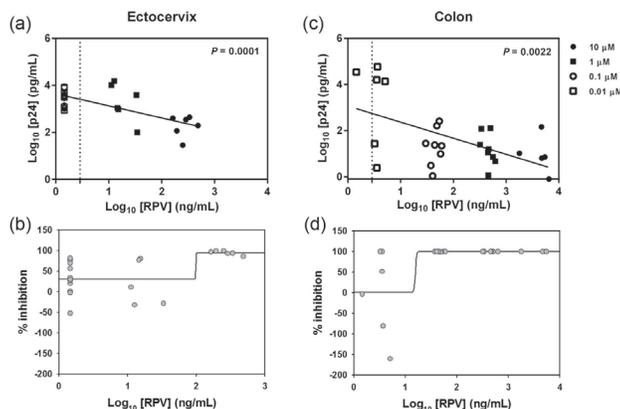
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**Background:** A long-acting injectable form of rilpivirine (RPV) is being evaluated in clinical trials for HIV prevention. Preclinical testing was done to define pharmacokinetic (PK) and pharmacodynamic (PD) activity of RPV in ectocervical and colonic tissues treated *in vitro* and to help inform PK data obtained from clinical trials.

**Methods:** *In vitro* 99% effective dose (ED<sub>99</sub>) and cytotoxic dose (CD<sub>99</sub>) of RPV against HIV-1<sub>bat</sub> was defined using a TZM-bl assay. RPV was evaluated for potency using polarized ectocervical and colonic explant tissues. Ten-fold dilutions of RPV, starting at 100  $\mu$ M, were applied either to the apical tissue surface with HIV or in the basolateral medium, 24 hours prior to HIV being applied to the apical tissue surface. Supernatants were collected over the culture period and assessed for HIV replication using a p24 ELISA. RPV was quantified in mucosal tissue using a validated liquid chromatography-mass spectrometry assay. PK/PD correlations were determined using GraphPad Prism. Non-linear Emax model with variable slope was used to evaluate concentration-response relationships using SigmaPlot.

**Results:** TZM-bl assay results showed RPV has an ED<sub>99</sub> of 8.27 nM and a CD<sub>99</sub> of 492 nM against HIV-1<sub>Bal</sub>. When applied to the apical surface at the same time as HIV-1<sub>Bal</sub>, 100 μM of RPV added to the cultures was needed to fully inhibit HIV infection in ectocervical tissue, while 10 μM was needed to inhibit HIV infection in colonic tissue. RPV added to the basolateral medium was more effective with 10 μM and 1 μM protecting ectocervical and colonic tissues, respectively. Improved activity was likely due to a longer pre-incubation with basolateral drug. To better estimate the amount needed for protection, RPV was quantified from ectocervical and colonic explant tissues treated basolaterally and significant inverse linear correlations (P < 0.001) with culture p24 were obtained. An Emax model showed RPV concentrations of >273 nM in ectocervical and >27.3 nM in colonic tissues were associated with HIV inhibition.

**Conclusions:** Our data show that RPV can suppress HIV infection in mucosal tissue, but higher levels of RPV are needed in female genital tissue than gastrointestinal tissue for protection. Based on our findings, rectal tissue RPV concentrations reached in clinical trials appears to be sufficient to block HIV infection, but at least 2-fold more drug is needed in female genital tissue to demonstrate similar inhibition. These data suggest targeted use of RPV for HIV prevention may be warranted



**875 A First-in-Human Trial of PC-1005 (MIV-150 and Zinc Acetate in a Carrageenan Gel)**

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**Background:** Pre-clinical data show PC-1005 to be potent against HIV, HSV-2 and HPV when applied vaginally or rectally. Primary objectives of this study were to assess the safety and pharmacokinetics (PK) of PC-1005 (comprised of the NNRTI MIV-150 and zinc acetate [ZA] in a carrageenan [CG] gel). Acceptability, adherence, and pharmacodynamics were also explored.

**Methods:** 20 healthy, abstinent women participated in a placebo-controlled, double-blind Phase 1 trial (RCT) at 1 US site, and were randomized (4:1) to apply 4 mL of PC-1005 or placebo vaginally once daily for 14d. The RCT was preceded by an open label safety run-in (OL) with 5 women applying PC-1005 once daily for 3d. Assessments included physical and pelvic exams, PK blood draws, safety labs, and colposcopy (with biopsies and cervicovaginal lavages [CVL] in the RCT). MIV-150 (plasma, CVL, tissue), zinc (plasma, CVL), and CG (CVL) concentrations were measured with LCMS-MS, ICP-MS, and ELISA, respectively. Antiviral activity against HIV, HSV-2 and HPV in CVL was measured using cell-based assays. Adherence and acceptability were assessed quantitatively via paper-based questionnaires. Safety, acceptability and adherence data were analyzed descriptively. PK parameters were calculated using non-compartmental techniques and actual sampling times. EC<sub>50</sub> values for CVL antiviral activity were calculated using a dose-response inhibition analysis.

**Results:** All 5 participants completed the OL period and applied 3/3 doses. 17 participants completed the RCT, 2 were lost to follow up (1 never dosed), 1 withdrew before dosing, and 16 applied ≥ 93% of doses. Participants were 30 years old, on average (range 19-44) and 52% were Black or African American. Acceptability was high; 94% of participants reported willingness to use the gel in the future. AE rates, most of which were mild and/or unrelated, were similar in both gel groups. MIV-150 was absorbed systemically at low levels without accumulation (Table 1). Plasma zinc concentrations were unchanged from baseline. 7/7 CVLs collected 4h post-dose demonstrated measurable anti-HIV and anti-HPV activity. High antiviral activity in baseline CVLs precluded assessment of anti-HSV-2 activity in cell-based assays.

**Conclusions:** PC-1005 gel used vaginally for 14d was well-tolerated, with low systemic absorption of MIV-150, and measurable HIV and HPV antiviral activity in CVL. These results warrant continued development of PC-1005 as a viable vaginal or rectal microbicide for prevention of HIV and other STIs.

**Table 1 Summary of MIV-150 Plasma Pharmacokinetic Parameters**

Parameter Statistics	Open Label (OL) Period		Randomized (RCT) Period		
	Day 1 (n=5)	Day 3 (n=5)	Day 1 <sup>a</sup> (n=14)	Day 8 <sup>b</sup> (n=14)	Day 14 <sup>b</sup> (n=6)
<b>C<sub>max</sub> (pg/mL)</b>					
Mean (CV%)	114 (34.7)	84.7 (48.1)	113 (37.7)	75.7 (38.8)	75.5 (23.1)
<b>T<sub>max</sub> (h)</b>					
Median (min - max)	3.92 (2.02-5.97)	3.92 (1.97-6.00)	2.95 (1.95-5.93)	3.98 (1.95-11.9)	4.96 (2.98-6.05)
<b>C<sub>min</sub> (pg/mL)</b>					
Mean (CV%)	ND	3.52 (52.7)	ND	13.3 (79.2)	9.70 (88.7)
<b>AUC<sub>0-24h</sub> (pg-h/mL)</b>					
Mean (CV%)	885 (25.9)	834 (25.0)	839 (36.7)	601 (37.0)	826 (38.9)
<b>AUC<sub>0-4h</sub> (pg-h/mL)</b>					
Mean (CV%)	906 (25.6)	ND	1173 (28.8)	ND	ND
<b>AUC<sub>0-8h</sub> (pg-h/mL)</b>					
Mean (CV%)	885 (25.9)	834 (25.0)	1126 (26.6)	847.3 <sup>c</sup>	827 (38.9)
<b>AUC<sub>0-12</sub> (pg-h/mL)</b>					
Mean (CV%)	753 (28.2)	646 (27.2)	818 (36.8)	601 (37.0)	586 (39.2)
<b>T<sub>1/2</sub> (h)</b>					
Mean (CV%)	3.89 (22.5)	4.82 (25.2)	4.44 (30.3)	4.20 <sup>c</sup>	5.51 (23.9)
<b>Cl/F (L/h)</b>					
Mean (CV%)	81.3 (22.5)	88.3 (22.9)	62.7 (34.8)	86.9 <sup>c</sup>	89.1 (35.9)
<b>R<sub>AUC</sub></b>					
Mean (CV%)	ND	0.942 (19.4)	ND	ND	0.837 (18.3)
<b>R<sub>AUC0-12</sub></b>					
Mean (CV%)	ND	0.858 (14.8)	ND	0.753 (26.4)	0.791 (34.3)
<b>R<sub>C<sub>min</sub></sub></b>					
Mean (CV%)	ND	0.743 (16.4)	ND	0.702 (23.2)	0.648 (37.4)

Note: Mean values are geometric means. <sup>a</sup>Includes participants randomized to Time 1 and Time 2 on Day 14. <sup>b</sup>Only includes participants randomized to Time 2 on Day 14. <sup>c</sup>Data is from 1 participant.

**876 CVLs From Women Vaginally Dosed With PC-1005 Inhibit Mucosal HIV-1 and HSV-2 Ex Vivo**

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**Background:** A recent Phase 1 trial demonstrated that PC-1005 gel containing 50µM MIV-150, 14mM zinc acetate dihydrate and carrageenan (CG) applied daily vaginally for up to 14 days is safe and well tolerated. Additionally, cervico-vaginal lavage samples (CVLs) collected 4h or 24h after last gel application showed MIV-150 and CG dose-dependent inhibition of HIV-1 and HPV in cell-based assays (Friedland et al, in preparation). We aimed to determine anti-HIV and anti-HSV-2 activity of CVLs in human ectocervical explants *ex vivo*.

**Methods:** CVLs collected at the baseline and 4h (n=6 PC-1005 and n=2 hydroxycellulose (HEC) placebo) or 24h (n=6 PC-1005 and n=1 HEC placebo) post last vaginal gel administration during Phase 1 trial were utilized. CG and MIV-150 concentrations in CVLs were measured by ELISA and LC-MS/MS, respectively. Human ectocervical explants were prepared from tissues received from National Disease Research Interchange. Explants (n=3 per condition) were incubated with CVLs (1:1) for 4h, washed with PBS and then challenged with 500 TCID<sub>50</sub> HIV-1<sub>Bal</sub> or co-challenged with 500 TCID<sub>50</sub> HIV-1<sub>Bal</sub> and 10<sup>6</sup> pfu HSV-2 per explant for ~18h, washed and then cultured for 14d. The activity of each baseline and corresponding post-gel exposure CVL was tested in tissues from the same donor. To determine contribution of CG to anti-HIV activity of CVLs, tissues were challenged with HIV-1<sub>Bal</sub> after exposure to baseline CVLs spiked with CG concentrations detected in the study (n=3 experiments). Infections were monitored by one step HIV *gag* RT-qPCR and HSV-2 *pol* qPCR on culture supernatants (individual replicate analysis). SOFT and CUM endpoint analyses were performed. Tissue viability post exposure to CVLs was tested using MTT assay. Log-normal generalized linear mixed models were used for statistical analysis.

**Results:** MIV-150 and CG in CVLs inhibited HIV and HSV-2 infection (single HIV challenge or co-challenge with HSV-2) in the explants in a dose-dependent manner (p≤0.01), with stronger inhibition using CVLs collected 4h post last gel administration. CG concentrations (>250 µg/ml; n=4 CVLs) could have contributed to the observed anti-HIV activity based on >55% HIV-1<sub>Bal</sub> infection inhibition in the CG spiking experiments.

**Conclusions:** The anti-HIV and anti-HSV-2 activity of CVLs in human ectocervical explants warrants the further development of PC-1005 gel as a broad-spectrum on demand microbicide.

**877 Transport of Drug and Virus in FRTs of Macaques Treated With a TDF Intravaginal Ring**

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**Background:** Prevention of HIV transmission using a tenofovir disoproxil fumarate (TDF)-eluting intravaginal ring (IVR) is a complex and poorly understood interplay between transport of drug and virus throughout and within the tissues of the female reproductive tract (FRT). Sufficiently high drug levels must be achieved both throughout and within the tissues of the entire FRT to prevent viral infection. This work demonstrates that not only is virus capable of infecting the ovaries, but that an IVR is also sometimes capable of achieving low drug concentrations there as well, highlighting the complexity of transport within the FRT and the need for further experiments and modeling.

**Methods:** Three pigtail macaques were treated with TDF-IVRs for 28 days, and vaginally challenged with a high dose of a replicative SIVmac239 virus and a single round non-replicative SIV-based vector expressing Luciferase and mCherry reporter genes. In order to identify infection events the isolated FRT was treated with luciferin to detect Luciferase activity using IVIS. TFV and TFV-DP concentrations in tissue were quantified using LC-MS/MS, with <sup>13</sup>C-labeled TFV used as an internal standard.

**Results:** Infection events were detected in both ovaries in two animals and in one ovary of the third macaque using IVIS. TFV levels were found to be variable throughout the FRT, with the highest concentrations found in the upper vagina/lower cervical area, near the site of the ring. The concentration of TFV in the FRT of the macaques were in the range of 40-28,000 fmol/mg of tissue and the concentration of TFV-DP in the FRT of the macaques were in the range of 3-6000 fmol/mg of tissue.

**Conclusions:** Infection events were seen in the ovaries of all three macaques, possibly reflecting the variability of ovarian drug levels (range of 7-30 fmol TFV-DP/mg of tissue) and virus transport.

**878 Impact of STI on Pharmacokinetics of Topical Tenofovir in Macaques**

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CDC, Atlanta, GA, USA

**Background:** Genital tract inflammation associated with sexually transmitted infections (STIs) can increase HIV risk and potentially reduce the efficacy of topically delivered tenofovir (TFV). We previously showed that co-infection of macaques with *Chlamydia trachomatis* (CT) and *Trichomonas vaginalis* (TV) increases SHIV infection risk by 2.5-fold but has little effect on the efficacy of a 1% TFV gel applied vaginally 30 minutes (100% efficacy) or 3 days (64% efficacy) before SHIV exposure. Here, we investigate the effect of CT/TV co-infection on systemic drug absorption from 1% TFV gel and on TFV diphosphate (TFV-DP) concentrations in vaginal lymphocytes.

**Methods:** Pigtailed macaques with regular menstrual cycles were co-infected with CT/TV and treated with 1% TFV gel vaginally once (n=6) or twice (n=6) per week for up to 2 complete menstrual cycles (10 weeks). Plasma TFV levels were measured 30 minutes after gel application and were compared to those seen in STI-naïve macaques that received the same gel formulation under the same dosing conditions. Cumulative TFV plasma exposure over a 28-day cycle was expressed as area under the curve 28 day (AUC<sub>28d</sub>). Intracellular TFV-DP concentrations were quantified in vaginal biopsies collected from 2 STI-infected and 2 uninfected macaques 2h after gel application. We also measured TFV-DP levels in vaginal lymphocytes collected 3 days after gel dosing in 4 STI-infected and 4 uninfected macaques.

**Results:** Co-infection with CT/TV increased plasma TFV concentrations over all phases of the menstrual cycle with AUC<sub>28d</sub> values that were ~2.7 times as high in STI-infected animals compared to uninfected macaques (1,877 and 685 ng\*d/ml, respectively; p = 0.02 Wilcoxon rank test). Median TFV-DP levels in vaginal biopsies collected 2 h after dosing were also higher in STI-infected [1,733 fmol/mg (range 152-8,277)] compared to uninfected [162 fmol/mg (range 43-648)] animals. Likewise, TFV-DP levels measured in vaginal lymphocytes at 3 days were higher in STI-infected [1,093 fmol/10<sup>6</sup> cells, range = 12 - 2,336] than in uninfected macaques [158 fmol/10<sup>6</sup> cells, range = 27-259].

**Conclusions:** We demonstrate increased vaginal absorption of TFV in the setting of STI co-infections, which may reflect increased tissue drug permeability. Both the modest 2.5-fold increase in susceptibility to SHIV by STIs and higher TFV-DP concentrations in vaginal tissue and lymphocytes likely explain the protection seen in macaques co-infected with STIs.

**879 A Long-Acting Biodegradable Subcutaneous Implant for Tenofovir HIV PrEP**

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**Background:** Antiretroviral (ARV) effectiveness for HIV pre-exposure prophylaxis (PrEP) is proven, but hinges on correct and consistent use. User compliance and therapeutic effectiveness can be improved by long acting drug delivery systems. Here we describe a thin-film polymer device (TFPD) as a biodegradable subcutaneous implant for PrEP. A thin-film polycaprolactone (PCL) membrane controls drug release from a reservoir, and release rates and device size are tunable, a key feature for an implant in the early stages of pre-clinical development. We have explored release of ARVs from various classes and will present data pertaining to development of a device to deliver Tenofovir Alafenimide Fumarate (TAF).

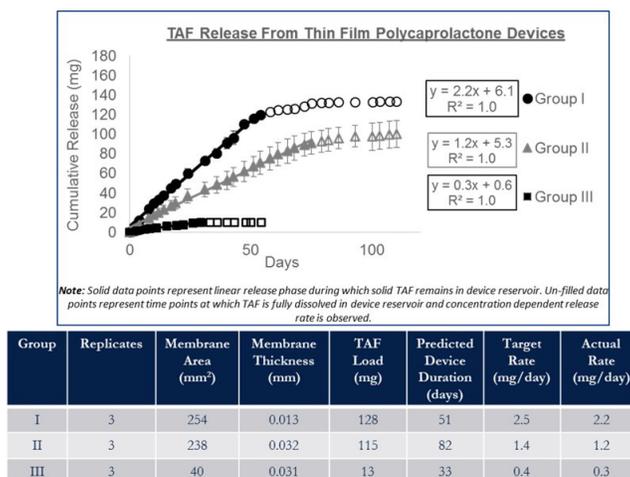
**Methods:** Devices were fabricated as a hollow rod with an open end using a wire-heat sealing apparatus and solvent cast PCL films. TAF was loaded into the reservoir with or without formulation excipients. Devices were sealed and incubated in PBS, pH7.4 at 37°C. For release rates we measured TAF concentration in release media over time. Sink

conditions were maintained by regularly replacing the media. Relationships between release rate and device parameters were evaluated using devices with 10-30 $\mu$ m thick membranes and 50-320 mm<sup>2</sup> surface areas. TAF chemical stability in the TFPD reservoir was evaluated by RP-HPLC.

**Results:** Based on published data for oral TAF, subcutaneous constant-rate release for HIV PrEP is estimated at < 2.8mg/day. To achieve membrane controlled release, TAF required additional formulation excipients such as PEG300 or hydroxypropyl- $\beta$ -cyclodextrin. The size and shape of the TFPD are tunable, achieving release rates ranging from 0.5-4.4 mg/day in devices no larger than a contraceptive implant.

A proportional relationship between membrane area and release rate was demonstrated. An inverse relationship between membrane thickness and release rate was observed for membranes between 10-15 $\mu$ m, with no further impact on release for membranes > 15 $\mu$ m. Prototype devices demonstrated linear release at 1.2mg/day for up to 90 days and at 2.2mg/day for up to 60 days. We achieved reproducibility in device design and performance with < 10% variability between replicate devices (Figure 1). TAF remained chemically stable in the device reservoir with < 3% change in purity at 50 days

**Conclusions:** We developed a biodegradable TFPD for subcutaneous delivery of TAF for HIV PrEP. The size, shape and release rate of the device are tunable over a > 8-fold range. This system is being further evaluated *in vivo*.



#### 880 Assessing Formulations of Tenofovir 1% Gel in HIV Seronegative Adults via RNA-Seq

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**Background:** Clinical trials employing rectal microbicides containing antiretroviral drugs have the goal of reducing risk of contracting HIV during sexual activity. The Combination HIV Antiretroviral Rectal Microbicide (CHARM)-01 study is a recent Phase 1, double-blinded, randomized, safety & acceptability, and pharmacokinetic study of rectally-applied tenofovir-based microbicides in healthy adults (aged 37.7 years  $\pm$  14.3) completed by the Microbicide Trials Network (MTN). With the hypothesis that gene expression changes in the local immune environment may hallmark the action of tenofovir and potentially alter risk of HIV infection, we used RNA-Seq to assess changes in the rectal mucosa caused by gel usage.

**Methods:** Three tenofovir 1% gel formulations were administered rectally: a vaginal formulation (VF, 3111 mOsmol/kg), a reduced-glycerin vaginal formulation (RGVF, 846 mOsmol/kg), and a rectal-specific formulation (RF, 479 mOsmol/kg). Participants received 4 mL of the gels: seven daily doses of RGVF, seven daily doses of RF, and six daily doses of placebo followed by one dose of VF, in a randomized sequence. We isolated total RNA from rectal biopsies from participants (n=14/group) and performed low input Illumina Truseq RNA-Seq on a HiSeq 2500 instrument. Top ranking differentially expressed genes by P value (P<0.05 in T or F tests) were forwarded to Gene Set Enrichment Analysis (GSEA) and Ingenuity Pathway Analysis.

**Results:** Multi Dimensional Scaling analysis of the top 500 genes by F-Test P value showed that RGVF had the most significantly different (P=0.001, Kruskal Wallis rank sum test) expression profile from baseline. RGVF increased T cell related proinflammatory responses (IFNG, IL12, CXCL9/10), complement (C2, CASPs), antiviral interferons (OAS1, IFIs/IFIs, MX1), and IL10. This signature was unique to RGVF, however a derivative signature of up- and down-regulated IFNG-response genes, SERPINS, and IL10 was also present in VF and RF. Bioinformatic deconvolution analysis showed that these signatures stemmed from general alterations in B cell and Monocyte/MDC gene activity.

**Conclusions:** Tenofovir may impact the inflammasome in the rectal mucosa and set a balance between antiviral IFNs and proinflammatory cytokine signaling. This balance may be delicate in determining HIV infection risk outcomes. The above biomarkers may help monitor and identify mechanisms and targets of protection or infection risk in future microbicide trials. Funding: MTN & NIH/NIAID/DAIDS IPCP Program (5U19AI082637).

#### 881 Understanding Pain and Anxiety Experienced Around Long-Acting Injectable PrEP

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**Background:** Long-acting injectable PrEP may improve adherence and acceptability compared to daily oral dosing. However acceptability may be limited by anxiety about injections and concerns about injection site reactions (ISR), especially pain. We interviewed a subset of participants of a Phase 2 trial of cabotegravir long-acting injection (CAB LA).

**Methods:** ÉCLAIR is a double-blind, randomized, multi-center study in 127 HIV-negative men at risk of HIV infection during which IM injections of 800mg CAB LA or PBO (saline) q12 weeks X 3 cycles were given. This substudy approached 48 participants who self-reported as MSM or male-to-female transgender women at 4 out of 10 sites and offered 30 randomly-selected individuals the opportunity to be interviewed. 2/28 who received placebo are excluded from results presented here. Interviewees included 27 MSM and one MTFT.

**Results:** 23/28 (82.1%) reported ISR with a mean pain score of 2.8 out of 5. Of those 23 subjects, 13 (56.5%) reported that pain lasted more than three days. In a subset of men who were asked more details about the timing of pain, 37.5% (6/16) reported no pain *during* injections and 31.3% (5/16) reported no pain *following* injection. 75% (21/28) stated the first injection hurt less than expected. Anxiety before the first injection was felt by 20/28 (64.3%), however this decreased to just 8/28 (28.6%) by subsequent injections. There was no correlation between anxiety and pain. Despite discomfort and anxiety cited by a large proportion, interest in the product remained high: out of the subset of participants queried, 93.8% (15/16) reported that if proven effective, they would definitely or very likely use CAB-LA. In addition, 62.5% (10/16) reported that they would prefer to use CAB-LA every twelve weeks to daily oral PrEP.

**Conclusions:** CAB-LA injections are acceptable in subset of ÉCLAIR participants who experienced two to three injections. The pain profile is less focused on the injection itself and more on the post-injection period. While there was significant anxiety around injections, this decreased as participants gained experience with injections. These findings suggest that patient education about CAB-LA should focus on informing patients about the true nature of pain associated with injections, and managing both expectations and anxiety. More research is required to validate these findings on a broader scale.

#### 882LB WITHDRAWN

#### 883LB HPTN 073: PrEP Uptake and Use by Black Men Who Have Sex With Men in 3 US Cities

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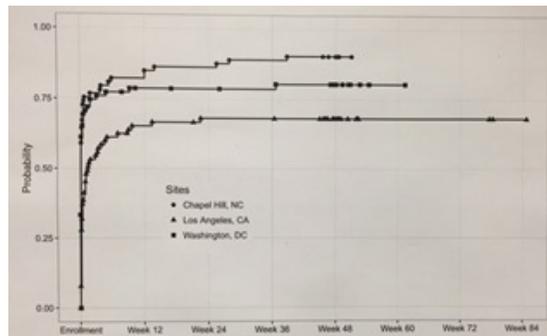
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**Background:** Among US subgroups, Black men who have sex with men (BMSM) remain at disproportionate risk of HIV acquisition. Comprising less than 0.4% of the U.S. population, they accounted for more than 20% of all new infections in 2013. Identifying effective and innovative methods to deliver effective prevention and halt the epidemic in this key population is an urgent public health priority. HPTN 073 is one of the first studies to evaluate pre-exposure prophylaxis (PrEP) in a US BMSM population.

**Methods:** HIV-uninfected BMSM were enrolled in three U.S. cities (Washington, DC., Los Angeles CA, & Chapel Hill, NC) All participants were offered once daily oral FTC/TDF combined with client-centered care coordination (C4)—a theory-based counseling approach to promote and support PrEP use, which combined service referral, linkage and follow-up strategies to assist participants in addressing unmet psychosocial needs. Each participant was offered PrEP and followed for a total of 12 months.

**Results:** 226 BMSM men were recruited; 209 (92%) completed 12 month of follow-up. 40% were <25 years, 27% were unemployed, 31% did not have health insurance. The median number of male partners in the prior 3 months was 3 (IQR 1-4), 33% reported a primary partner and 73% casual male partners. PrEP was accepted by 178 (79%) of study participants (see Figure); 68% remained on PrEP at 26 weeks. Self-reported adherence above 50% was 85% at 4 weeks and 78% at 26 weeks. 23/24 (96%) men reporting an HIV+ primary partner and 104/120 (86%) of men reporting casual partners with unknown or HIV+ status accepted PrEP. Those agreeing to take PrEP utilized a median of 6 C4 sessions (range 3–8) compared to men not accepting PrEP (median 4 range 2-6). Among the 178 men who ever accepted PrEP, 5 HIV infections occurred in 172 person years (PY) (incidence=2.9 95%CI(0.9-6.8)) compared to 3 in 39 PY (incidence=7.7 95%CI(1.6-22.5)) in men who never accepted PrEP. Of the 5 seroconverters who ever took PrEP, 2 had discontinued PrEP at 50 and 272 days prior to seroconversion.

**Conclusions:** Providing theory-based culturally tailored programs for BMSM can potentially increase their ability to establish and maintain adherence and prevent HIV in this highly impacted group. HPTN 073 demonstrated high uptake of PrEP in BMSM utilizing a novel coordinated counseling model that was highly acceptable, and led to data that could support a reduced rate of HIV-infection for BMSM on PrEP. These findings help address a vital US public health gap in HIV prevention.



#### 884 HPTN 067/ADAPT: Predictors of Coverage of Sex Events in PrEP Regimens, Thai MSM-TGW

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**Background:** We sought to identify baseline predictors of pre-exposure prophylaxis (PrEP) coverage of sex events in HPTN 067, a phase 2, open-label feasibility study of daily and non-daily regimens of FTC/TDF PrEP among Thai men who have sex with men (MSM), and transgender women (TGW), completed in Bangkok, 2012–2014.

**Methods:** We randomly assigned participants to one of three self-administered dosing regimens for 24 weeks: daily, time-driven twice weekly with a post-sex dose, or event-driven before and after sex. Bangkok was one of three study sites, one of which enrolled women. Alcohol (AUDIT score) and substance use were assessed by computer-assisted self-interview. Using sex act as the unit of analysis we defined coverage as taking  $\geq 1$  tablet in the four days before sex and  $\geq 1$  tablet within 24 hours after sex. We used general estimating equations for clustered data to evaluate predictors associated with coverage.

**Results:** Among 178 MSM and TGW the proportions of sex acts that were covered by PrEP were similar in the daily and time-driven arms (85% vs 84%,  $p=0.79$ ). Participants reported use of the following substances at baseline: stimulants (e.g. amphetamine type) (8.4%), marijuana (2.8%), opiates (1.7%), and other drugs (23%). AUDIT scores indicated moderate alcohol use in 20.8% and high alcohol use in 6.7%. Proportion of covered sex acts by those reporting stimulant use (coverage: 74%) was similar to those reporting other drug use (non-alcohol) (76%,  $p=0.80$ ), but lower than those reporting no substance use (85%,  $p=0.04$ ). In a multivariable model, age 25–35 years (adjusted odds ratio [aOR] 2.34, 95% CI 1.37–3.97,  $p=0.002$ ), completion of college (aOR 1.74, 95% CI 1.04–2.92,  $p=0.03$ ), along with moderate (AUDIT Score 7–12: aOR 1.75, 95% CI 1.15–2.66,  $p<0.01$ ) to high levels (AUDIT Score  $\geq 13$ : aOR 2.86, 95% CI 1.19–6.86,  $p=0.02$ ) of alcohol use, were associated with higher coverage. A higher number of reported sex events (13–80 over three months at baseline: aOR 0.57, 95% CI 0.32–1.00,  $p=0.05$ ) and use of stimulant drugs (aOR 0.47, 95% CI 0.24–0.93,  $p=0.03$ ) were significantly associated with lower coverage.

**Conclusions:** Higher coverage of sex acts with PrEP was associated with higher education, and higher AUDIT scores at baseline, after adjusting for age. Use of stimulants and higher sexual frequency in the past 3 months was associated with lower coverage. Regardless of substance use, the majority of Thai MSM/TGW followed the regimen dosing schedules to provide coverage of sexual exposures with PrEP.

**Table.** Univariate and multivariate analysis of factors associated with higher coverage of sex events, Bangkok HPTN 067/ADAPT study (N=178)

Characteristic	OR	p value	Adjusted OR*	95% CI	p value
<b>Age (years)</b>					
<25			Ref	--	-
25-35	1.68	0.03	2.34	1.37-3.97	0.002
>35	1.13	0.78	1.68	0.95-2.97	0.07
<b>Education</b>					
College completion	1.65	0.21	1.74	1.04-2.92	0.03
<b>Sex events/3 months</b>					
0-4			Ref	--	
5-12	0.66	0.11	0.65	0.35-1.21	0.18
13-80	0.55	0.04	0.57	0.32-1.00	0.05
<b>AUDIT score†</b>					
0-6			Ref	--	
7-12	1.49	0.07	1.75	1.15-2.66	<0.01
>13	1.31	0.47	2.86	1.19-6.86	0.02
<b>Reported drug use type</b>					
No drug use			Ref	--	
Stimulants with or without others	0.52	0.04	0.47	0.24-0.93	0.03
Other illicit drugs only	0.59	0.15	0.76	0.49-1.15	0.20

\* Characteristics considered in the model: study arm, age, employment, education, alcohol use (AUDIT), drug use type, depression score, number of sex events reported in the past 3 months, number of sex partners reported in the past 3 months, and reported condomless sex  
 † The Alcohol Use Disorders Identification Test (AUDIT) is a simple ten-question test developed by WHO to determine if a person may be at risk for alcohol abuse problems.

**885 HPTN 067-ADAPT: Factors Associated With PrEP Coverage Among New York City MSM and TGW**

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**Background:** New York City participants in the completed HPTN 067 study were more likely to have coverage of sex events (adherence to pre- and post-sex PrEP) when assigned to daily PrEP compared to non-daily regimens. Understanding factors associated with coverage can inform future PrEP support interventions.

**Methods:** Participants in this phase 2, open-label, randomized clinical trial were assigned 1:1:1 to 24 weeks of FTC/TDF PrEP dosed: daily (D), twice weekly plus a post-sex dose (time-driven [T]), or pre- and post-sex doses (event-driven [E]). Coverage of sex events (yes/no) was defined based on pre-clinical research as PrEP taken within 4 days pre- and 24 hours post-sex. Coverage was assessed via electronic monitoring adjusted by self-reported sexual and pill taking behavior collected in weekly interviews. Demographics were collected by staff, other data (including AUDIT to assess alcohol use and CES-D to assess depression) were collected via computer assisted self-interview (Table). Logistic regression for clustered data (clustering on participant) was used to estimate odds ratios and adjusted odds ratios. Characteristics significant at p<0.05 were retained in the final model.

**Results:** Participants (N=179) were: 98% men who have sex with men (MSM), 2% transgender women (TGW); median age 30 years; 60% black, 11% white, 24% Hispanic, 6% other. Arm D participants had significantly higher coverage of sex acts (66% D, 47% T, 52% E; p=0.03). In univariate analyses (Table), other factors associated with better coverage included older age, employment, higher education, and higher PrEP information, motivation, and behavioral skills. Factors significantly associated with lower coverage included heavy alcohol use, black race, depression, and opiate use. In multivariate analyses (Table), significant associations with better coverage included: arm D (aOR=0.49 95%CI 0.29-0.83 for arm T vs. D; aOR=0.62, 95%CI 0.38-1.01 for arm E vs. D), older age (aOR=1.03, 95%CI 1.01-1.05), employment (aOR=1.67, 95%CI 1.07-2.59), and higher PrEP motivation (aOR=1.03, 95%CI 1.00-1.05). Black race vs. white (aOR=0.49, 95%CI 0.25-0.97) or Hispanic (aOR=0.64, 95%CI 0.31-1.30), and heroin use (aOR=0.34, 95%CI 0.17-0.69) remained significantly associated with lower coverage.

**Conclusions:** This analysis identified factors that may require interventions to optimize adherence to PrEP, including younger age, unemployment, lack of motivation for PrEP, and heroin use. Further study is needed to assess determinants of racial differences.

Characteristic	OR	p value	Adjusted OR	95% CI	p value
Age	1.03	<0.01	1.03	1.01-1.05	0.001
Race/Ethnicity		0.08			0.02
White	Ref		Ref		
Black	0.35	0.01	0.49	0.25-0.97	0.04
Hispanic	0.47	0.10	0.64	0.31-1.30	0.22
Higher than high school education	2.09	0.002	NA		
Employment	2.39	0.006	1.67	1.07-2.59	0.03
Depression	0.54	<0.05	NA		
Hazardous/harmful alcohol use	0.49	0.006	NA		
Reported heroin use	0.45	0.06	0.34	0.17-0.69	0.005
Study Arm		0.04			0.03
Daily	Ref		Ref		
Time-driven	0.50	<0.05	0.49	0.29-0.83	0.008
Event-driven	0.53	0.01	0.62	0.38-1.01	0.05
PrEP Information	1.26	<0.001	NA		
PrEP Motivation	1.04	0.008	1.03	1.00-1.05	0.02
PrEP Behavioral Skills	1.04	0.004	NA		

**886 On Demand PrEP With Oral TDF-FTC in the Open-Label Phase of the ANRS IPERGAY Trial**

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**Background:** On demand PrEP with oral TDF-FTC has been shown to reduce the incidence of HIV-1 infection in high risk MSM in the ANRS IPERGAY trial from 6.60 per 100 participants-years (py) in the placebo arm to 0.91 per 100 py in the TDF-FTC arm. However, because the placebo arm was discontinued early, the cumulative follow-up time with TDF/FTC was only 219 py and the long term efficacy and safety of this strategy remains to be demonstrated.

**Methods:** High risk adult MSM who were followed or being screened in the ANRS IPERGAY trial at the time of the discontinuation of the placebo arm (November 2014) were offered to continue follow-up every two months with open-label TDF/FTC. The primary study objectives of this open-label phase were to assess study retention, HIV incidence, safety and changes in sexual behaviour.

**Results:** In November 2014, among the 400 pts initially enrolled in the study, 336 (84%) were eligible and all but 3 (99%) were enrolled in the open-label phase. Twenty-nine new pts were also enrolled in this open-label study. Overall, 362 pts were enrolled for a cumulative follow-up time of 248 py until September 14, 2015. Study retention was good with only 13 pts discontinuing follow-up (3.6%). During follow-up, only a single individual who had discontinued PrEP acquired HIV-1 infection. The incidence of HIV-1 infection in this open-label phase was 0.40 per 100 py (95%CI: 0.01-2.25). Pts used a mean of 18 pills/month (SD:8.7). Overall, 33% of pts acquired a new STI. There was no significant changes between the double-blinded phase and the open-label phase in the median number of sexual intercourses or sexual partners, but there was a significant decrease in condom use for receptive anal intercourse ( $p=0.01$ ). Safety was good with a low rate of serious adverse events (4%). One pt discontinued TDF-FTC because of an increase in serum creatinine level. Drug-related gastrointestinal adverse events (nausea, diarrhea, abdominal pain) were reported in 10% of pts.

**Conclusions:** Open-label on demand PrEP with oral TDF-FTC remains highly effective to prevent HIV-infection in high risk MSM and has a good safety profile.

#### 887 PrEP and Condom Use in High Risk MSM in the ANRS IPERGAY Trial

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**Background:** Men who have sex with men (MSM) constitute a high-risk group in France's HIV epidemic. In the setting of the follow-up in the ANRS IPERGAY PrEP trial, we aimed to identify behavioural trajectories over time for PrEP and condom use and to investigate links between these trajectories, using a tailored methodological framework.

**Methods:** ANRS IPERGAY was a randomized double-blind trial of sexual activity-based PrEP in high-risk MSM, assigned to TDF-FTC or placebo. Online questionnaires collecting sexual behaviour and PrEP adherence data during the most recent episode of sexual intercourse were completed every 2 months. Only MSM reporting anal sex were included in this analysis. A longitudinal multi-trajectory model was constructed for two outcomes: PrEP use (correct/sub-optimal versus no PrEP), and condom use for anal sex (yes/no). Multivariate analyses were performed on identified trajectories using sociodemographic, socioeconomic, and behavioural data.

**Results:** 332 participants (47.9% placebo; 52.1% PrEP) who had anal sex at least once during follow-up provided information on PrEP (1115 questionnaires) and/or condom (1935 questionnaires) use during the most recent episode of sexual intercourse. Four trajectories of PrEP use over time were identified among participants: "systematic users (PrEP-SU)" (39.5%), "high-level progressive users (PrEP-HLU)" (31.1%), "declining users (PrEP-DU)" (13.3%), and "low-level users (PrEP-LLU)" (16.1%). For condom use, common behaviour patterns identified two groups: "high-level users (C-HLU)" (29.9%) and "low-level users (C-LLU)" (70.1%). Joint trajectory modelling identified four groups: among C-HLU, 78% were PrEP-SU/PrEP-HLU, while 22% were PrEP-DU/PrEP-LLU. Among C-LLU, 68% were PrEP-SU/PrEP-HLU, whereas 32% were PrEP-DU/PrEP-LLU. The latter group (most-at-risk group), compared most protected group (C-HLU/PrEP-SU) included more frequently older participants (OR=1.05 per each additional year,  $p<0.001$ ) with low-education levels (OR=1.91,  $p=0.02$ , ref.  $\geq$  high school), participants with unknown partners (OR=2.82,  $p<0.001$ ), those sexually dissatisfied (OR=2.09,  $p<0.001$ ), and a trend appears for those practicing active anal sex (vs. receptive,  $p=0.08$ ).

**Conclusions:** In the ANRS IPERGAY trial, most high-risk MSM used either PrEP and/or condoms during the most recent anal intercourse. Special attention must be paid to MSM with low condom use who do not compensate by using PrEP, representing 22% among the 332 participants included in this analysis.

#### 888 Increasing PrEP Use Among Men Who Have Sex With Men, New York City, 2013-2015

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**Background:** Pre-exposure prophylaxis (PrEP) is effective for HIV prevention, yet has been underutilized. Since 2012, the New York City (NYC) Department of Health and its partners have launched programs to support PrEP uptake. Using data from routine behavioral surveillance among men who have sex with men (MSM), we examined recent trends and associations with PrEP use.

**Methods:** Data were derived from annual surveys conducted in-person and online, 2013-2015. Eligible respondents were NYC residents, who were born male, aged 18-40, and reported anal sex with a man (past 6 months). This analysis excluded those who reported being diagnosed with HIV. PrEP use was defined as use in the past 6 months. Demographic factors examined included age (18-29/30-40 years), race/ethnicity (black/Hispanic/white/other), education (college degree) and insurance status. Behavioral factors were condomless sex or known HIV-positive partner at last sexual encounter and number of condomless partners (3 or more) and/or post-exposure prophylaxis (PEP) use in the past 6 months. Using logistic regression, we assessed associations between PrEP use and year, factors, and year-factor interaction terms. Those associated bivariately ( $p<0.05$ ) were added to a multivariate model with age, race/ethnicity, insurance, survey type (in-person/online) and year.

**Results:** Among 1595 respondents, the majority were aged 18-29 (63%), black (24%) or Hispanic (32%), educated (60% had a college degree or higher) and insured (83%). Report of PrEP use was 2.1%, 3.2% and 14.8% in 2013, 2014 and 2015; this increase was significant in the multivariate model ( $p<0.001$ ). Among demographic factors examined, only being insured was bivariately associated with PrEP use (OR 2.5, CI 1.1-5.5); it was not significant in the multivariate model. PrEP use was associated with condomless sex (adjusted odds ratio (aOR) 3.8, 95% confidence interval (CI) 2.4-6.1) and sex with a known HIV-positive partner (aOR 3.0, CI 1.3-6.8) at the last encounter; and  $\geq 3$  condomless partners (aOR 2.8, CI 1.8-4.4) and PEP use (aOR 26.9, CI 12.8-56.3) in the past 6 months. None of the associations with PrEP differed by year (interaction term  $p>0.05$ ).

**Conclusions:** Findings suggest PrEP use is increasing among MSM in NYC. Use appears to be greater among those with higher behavioral risk, consistent with recommendations. The association of use with insurance status underscores the importance of addressing financial barriers. Monitoring for disparities will be critical as PrEP use increases.

#### 889 Awareness and Use of PrEP Appear to Be Increasing Among Internet Samples of US MSM

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**Background:** There has been increasing traditional and social media coverage of pre-exposure prophylaxis using combination emtricitabine/tenofovir (FTC/TDF) to prevent HIV infection (PrEP). We sought to describe changes in awareness of PrEP, willingness to use PrEP, and use of PrEP among internet-using men who have sex with men (MSM) from the time before FDA-approval of FTC/TDF for PrEP in July 2012 through March 2015.

**Methods:** Data were from 3 nationwide cross-sectional internet surveys of MSM living in the United States. The analysis sample included men who reported sex with a man in the past 12 months and did not report being HIV-positive. Because the percentage reporting awareness of PrEP changed non-linearly over time within survey cycle, we aggregated data into groups of months that spanned cycles to perform a segmented regression assessing the time trend for percentages of participants reporting awareness of, willingness to use, and actual use of PrEP. Multivariate models controlled for source of recruitment (a geo-location based sexual networking site versus other websites), race, educational attainment, income and risk behaviors included as indicators for PrEP in CDC guidelines ( $\geq 10$  partners vs.  $\leq 9$ , and bacterial STI diagnosis in the prior 12 months.) For awareness and use of PrEP we used a Poisson risk model with 3 indicator variables for time (See Table). We used a log-binomial model of percentage willing to use PrEP because the Poisson model gave estimates of  $> 100\%$  willingness for some subgroups.

**Results:** The total sample included 10,097 MSM. Awareness, willingness to use and actual use increased significantly over time, with the significant increases in awareness over time and in use in the most recent time period, November 2014-March 2015, compared to May 2012-April 2014 (See Table). The percentage of MSM willing to use PrEP increased

from the earliest time point but plateaued after April 2014. Awareness of, willingness to use, and use of PrEP were higher among higher risk MSM, including those recruited from a geospatial sexual networking application, those reporting a bacterial STI, and those with ≥ 10 partners in the last 12 months (See Table).

**Conclusions:** Awareness and use of PrEP are increasing among internet-using MSM in the US. Though it is encouraging that more higher risk MSM are aware of, willing to use, and have used PrEP, there remains a large gap between the number of MSM who report being willing to use PrEP and those who have actually done so.

Table: Changing prevalence of awareness, willingness to use, and actual use of PrEP over time among US MSM participating in online surveys

Variable	Category	N (Total sample = 10,097)	Heard of Prep		Willing to use Prep		Used Prep in the past 12 months	
			Unadjusted Prevalence	Adjusted <sup>1</sup> Prevalence ratio (95% CI) <sup>2</sup>	Unadjusted Prevalence	Adjusted <sup>2</sup> Prevalence ratio (95% CI)	Unadjusted Prevalence	Adjusted <sup>1</sup> Prevalence ratio (95% CI)
Time Period	May 2012-April 2014	5289	44.7%	Ref <sup>3</sup>	39.2%	Ref	0.5%	Ref
	May 2014-October 2014	2804	59.0%	1.16 (1.10-1.23)	50.3%	1.13 (1.07-1.20)	2.4%	1.51 (0.78-2.96)
	November 2014-March 2015	2004	68.0%	1.33 (1.26-1.40)	49.5%	1.12 (1.06-1.19)	4.9%	2.90 (1.56-5.40)
Recruitment Site	Geo-Spatial Sexual Networking App	1644	71.0%	1.10 (1.05-1.15)	60.7%	1.17 (1.10-1.24)	6.6%	1.69 (1.22-2.32)
	Other	8453	50.4%	Ref	41.0%	Ref	1.2%	Ref
Bacterial STI in last 12 months	Yes	661	71.4%	1.19 (1.12-1.28)	65.5%	1.21 (1.14-1.28)	9.8%	2.8 (2.05-3.83)
	No	9436	52.8%	Ref	42.8%	Ref	1.5%	Ref
≥ 10 sex partners in last 12 months	Yes	2036	69.2%	1.21 (1.16-1.27)	60.8%	1.34 (1.28-1.41)	6.7%	3.2 (2.23-4.60)
	No	8034	50.1%	Ref	40.3%	Ref	0.9%	Ref

1. Estimates from a Poisson risk model adjusted for race, educational attainment, income and covariates listed in the table.  
 2. Estimates from a log-binomial risk model adjusted for race, educational attainment, income and covariates listed in the table.  
 3. CI= Confidence interval; Ref=Reference category for prevalence ratio comparison.

**890 Increasing HIV Suppression, PrEP Use, and STDs in Boston MSM Accessing Primary Care**

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**Background:** Recent studies suggest that early HAART initiation and PrEP could decrease HIV incidence, but the measurement of impact in primary care settings has been limited.

**Methods:** Fenway Health (FH), the largest 1<sup>o</sup> care center for men who have sex with men (MSM) in Massachusetts (MA) has used an electronic medical record since 1997 (Centricity™), facilitating analyses of secular trends related to HIV census, HAART use, PrEP initiation and incident STDs. Time trend analyses were performed using Spearman's rank test for correlation and pairwise comparisons were made with Fisher's exact chi-square test.

**Results:** Between 2004 and 2014, HIV+ pts in 1<sup>o</sup> care at FH increased from 1083 to 2101 (p<0.001). In 2004, 922 HIV+ pts were newly diagnosed in MA, compared to 698 in 2013 (p<0.001), and the % MA HIV+ diagnosed at FH increased from 6% to 14%. At FH, 58 pts initiated HAART in 2004 and 121 in 2014 (p<0.001). The median CD4 at the time of HAART initiation was 238 cells/mm<sup>3</sup> in 2004, and 464 in 2014 (p<0.001). In 2004, 68% of newly diagnosed pts initiated HAART within the first year of care, compared to 97% in 2014 (p<0.001). During the same period, the % of virologically suppressed pts rose from 57 to 86% (p<0.001) (with 88% suppression among those using HAART). PrEP was first used by 5 pts outside of a clinical trial in 2011, while in 2014, 537 pts initiated PrEP (p<0.001), and in 2015, 589 began PrEP (as of 8/31), with more than 83% of PrEP initiators still using PrEP. Between 2011 and 2015, 5 MSM pts who initiated PrEP became HIV+ (<0.5%) compared to 93 (~2.2%) of more than 4,000 HIV- MSM who did not use PrEP (p=0.006). In 2005, 162 pts were diagnosed with syphilis or rectal or urethral gonorrhea (GC) or chlamydia (CT), while in 2014, 1145 pts tested + for at least one infection (p<0.001); and as of 8/31/15, it was 918. Since 2005, 1/2 of new syphilis diagnoses were in HIV+ patients, while 80% of incident GC/CT infections were in HIV- MSM. More than one third (36%) of MSM who initiated PrEP in 2014 had a recent bacterial STD.

**Conclusions:** While the HIV+ census at FH has increased over the past decade, earlier treatment initiation has been associated with improved virologic suppression. PrEP use has significantly increased among HIV- MSM, and HIV incidence appears lower in PrEP users than non-users. But, bacterial STD rates significantly increased for HIV+ and - MSM. HIV spread may be slowing among MA MSM, but ongoing screening for bacterial STDs is required, given their significant co-prevalence.

Variables by year	2004	2005	2011	2013	2014	P value
N HIV+ patients newly diagnosed in Massachusetts (MA)	922	902	697	698		<0.001
% of MA HIV+ patients diagnosed at FH	6.0%	8.1%	10.6%	14.2%		=0.002
N primary care HIV+ patients at FH	1083	1143	1775	1839	2101	<0.001
N HIV+ FH pts initiating HAART each year	58	48	108	124	121	<0.001
Median CD4 of FH patients at HAART initiation	238	241	399	470	464	<0.001
% HIV+ FH pts virologically suppressed	57%	58%	82%	80%	86%	<0.001
N FH pts initiating PrEP	0	0	5	102	561	<0.001
N FH pts diagnosed with Gonorrhea, Chlamydia, or Syphilis		162	342	725	1145	<0.001

**891 Missed Opportunities to Prescribe PrEP by Primary Care Physicians in Saint Louis**

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**Background:** Current CDC guidelines recommend delivery of HIV pre-exposure prophylaxis (PrEP) by primary care physicians (PCP) for high-risk individuals. We describe barriers to obtaining PrEP from PCPs among individuals who sought PrEP at an infectious diseases specialty clinic.

**Methods:** From July 2014 - September 2015, we conducted an intake survey among 69 patients seeking PrEP from the Washington University in St. Louis (WUSTL) HIV clinic as part of a prospective observational cohort. Survey questions included demographics, sexual behaviors, and whether patients had a PCP. Participants with a PCP were asked why they did not seek or obtain PrEP services from their PCP.

**Results:** Participant median age was 29 years (IQR 26-35), 62% were white, 68% were college graduates, 86% were MSM, 33% had a known HIV+ partner, 74% reported condomless sex in the last 3 months, and 21% reported a sexually transmitted disease in the last 12 months. 71% had a PCP; of these, 46% reported feeling uncomfortable discussing their sexual practices with their PCP. Of those comfortable, 67% asked their provider and were not prescribed PrEP. Overall, 47% asked their PCP for PrEP before coming to WUSTL, but were not prescribed. Commonly cited reasons cited for PCPs not prescribing PrEP included PCPs' concerns that PrEP was experimental, PCPs not feeling comfortable with the medication, and PCP perceptions that patients did not need PrEP beyond appropriate condom use. As part of provision of PrEP, HIV physicians referred 59% PrEP seekers to a new provider with whom they could feel more comfortable discussing their sexual practices. These referrals were not only for MSM but for heterosexual couples that did not feel comfortable discussing their HIV positive partner's status with their current providers.

**Conclusions:** In order for successful PrEP implementation in the US, PrEP should be prescribed as part of routine care by PCPs. PCP training needs to go beyond the science of PrEP and include creating an environment where sexual practices can be communicated in a culturally sensitive manner. Unless these discussions can take place, the role for PrEP

prescribing, even for high-risk and obviously eligible patients, may remain in the hands of HIV specialists, which is contrary to implementation goals. During initial implementation of PrEP, an additional role of HIV physicians may be to help PrEP seekers find a comfortable primary care home

**892 Early Adopters and Incident PrEP Prescribing in a Detailing Campaign, 2014-2015**

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**Background:** Pre- and post-exposure prophylaxis (PrEP and PEP) for HIV are effective yet under-prescribed. The New York City (NYC) Health Department conducted a public health detailing campaign October 2014–April 2015. Representatives visited primary care (PC) and infectious disease (ID) providers to promote prescribing PrEP and PEP, focusing on practices that had recently diagnosed HIV among at-risk populations. Initial and follow-up visits (~5–8 weeks later) consisted of short, individual-level presentations. We examined characteristics associated with PrEP prescribing at initial visit (early adopter) and with prescribing at follow-up visit (incident prescriber).

**Methods:** We included potential prescribers [MDs, nurse practitioners (NPs), and physician assistants (PAs)] reached for both initial and follow-up visits. Providers were identified as early adopters or incident prescribers based on self-report of ever prescribing PrEP at initial or at follow-up only, respectively. Characteristics examined were provider specialty/training [PC-MD, ID-MD or NP/PA]; practice characteristics, including type [hospital-affiliated (HA), private practice (PP), community health clinic (CHC)]; location (Manhattan vs. other); neighborhood HIV diagnosis and poverty rates; report of prescribing post-exposure prophylaxis (PEP); and length of initial visit (min). Multivariate models were constructed using generalized estimating equations. Adjusted odds ratios (aOR) and 95% confidence intervals (CI) were reported with all characteristics adjusted for each other except PEP; PEP was adjusted for all others.

**Results:** At initial visit, 18% (155/881) of providers at 492 facilities were early adopters. Among all others, 13% (89/709) of providers at 412 facilities reported incident prescribing. Early adoption was associated with ID-MD (Table); CHC practice type vs. PP (aOR 1.9, CI 1.1–3.2) and vs. HA (aOR 2.5 CI 1.4–4.5); Manhattan location (aOR 4.2 CI 2.5–7.2); and PEP prescribing (Table). Incident prescribing was associated with ID-MD; previous and incident PEP prescribing; and initial visit length ≥10 min, with no additional increase seen ≥20 (Table).

**Conclusions:** We observed early adoption and incident PrEP prescribing at NYC practices serving at-risk and potentially low-income populations. Prescribing PEP may be an important step for newly prescribing PrEP, supporting the promotion of PrEP and PEP in tandem. Detailing may have influenced new PrEP prescribing, particularly if the initial presentation was ≥10 min.

TABLE. Select Characteristics Associated with PrEP Prescribing among Providers Reached by a Public Health Detailing Campaign, New York City, 2014-15

Characteristic	Early Adoption <sup>a</sup>			Incident Prescribing <sup>a</sup>		
	n/N	%	Adjusted OR (95% CI) <sup>b</sup>	n/N	%	Adjusted OR (95% CI) <sup>b</sup>
<b>Overall</b>	155/881	18		89/709	13	
<b>Provider specialty/training</b>						
Primary care MD(PC-MD)	66/509	13	Ref	45/434	10	Ref
Infectious disease MD (ID-MD)	62/237	26	2.3 (1.4 - 3.9)	37/171	22	2.3 (1.3 - 4.3)
Nurse practitioner/physician assistant (NP/PA)	27/135	20	1.2 (0.8 - 2.0)	7/104	7	0.5 (0.2 - 1.1)
ID vs. NP/PA			1.9 (1.1 - 3.2)			4.6 (1.9 - 11.0)
<b>Previously prescribed PEP</b>						
No	15/603	2	Ref	51/575	9	Ref
Yes	137/269	51	34.7 (18.6 - 64.6)	38/128	30	3.5 (2.2 - 5.6)
<b>New PEP prescribing reported at follow-up</b>						
No				47/619	8	Ref
Yes				42/80	53	10.3 (5.4 - 19.6)
<b>Initial visit length (min)</b>						
<10				6/122	5	Ref
≥10-<20				30/235	13	3.2 (1.2 - 8.1)
≥20				53/352	15	3.3 (1.3 - 8.3)
≥20 vs. <10						1.0 (0.6 - 1.9)

<sup>a</sup>Early adoption= PrEP prescribing at initial visit; incident prescribing= PrEP prescribing at follow-up visit with early adopters excluded

<sup>b</sup>Adjusted for provider training, practice type, borough/county, neighborhood-level HIV diagnosis (top 3 quartiles vs. lowest) and poverty rate (≥10% living below federal poverty level vs. <10%) and detailing phase (10/14-1/15 vs. 2/15-4/15)

<sup>c</sup>Adjusted for provider training, practice type, borough/county, neighborhood HIV diagnosis (top 3 quartiles vs. lowest; by ZIP code) and poverty rate (≥10% living below federal poverty level vs. <10%; by ZIP code), detailing phase (10/14-1/15 vs. 2/15-4/15), initial visit length, and time between initial and follow-up visit (days)

**893 Correlates of Uptake of HIV Prevention Interventions Among Black MSM in DC, 2013-2014**

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**Background:** Eliminating racial HIV disparities among MSM will require a greater uptake of HIV prevention interventions among Black MSM (BMSM), the group with the highest HIV incidence in the US. However, interventions such as PrEP necessitate engagement in a health care system that often does not meet the needs of BMSM. This study examined correlates of the uptake of HIV prevention interventions among BMSM.

**Methods:** We interviewed two non-clinic-based samples of BMSM in Washington, DC: (1) peer-referred men who were inadequately engaged in health care and/or reported barriers to care (n=75) and (2) an Internet-based sample recruited irrespective of health care characteristics (n=93). Participants reported on their uptake of HIV prevention interventions in a computer-assisted self-interview. A randomly selected subsample of those with barriers to care provided ethnographic data on health care experiences in a qualitative interview (n=30). Correlates of uptake of interventions were assessed using Chi-square tests.

**Results:** Of 168 total BMSM, 61% were <30 years old, 86% had health insurance, and 81% were HIV-negative, 54% of whom were offered an HIV test at their last health care visit. Among HIV-negative BMSM in the first sample with barriers to care, a higher proportion of those who sought care at community-based clinics received HIV prevention interventions (testing, counseling, or PrEP) at these visits (90%) compared to those who accessed primary (53%) or acute care (44%) settings (p=0.005). In the Internet-based sample, PrEP uptake was positively associated with having accessed a community-based clinic but not a primary or acute care setting in the last year (OR= 4.7; 95% CI: 1.6-13.9), and was negatively associated with having private health insurance (OR=0.23; 95% CI: 0.08-0.92). In qualitative interviews, BMSM expressed preferences for receiving interventions at community-based clinics that were known to have culturally competent providers despite also often having access to private primary care providers.

**Conclusions:** In a non-clinic-based sample of BMSM, reported uptake of HIV prevention interventions was highest in community-based clinics that were culturally sensitive to the unique health needs of BMSM. Having access to health insurance and to health care does not necessarily facilitate the uptake of HIV prevention interventions for BMSM. It is critical that all health care encounters regardless of the setting support the uptake of prevention interventions for those at highest risk of HIV.

**894 HIV Preexposure Prophylaxis: Adherence and Discontinuation in Clinical Practice**

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**Background:** High adherence was critical to the efficacy of daily oral emtricitabine/tenofovir (FTC/TDF) preexposure prophylaxis (PrEP) in clinical trials. Low adherence or early discontinuation may reduce the effectiveness of PrEP in clinical practice.

**Methods:** We conducted a cohort study of Kaiser Permanente Northern California members initiating PrEP from July 2012 through December 2014. Follow-up was from the first dispensing of FTC/TDF until the earliest of PrEP discontinuation (i.e.,  $\geq 120$  days without medication), health plan disenrollment, HIV seroconversion, death, or end of study (June 2015). Refill adherence was calculated by dividing days' supply dispensed by total days between first and last FTC/TDF fill during follow-up among patients with  $\geq 2$  fills. We used chi-square tests to examine low adherence ( $< 60\%$ , consistent with taking  $< 4$  of 7 doses per week) by age, gender, and race/ethnicity. Multivariable log-binomial regression was used to estimate risk ratios (RRs) for factors associated with discontinuation.

**Results:** Among 972 individuals who initiated PrEP, there were 850 person-years of follow-up, with a mean of 0.9 years per person. The mean age at PrEP initiation was 37 years (range 18-68), and 98% of PrEP users were men. The majority were White (65%), followed by Hispanic (11%), Asian (9.7%), and Black (4.0%). Among 915 individuals with  $\geq 2$  fills, mean adherence was 92% (median 97%; interquartile range: 90%-100%), with  $> 80\%$  adherence in all demographic subgroups. Only 27 (3.0%) PrEP users had  $< 60\%$  adherence, with a higher proportion with low adherence in patients aged  $< 30$  vs.  $\geq 30$  years (5.7% vs. 2.0%,  $P=0.005$ ) and in Blacks/Hispanics vs. other racial/ethnic groups (6.6% vs. 2.3%,  $P=0.007$ ); the rarity of low adherence precluded multivariable analysis of this outcome. PrEP was discontinued by 219 (23%) individuals. There were no differences in discontinuation by age or race/ethnicity, but women were over twice as likely to discontinue than men (RR 2.4, 95% confidence interval: 1.6-3.6;  $P<0.001$ ). There were no seroconversions during PrEP use; however, there were 2 new HIV infections in Black and Hispanic men aged  $< 30$  years who had discontinued PrEP.

**Conclusions:** There were no HIV infections among active PrEP users during 850 person-years of follow-up, which is consistent with the high adherence observed in this population. Given the two seroconversions after PrEP discontinuation, there is a critical need for strategies to support continuation of PrEP throughout periods of HIV risk.

### 895 National HIV Incidence Estimates Among STI Clinic Attendees in England, UK

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**Background:** Currently, in England, national estimates for HIV incidence exist only for MSM. These are based on back-calculation and simulation models, both of which do not take migration into account which is necessary in particular for ethnic minority populations. Here we used a recent infection testing algorithm (RITA), consisting of biomarker, epidemiological and clinical information to examine national trends in incidence for all persons attending sexual transmitted infection (STI) clinics, where four of five people with HIV are diagnosed.

**Methods:** For each year, national HIV case report information from between 125 and 150 of the 210 STI clinics in England was linked to biological and testing information. The AxSYM assay, modified to determine antibody avidity, was used to classify HIV infections as likely recently acquired. A recency index cut-off of 0.8 was used, giving an estimated mean duration of recent infection of 181 days. All cases with a viral load  $< 400$  copies/mL or on ART or with an AIDS diagnosis were classified as longstanding infections. We calculated a 1.9% proportion false recent using known longstanding infections and estimated HIV incidence using the WHO formula for cross-sectional studies.

**Results:** From 2009 to 2013, between 161,000 and 231,000 heterosexuals (including between 9,700 and 26,000 black Africans) and 19,000 and 55,000 MSM attending STI clinics each year were included in analyses. National estimates of HIV incidence among heterosexuals remained stable between 0.03% (95% C.I. 0.02%-0.05%) and 0.05% (0.03%-0.05%), whilst among black African heterosexuals it was 4-5-fold higher, increasing slightly (although non-significantly) from 0.15% (0.05%-0.26%) in 2009 to 0.19% (0.04%-0.34%) in 2013. Incidence among MSM was highest and increased (non-significantly) from 1.24% (95% C.I. 0.96-1.52%) to 1.46% (95% C.I. 1.23%-1.70%) after a peak of 1.52% (95% C.I. 1.30%-1.75%) in 2012.

**Conclusions:** These are the first national HIV incidence estimates for both heterosexual and MSM populations in the UK attending STI clinics. They show MSM and black Africans remain disproportionately at risk of HIV infection. Our novel method based on a biomarker and surveillance data provides timely and accurate HIV incidence estimates which are critical in monitoring the population impact of prevention programmes including pre-exposure prophylaxis.

### 896 High HIV Incidence in Men Attending New York City LGBT and STD Clinics, 2009-2012

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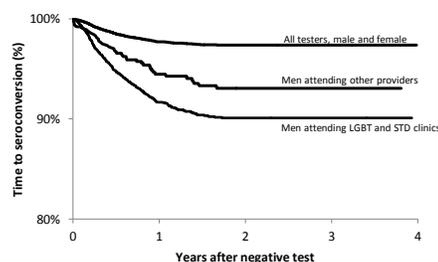
**Background:** HIV incidence is notoriously difficult to measure accurately using cross-sectional laboratory-based algorithms and/or statistical modeling. The ideal method – direct measurement of seroconversions in a cohort of seronegative persons followed over time – is logistically challenging, expensive and difficult to implement on a population basis.

**Methods:** We assembled a cohort of initially seronegative persons undergoing HIV diagnostic testing at the public health laboratory (PHL) in New York City. Persons were followed from the date of their first negative test on or after January 1, 2009, through the date of their first positive test, death, or December 31, 2012. Seroconversions were identified in the PHL database and through matching with the HIV surveillance registry.

**Results:** Of the 74,463 unique individuals with an initially negative test result, there were 18,197 persons, including 12,854 women (99% aged 15-44) and 5,246 men, with at least one subsequent negative or positive test at the PHL, or at least one subsequent positive test at another venue. The repeat tester cohort had 34,445.9 total person-years of follow-up, 447 seroconversions (56.2% at the PHL and 43.8% at other sites) and an incidence rate of 1.30/100 py (1.18, 1.42). Incidence was elevated in men overall (5.7/100 py [95% CI 5.2, 6.2]); adjusted hazard ratio for male-to-female 43.9 [95% CI 29.9, 64.4]) and highest among men whose specimens were submitted from clinics serving the LGBT community (14.2/100py [10.1, 18.2]), men attending STD clinics (5.8/100 py [5.1, 6.4]) and young (aged  $< 30$ ) black (8.5/100py [7.0, 10.1] and white men (6.3/100 py [4.9, 7.8]).

**Conclusions:** Incidence in men attending LGBT clinics was 12 times higher than incidence in all repeat testers and 2.5 times higher than incidence in men overall and men tested in STD clinics. The high male-to-female hazard ratio likely reflects the inclusion of many women tested during pregnancy. Incidence was also elevated among young (aged  $< 30$ ) black and white men. Data from this large cohort can be used to target local prevention resources, including PrEP and nPEP, to appropriate venues and populations.

Figure: Time to seroconversion – all testers, male and female, and men attending LGBT and STD clinics vs. men attending all other providers



**897 Longitudinal Analysis of Sexual Networks of US Black MSM in HPTN 061**

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**Background:** Black men who have sex with men (BMSM) have been disproportionately affected by HIV in the U.S., which is not explained by differences in individual behavioral risks. Sexual networks of BMSM may place them at increased HIV risk compared to non-BMSM. Sexual networks are rarely static, and longitudinal data are lacking on how changes in BMSM sexual networks over time influence HIV and sexual risk behaviors.

**Methods:** In this longitudinal investigation of BMSM sexual networks, we utilized self-reported egocentric network data collected over 12 months from BMSM in HPTN 061, a feasibility study of a multi-component HIV prevention intervention in 6 U.S. cities. At months 0 (M0), 6 (M6), and 12 (M12), BMSM completed a network inventory by describing their social and sexual network members from the prior 6 months. Sexual network composition, size, density (extent to which members had sex with one another), and serodiscordant/serostatus unknown condomless sex from M6 and M12 at the participant and partner levels were compared to M0 data using paired t-tests or McNemar's test.

**Results:** Of 1,553 men at M0, 348 (22%) were HIV-positive by HIV testing. At M0, mean sexual network size was 3.3 (SD 2.2) and mean sexual network density was 4.6% (SD 16.4%). 56% reported having exclusively Black partners in the last 6 months, 86% had overlap of social and sexual networks, 22% reported having an HIV-positive male partner, and 48% reported serodiscordant/serostatus unknown condomless sex. Using partner-level M0 data, 27% of partners had ≥2 age category difference between partner and participant. Compared with M0, the following significantly decreased over time: mean sexual network size (2.4 at M6, 2.0 at M12), mean sexual network density (2.5% at M12 only), overlap of social and sexual networks (76% at M6, 70% at M12), and condomless sex (26% at M6, 23% at M12) (all p-values<0.001). No significant changes in having exclusively Black sex partners, having HIV-positive male partners, and age difference between partners and participants were noted over time.

**Conclusions:** The sexual networks of BMSM in HPTN 061 were dynamic over 12 months. While decreases in sexual network size and density may reflect decreased HIV transmission and acquisition risk, decrease in overlap between social and sexual networks over time, with decreased social support, is concerning. Future studies should examine how BMSM sexual networks change over time and how these changes influence HIV infection and sexual risk behaviors.

**898 Does Size Really Matter? Sensitivity Analysis of Number of Seeds in an RDS Study**

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**Background:** Respondent-driven sampling (RDS) is an increasingly used peer chain-recruitment method to sample "hard-to-reach" populations for whom there are no reliable sampling frames. Implementation success of RDS varies; one potential negative factor being the number of seeds used. We conducted a sensitivity analysis on estimates produced using various RDS-weighting procedures and sample cuts based on data from a study of men who have sex with men in Vancouver, Canada.

**Methods:** Participants were recruited from 2012-2014 using RDS, and had to be aged ≥16 years of age and report recent sex with another man. The study protocol included a self-completed questionnaire on demographics, sexual behavior and substance use. To conduct this analysis, we used various sample cuts, starting with all participants and subsequently removing unproductive seeds (0 recruitment waves), chains of ≤1 recruitment waves, and chains of ≤2 recruitment waves. Using the RDS Analyst 0.52 and SAS 9.4, we calculated estimates for three different outcomes for each sample cut and three different RDS weighting procedures: RDS-I (Salganik-Heckathorn), RDS-II (Volz-Heckathorn), and RDS-SS (Giles). We also assessed seed dependence with bottleneck analyses and convergence plots.

**Results:** Overall, 719 participants were recruited, which included 119 seeds and a maximum of 16 recruitment waves (mean recruitment chain length = 1.75). The sample of >0 recruitment waves removed all unproductive seeds (n=50/119, 42.0%), resulting in 69 chains (mean length = 3.0). The sample of >1 recruitment waves removed 125 seeds or recruits (17.4% of overall sample), resulting in 37 chains (mean length = 4.8). The final sample of >2 recruitment waves removed a further 182 seeds or recruits (25.3% of overall sample), resulting in 25 chains (mean length = 6.1). Based on these various samples, Table 1 provides estimates for three key study outcomes: HIV serostatus, high risk sex (condomless anal intercourse with HIV discordant/unknown status partner), and injecting drugs. Convergence plots and bottleneck analyses were satisfactory.

**Conclusions:** For each outcome and within each sample cut, the crude proportions fell within 95% confidence intervals of all RDS-weighted estimates. All RDS-weighted estimates were similar and fell within the 95% confidence intervals of each other. Although potentially costly and time consuming, our results indicate that RDS studies are not negatively affected by large numbers of unproductive or lowly productive seeds.

**Table 1. Three key study outcomes using various sample cuts and RDS-weights**

		Crude %	RDS I % (95% CI)	RDS II % (95% CI)	RDS SS % (95% CI)
<b>HIV-positive serostatus</b>	Overall (n=719)	27.7	22.5 (19.4-25.6)	26.7 (20.7-32.7)	26.7 (20.7-32.7)
	>0 Wave (n=669)	28.3	22.9 (19.7-26.2)	27.7 (21.5-33.9)	27.7 (21.4-34.1)
	>1 Wave (n=594)	30.0	24.2 (20.6-27.7)	29.3 (22.6-36.0)	29.4 (22.6-36.1)
	>2 Wave (n=537)	30.0	23.8 (20.1-27.5)	29.1 (22.1-36.1)	29.1 (22.1-36.2)
<b>High risk sex in past 6 months</b>	Overall (n=719)	37.3	34.9 (31.1-38.8)	33.6 (27.6-39.6)	33.7 (27.7-39.7)
	>0 Wave (n=669)	38.4	35.5 (31.5-39.5)	35.2 (28.9-41.5)	35.3 (29.0-41.6)
	>1 Wave (n=594)	38.0	36.0 (31.7-40.3)	35.8 (29.3-42.4)	35.8 (29.2-42.4)
	>2 Wave (n=537)	38.7	37.0 (32.4-41.5)	36.4 (29.5-43.3)	36.5 (29.6-43.3)
<b>Injected drugs in past 6 months</b>	Overall (n=719)	7.1	8.8 (5.5-12.1)	7.3 (3.9-10.7)	7.3 (3.9-10.7)
	>0 Wave (n=669)	7.6	9.1 (5.8-12.4)	8.1 (4.4-11.8)	8.1 (4.3-11.9)
	>1 Wave (n=594)	8.1	8.7 (5.7-11.7)	8.0 (4.7-11.3)	8.0 (4.7-11.3)
	>2 Wave (n=537)	8.0	8.7 (5.5-11.9)	8.3 (4.8-11.8)	8.3 (4.8-11.9)

**899 Respondent-Driven Sampling: An Epidemiological Tool With Interventional Potential**

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**Background:** To achieve the UNAIDS 90-90-90 target, key populations, such as men who have sex with men (MSM) and people who inject drugs (PWID) in low- and middle-income countries, must be reached. Respondent-driven sampling (RDS), a chain-referral strategy, is an established epidemiologic tool to characterize epidemics in hidden populations. We explore the ability of RDS to identify unaware HIV-positive persons over successive recruitment waves and its ability to reach people in all regions of medium to large cities in India.

**Methods:** We conducted RDS surveys in 27 Indian cities (12 MSM, 15 PWID). Individuals were eligible if they were age ≥ 18 years and reported recent MSM behavior (MSM sites) or drug injection (PWID sites). We initiated each RDS with 2-3 "seeds" per site, gave participants two coupons to recruit network members, and continued recruitment until 1000

subjects were enrolled. Participants completed a survey and were tested for HIV. We assessed the ability of RDS to identify HIV-positive persons unaware of their status across recruitment waves and created zip code maps of recruitment using ArcGIS.

**Results:** We recruited 26,503 participants (12,022 MSM and 14,481 PWID) from 27 cities, over a median (range) of 112 days (52–200) and 21 recruitment waves (11–50). Of 4,065 HIV-positive persons identified, 2,325 (57%) were unaware of their status. While HIV prevalence was relatively stable across recruitment waves (~15%), the percentage of HIV-positive persons unaware of their status increased from 47% in waves 1-5 to 78% in waves >25 ( $p < 0.001$ ), suggesting identification of persons with poor service access as recruitment progressed deeper within networks. Further, despite recruiting from a single venue in each city, RDS reached individuals across all zip codes, with most zones reached within 5 recruitment waves (Figure). For example, in Chennai, participants were recruited from a median of 4.2 km from the study site, with 50 participants recruited from >20 km away.

**Conclusions:** Beginning with 2 or 3 “seeds” and recruiting from a single venue, RDS demonstrated the ability 1) to efficiently identify HIV-positive persons who were unaware of their status (with an increasing likelihood of identifying such persons in later recruitment waves), and 2) to reach MSM and PWID throughout all geographical regions in medium to large cities. Combined with evidence-based linkage strategies, RDS has the potential to improve the care continuum in key populations in low- and middle-income countries.

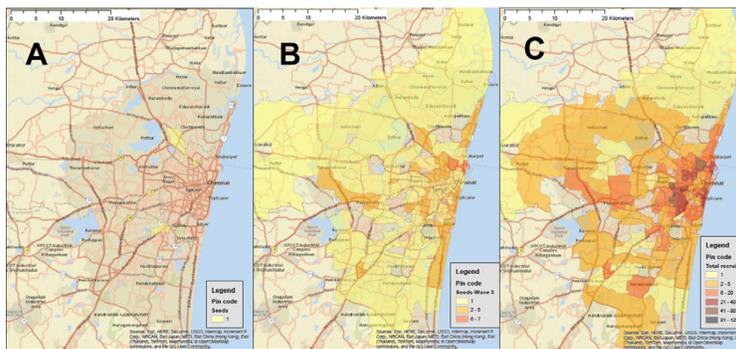


Figure. Zip code locations of “seeds” (A), waves 1-5 (B), and complete recruitment (C) of RDS in Chennai, India

#### 900 10 Years of HIV and STI Testing at Silom Community Clinic (SCC) in Bangkok, Thailand

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<sup>1</sup>CDC, Atlanta, GA, USA; <sup>2</sup>Emory Univ Rollins Sch of PH, Atlanta, GA, USA; <sup>3</sup>Thailand Ministry of PH US CDC Collab, Nonthaburi, Thailand **Background:** The Silom Community Clinic (SCC) conducts voluntary counseling and testing (VCT) for men who have sex with men (MSM) and transgender (TG) women in Bangkok, Thailand. We describe 10 years of human immunodeficiency virus (HIV) and sexually transmitted infection (STI) prevalence, and risk factors for prevalent HIV infection.

**Methods:** We offered all clients attending SCC from 2005–2015 testing for HIV, syphilis, and hepatitis B virus (HBV). VCT HIV testing followed a 3 step algorithm using rapid HIV tests on blood specimens. Syphilis testing included non-treponemal (rapid plasma reagin, RPR) and treponemal-specific testing; RPR titer >1:8 or reactivity on treponemal test was considered syphilis infection. Hepatitis B testing included hepatitis B surface antibody. We assessed HIV and syphilis prevalence over 10 years using a Cochran-Armitage test for trend, and risk factors for prevalent HIV and STIs at the baseline visit using bivariate and multivariable logistic regression analysis.

**Results:** There were 8,945 unique clients attending VCT from Sept, 2005– May, 2015; the mean age was 28.2 years and 3905 (43.7%) had been tested before for HIV. At the first visit for VCT, 1217 (13.6%) did not have an HIV test done. Most (67.9%) had a least one follow-up visit and 1972 (22.1%) had more than 6 visits. Overall, 2390 (30.9%) tested positive for HIV, 1159 (15.0%) tested positive for syphilis, and 3587 (47.8%) tested positive for HBV surface antibody. HIV and syphilis prevalence changed significantly by year ( $p < 0.01$ ), with an increase from 12.7% to 24.5% in syphilis in the last 5 years ( $p < 0.01$ ). Risk factors in multivariable analysis for prevalent HIV infection were age  $\geq 25$  years (25–29 years: aOR 1.5, 95% CI 1.3–1.7;  $\geq 30$  years: aOR 1.6, 95% CI 1.4–1.8), testing positive for HBV surface antibody (aOR 1.3, 95% CI 1.2–1.5), syphilis (aOR 5.9, 95% CI 5.1–6.9), and having moved to Bangkok since birth (aOR 1.5, 95% CI 1.3–1.7). A protective factor for prevalent HIV infection was past HIV testing (aOR 0.4, 95% CI 0.4–0.5).

**Conclusions:** Ten years of comprehensive HIV and STI testing of MSM and TG women in Bangkok, Thailand demonstrate nearly 1 in 3 clients present with HIV infection; significant increases in syphilis in the last 5 years have occurred. Risk factors for prevalent HIV may support targeted efforts to identify MSM and TG women with HIV who would benefit from treatment as prevention.

#### 901 Why Are Trends in HIV Diagnoses in Sub-Saharan African Migrants in Europe Changing?

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**Background:** Sustained declines in HIV diagnoses in migrants from Sub-Saharan Africa (SSA) have been reported in countries of the European Union/Economic Area (EU/EEA) but reasons for the decline are not well understood. We aim to describe whether declines are homogeneous for migrants from different regions within SSA and if they are associated with delays in HIV testing.

**Methods:** HIV reports to the European Surveillance System (TESSy) from 30 EU/EEA countries from 2004 till 2013 were analysed. Cases from SSA were further divided into UN regions: Western, Central, Eastern and Southern Africa. Differences in CD4 counts at HIV diagnosis over time for each SSA region were used as a measure of HIV testing delay; these were analyzed using median regression adjusting for transmission category, age and sex.

**Results:** Of 252 609 cases reported from 2004 to 2013 with data on country of origin, 57 405 (23%) were from SSA; 35% from Western SSA, 32% from Eastern, 23% from Central, 5% from Southern SSA, 5% from unknown SSA regions. HIV had been acquired heterosexually in 88% of cases. HIV reports declined from 2004 to 2012 (2013 data removed when analysing trends due to reporting delay) both in absolute (and relative) terms by 2824 (37%) for SSA globally; 60 (3%) for Western, 1571 (57%) for Eastern, 563 (33%) for Central, and 250 (59%) for Southern SSA. Variations within EU/EEA countries were observed. Declines were more pronounced in women. Median CD4 count at diagnosis in the 33 129 SSA migrants with this information showed steady increases from 241 cells/mm<sup>3</sup> in 2004 to 280 cells/mm<sup>3</sup> in 2012 for all SSA migrants. Increases were seen for the different regions (less pronounced in Western SSA origin), in univariate and multivariate analyses (Table). Results were largely unchanged taking into account reporting heterogeneity of HIV cases and CD4 counts.

**Conclusions:** The decreases of HIV reports in migrants from SSA in the EU/EEA from 2004 to 2012 are influenced most by declines in cases from Central and Eastern SSA, while cases in migrants from Western SSA are stable. Median CD4 count has increased over the years and for all SSA regions. These results do not suggest increases in delayed HIV testing. Other explanations for the different trends are changes in migratory flows into the EU/EEA – decreasing numbers from Central and Eastern SSA and increasing from Western SSA – and the impact of changes in HIV incidence in the SSA regions of origin of these migrant populations.

**Table: Univariate and multivariate analyses of median regression of the differences of median CD4 cell counts in migrants originating from SSA, 2004-2012**

	WESTERN		CENTRAL		EASTERN		SOUTHERN	
	Median difference (95% CI)		Median difference (95% CI)		Median difference (95% CI)		Median difference (95% CI)	
	Univariate	Multivariate	Univariate	Multivariate	Univariate	Multivariate	Univariate	Multivariate
2004	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
2005	-17 (-48; 14)	-15 (-44; 14)	13 (-18; 44)	5 (-25; 35)	3 (-14; 20)	2 (-14; 18)	23 (-22; 68)	31 (-11; 73)
2006	-37 (-68; -6)	-26 (-56; 4)	8 (-24; 40)	5 (-26; 36)	-8 (-26; 10)	-6 (-23; 11)	40 (-5; 85)	43 (1; 85)
2007	-27 (-58; 4)	-10 (-39; 19)	38 (6; 70)	28 (-3; 59)	3 (-16; 22)	5 (-12; 22)	53 (6; 100)	59 (15; 103)
2008	0 (-28; 28)	19 (-7; 45)	48 (20; 76)	45 (18; 72)	-3 (-22; 16)	14 (-3; 31)	10 (-39; 59)	2 (-44; 48)
2009	21 (-7; 49)	31 (5; 57)	52 (24; 80)	48 (22; 75)	5 (-14; 24)	19 (1; 37)	20 (-31; 71)	23 (-25; 71)
2010	7 (-20; 34)	26 (0; 52)	38 (10; 66)	42 (16; 69)	25 (5; 45)	35 (16; 54)	30 (-25; 85)	35 (-17; 87)
2011	9 (-18; 36)	21 (-4; 46)	62 (34; 90)	56 (29; 83)	33 (12; 54)	37 (17; 57)	35 (-23; 93)	46 (-8; 100)
2012	13 (-14; 40)	26 (0; 52)	67 (39; 95)	58 (31; 86)	26 (3; 49)	42 (21; 63)	110 (50; 170)	96 (39; 153)

**902 Migration, HIV Infection, and Combination HIV Prevention Access in Rakai, Uganda**

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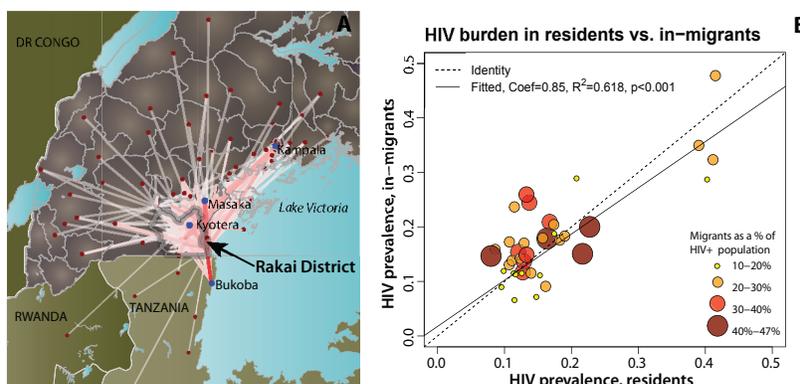
<sup>1</sup>Johns Hopkins Univ, Baltimore, MD, USA; <sup>2</sup>Rakai Hlth Scis Prog, Kalisizo, Uganda; <sup>3</sup>Johns Hopkins Univ Sch of Med, Baltimore, MD, USA; <sup>4</sup>Johns Hopkins Bloomberg Sch of PH, Baltimore, MD, USA; <sup>5</sup>Rakai Hlth Scis Prog, Entebbe, Uganda; <sup>6</sup>Rakai Hlth Scis Prog, Baltimore, MD, USA

**Background:** Migration has been historically associated with HIV spread in sub-Saharan Africa (SSA); however, the geography of local migration networks and the extent to which migrants contribute to total HIV burden and access HIV treatment and prevention services at the community- level are largely unknown.

**Methods:** We surveyed 22,533 individuals aged 15-49 residing in 38 communities in the Rakai District, Uganda from August 2011 to January 2015 through the Rakai Community Cohort Study (RCCS). During the RCCS household census, persons who recently migrated into study communities since the prior survey (~1 year) were identified and their place of origin recorded and then geocoded to assess geographic flow of migrants. Consenting participants were HIV tested and interviewed to ascertain demographic, behavioral, and health data, including self-reported ART use and male circumcision status. The proportions of total HIV burden and newly detected cases attributable to in-migration were estimated and the predominant geographic sources of migrant-introduced HIV infections were identified.

**Results:** We identified 5,533 individuals who recently migrated into RCCS study communities. These persons comprised 25% of the surveyed population and 23% of all persons living with HIV (ranging from 10-47% of total HIV burden across communities). Additionally, 49% (935/1918) of all newly identified HIV cases were introduced by migrants. Migrants were predominately female (n=3,603, 63%), and mostly came from within the Rakai District (61%), the neighboring Masaka District (17%), or Kampala (6%); however, the overall migration network was geographically broad, spanning multiple districts and national boundaries (Fig1A). HIV prevalence did not vary between in-migrants and the resident population, though HIV-infected migrants tended to move to communities with higher prevalence (p<0.001; Fig1B). Male in-migrants were significantly less likely to be circumcised (RR=0.71, 95%CI: 0.66-0.77), and HIV-infected in-migrants were significantly less likely to use ART (RR=0.51, 95%CI: 0.45-0.58) compared to residents, irrespective of age or gender.

**Conclusions:** Recent in-migrants account for a substantial proportion of total HIV burden in Ugandan communities. Large influxes of HIV-infected persons who are not linked to care and preferentially migrating to communities with higher HIV prevalence underscores the need for continuous HIV surveillance at the local level and novel, targeted interventions for mobile populations in SSA.



**903 HIV Epidemiology and Service Uptake Among Fisherfolk in Asembo, Western Kenya, 2015**

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**Background:** In 2012, Nyanza region had the highest HIV prevalence in Kenya among persons aged 15-49 years of age (15.1% vs 5.6% nationwide). Fisherfolk, people who catch, sell, process fish and their spouses are a priority population in HIV transmission dynamics. We described HIV prevalence and HIV health service utilization among the fisherfolk in Asembo, of Nyanza region in rural western Kenya.

**Methods:** A cross-sectional bio-behavioural census survey was conducted at Lake Victoria beaches and adjacent villages in Asembo between August 2014 and March 2015. Participants were interviewed on HIV risk behavior, service utilization and offered HIV testing. Self-reported HIV status was documented if testing was declined. Logistic regression was used to determine factors associated with a positive HIV status.

**Results:** Of 4,050 participants interviewed, 940 (23%) were fisherfolk. The median age of fisherfolk was 32 years, 30% were aged 30-39 years; 62% were female. Among fisherfolk 71% were married, almost one-third (31%) of fisherfolk were fishmongers, 28% fishermen, 21% spouses of fishermen, 9% fish processors, 2% boat managers, with 9% in other cadres. Only 12% of fisherfolk reported having migrated out of Asemo for  $\geq 1$  month.

Of the 911 fisherfolk with HIV status available, 23.4% (95% CI 21-26) were HIV positive (74% self-reported and 26% tested). HIV prevalence was 26.8% among females and 17.8% among males, 35% among 30-39 year olds, 33.3% among boat managers and 32.1% among fish processors. Fisherfolk who were currently or previously married were significantly more likely to be HIV positive compared to those who were single (AOR 2.3, 95% CI 1.1-4.9; AOR 6.7, 95% CI 2.9-15.7 respectively). Compared to fisherfolk aged 30-39 years, those aged 13-19, 20-29, and  $\geq 50$  years were significantly less likely to be HIV positive (AOR 0.1, 95% CI 0.0-0.4; AOR 0.5, 95% CI 0.3-0.7; AOR 0.2, 95% CI 0.1-0.4, respectively). Migration was not associated with HIV positivity.

Circumcision was reported by 52% (95% CI 46-57) of male fisherfolk. Among the 183 HIV positive fisherfolk, 53% (95% CI 45-60) reported being currently in HIV care.

**Conclusions:** HIV prevalence among the Asemo fisherfolk was high among women; persons currently or previously married and those in their thirties were more likely to be infected compared with others. Given that only about half of HIV infected persons reported being in care, targeting and adapting treatment and prevention interventions to the needs of this key population is critical.

#### 904 Young HIV+ Adults in Botswana Less Likely to Seek Treatment or Be Virologically Suppressed

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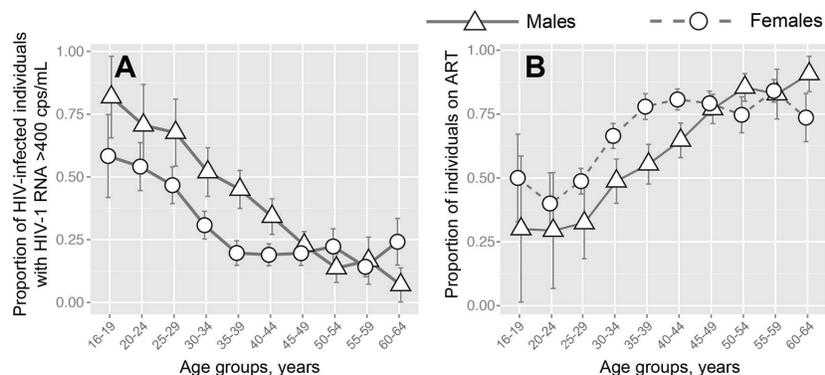
<sup>1</sup>Harvard Sch of PH, Boston, MA, USA; <sup>2</sup>Botswana Harvard AIDS Inst Partnership, Gaborone, Botswana; <sup>3</sup>Massachusetts General Hosp, Boston, MA, USA; <sup>4</sup>Brigham and Women's Hosp, Harvard Med Sch, Boston, MA, USA

**Background:** The scale-up of ART programs has been successful in Botswana. However the nature and role of populations with detectable plasma HIV-1 RNA remain unclear.

**Methods:** As part of the Botswana Combination Prevention Project (an ongoing cluster-randomized HIV combination prevention trial in Botswana), a baseline household survey was performed in 24 rural communities November 2013–August 2015. Residents 16–64 years old were targeted in a random sample of 20% of households in each community. For this analysis, detectable HIV-1 RNA was defined as  $>400$  cps/mL. Women under 30 y.o. and men under 35 y.o. were defined as young adults.

**Results:** 2,727 HIV-infected residents enrolled; 1,915 (70%) were currently on ART. A high proportion of all HIV-infected individuals (72%), including 96% of individuals on ART, were virologically suppressed. Twenty eight percent (95% CI: 25%–30%) of individuals had detectable HIV-1 RNA. Younger age was associated with having detectable HIV-1 RNA ( $p=0.0001$ , Fig. A) and with not being on ART ( $p<0.0001$ , Fig. B). Young HIV-infected men and women were approximately 23% less likely to be on ART (RR: 0.77; 95% CI: 0.70–0.85;  $p<0.0001$ ). Among the 495 HIV-positive young men and women, 252 (51%) had a detectable HIV-1 RNA. The median HIV-1 RNA level in all individuals with detectable HIV-1 RNA was 4.2 (IQR 3.6–4.8)  $\log_{10}$  cps/mL and was higher in men than in women ( $p<0.0001$ ), but did not differ by age group ( $p=0.19$ ). Seventeen percent of all HIV-infected individuals and 61% of persons who were not virologically suppressed had HIV-1 RNA  $>10,000$  cps/mL. Eight percent of all individuals infected with HIV for over 12 months and 55% of persons not virologically suppressed had HIV-1 RNA  $>10,000$  cps/mL. A relatively high proportion of ART-naïve individuals (15%) had HIV-1 RNA  $<400$  cps/mL. We cannot exclude that at least some of these individuals were taking ARV.

**Conclusions:** In rural communities in Botswana, a high proportion (72%) of all HIV-infected individuals had HIV-1 RNA  $\leq 400$  cps/mL. However, young HIV-infected adults were less likely to be on ART and the majority of them had detectable HIV-1 RNA. Targeted interventions should be tailored to improving HIV care-seeking behavior among young adults in Botswana. Young adults in Botswana may be contributing disproportionately more to HIV-transmission networks. Research on dynamics of HIV transmission networks among young adults may inform prevention programs.



#### 905 High Risk of HIV Transmission and Acquisition Among Older South Africans

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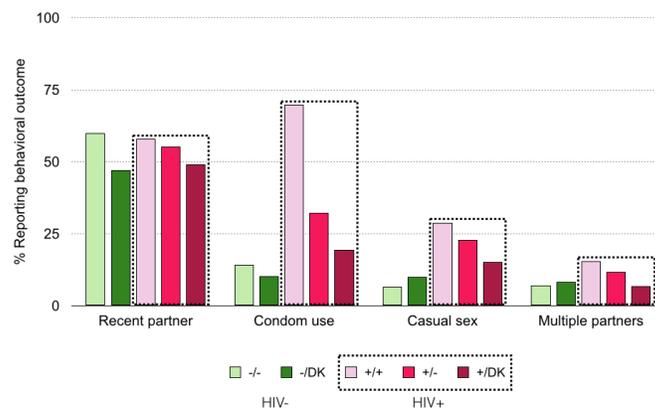
**Background:** HIV risk in older adults in sub-Saharan Africa is understudied. Sexual behavior and HIV status are likely associated in this population because risky sexual behaviors increase the risk of acquiring HIV, and because HIV testing and counseling services could prompt safer sexual behaviors to avoid subsequent transmission. Exploring these associations will help to understand the potential drivers of sexual transmission of HIV in older adults, and will provide preliminary insight into the importance of HIV testing and counseling for secondary prevention in this understudied population.

**Methods:** Using baseline data from the Health and Aging in Africa: Longitudinal Studies of INDEPTH Communities, we examined the relationship between HIV status, status awareness, and sexual behavior in older adults in a population-based sample of 4714 men and women over age 40 in the rural Agincourt sub-district of South Africa. We classified participants into one of five HIV status categories based on laboratory-confirmed HIV status, and whether self-reported HIV status (positive, negative, or unknown) was concordant with the laboratory-confirmed status. We compared prevalence of recent sexual activity, condom use, casual sex, and multiple partnerships across each HIV status category using log-linear regression models, adjusting for key socio-demographic characteristics (age, education, and marital status).

**Results:** HIV prevalence was very high in this population (22.2%; 95% CI: 20.7, 23.7) and nearly half of the laboratory-confirmed HIV cases were not aware of their status (44%). Recent sexual activity was common and represented in similar proportions across all HIV- and HIV+ categories. Those who tested HIV+ were more likely to report condom use with their most recent partner, compared to those who tested HIV-; however, the magnitude of effect was much stronger among those who were aware of their positive status (aPR: 4.23; 95% CI: 3.47, 5.15). Importantly, those who tested HIV+ were also more likely to report recent casual sex and multiple partnerships, compared to those who tested HIV-.

**Conclusions:** Older HIV+ adults in a high HIV prevalence community in rural South Africa report sexual behaviors that are consistent with HIV transmission; similarly, older HIV- adults report sexual behaviors that are consistent with HIV acquisition. HIV prevention initiatives that target older adults are urgently needed to reduce both HIV transmission and acquisition in this and similar communities in sub-Saharan Africa.

**Figure 1.** Prevalence of recent sexual activity, condom use, casual sex, and multiple partnerships by HIV status category



-/+ = Those who tested HIV- and self-reported knowing their status as HIV-  
 -DK = Those who tested HIV- and self-reported that they did not know their status  
 +/+ = Those who tested HIV+ and self-reported knowing their status as HIV+  
 +/- = Those who tested HIV+ and self-reported knowing their status as HIV-  
 +DK = Those who tested HIV+ and self-reported that they did not know their status  
**Recent partner** = self-report of at least one sex partner in the last 24 months  
**Condom use** = self-report of at least some condom use with most recent sex partner  
**Casual sex** = self-reported categorization of most recent sex partner as 'casual' or 'anonymous' as opposed to 'regular'  
**Multiple partners** = self-report of more than one sex partner in the last 24 months

#### 906 Factors Associated With Misreporting HIV Status Among MSM From Baltimore

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**Background:** Unrecognized HIV infection and antiretroviral (ARV) drug use are critical indicators for monitoring the HIV epidemic, but frequently rely on self-report. We tested for presence of ARVs in HIV+ men who have sex with men (MSM) and analyzed socio-demographic factors associated with misreporting their HIV status.

**Methods:** From the 2008 MSM cycle of National HIV Behavioral Surveillance in Baltimore, sera from 147 HIV+ MSM were tested for the presence of ARVs using liquid chromatography-tandem mass spectrometry (API 4000 Triple Quadruple Mass Analyzer) which detects 20 antiretroviral (ARV) drugs. Factors associated with misreported unrecognized HIV infection were analyzed.

**Results:** The prevalence of unrecognized infection, calculated as those with no self-reported knowledge of prior HIV infection divided by the total that tested HIV positive, was 74%. No error in ARV reporting was detected among self-reported HIV-positive participants. Of 109 participants originally classified with unrecognized HIV infection, 33% (36/109) had at least one ARV drug detected and 31% (34/109) had detectable regimens consistent with ARV therapy. Compared to those with no ARV detected, misreporters were significantly older (mean 38.4 vs. 29.6,  $p < 0.0001$ ) and less likely to report binge drinking (31% vs. 55%,  $p = 0.017$ ). No socio-economic differences were significant in multivariate models. Compared to those who self-reported HIV positive status and ARV use, misreporters were significantly more likely to be unemployed (39% vs. 9%,  $p = 0.032$ ) and marginally more likely to be recently homeless (33% vs. 9%,  $p = 0.058$ ) and recently arrested (17% vs. 0%,  $p = 0.073$ ). No differences in sexual or drug use behaviors were observed. Recalculated prevalence of unrecognized HIV infection among MSM with HIV was 49.7%; 27.8% among NH white ( $n = 18$ ) and 51.4% among NH black participants ( $n = 111$ ), with decreasing prevalence by age.

**Conclusions:** These analyses provide new insight into the extent of under-reporting of known HIV infection and ARV status in behavioral surveillance. The high rate of unrecognized HIV infection among Baltimore MSM, with nearly half of HIV+ individuals who knew their status misreporting their status, is alarming. Further qualitative research may help to understand and contextualize misreported HIV status in future behavioral surveys.

#### 907 Validation of Self-Reported HIV Status Among Older Adults in Rural South Africa

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**Background:** Little is known about willingness to disclose HIV status among the growing number of HIV positive older adults in South Africa. Estimates of sensitivity and specificity of self-reported status in this population are unavailable but important for researchers and policy makers. We validate self-reported HIV status and explore factors associated with accuracy of self-report.

**Methods:** We analyzed data from the Health and Aging in Africa: Longitudinal Studies of INDEPTH Communities (HAALSI) baseline survey, an observational cohort of randomly sampled adults age 40+ years in a poor, rural community in Agincourt, South Africa. Self-reported HIV status and prior HIV testing was obtained through structured interviews by local field workers. Dried blood spots (DBS) were collected at time of interview and HIV enzyme-linked immunosorbent assays for HIV status were conducted. We calculated sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for self-reported HIV status compared to "gold standard" DBS results, and stratified findings by demographic characteristics. Logistic regression explored associations between demographic characteristics and sensitivity of self-report.

**Results:** 3,426 individuals (94.4% of respondents) had DBS results available and were included in analysis. HIV prevalence was 21.8% from DBS and 12.5% from self-report. Sensitivity of self-report was 53% (95% CI: 49%-57%), specificity 99% (95% CI: 99%-99%), PPV 94% (95% CI: 91%-96%) and NPV 88% (95% CI: 87%-89%). Among those who reported knowing their HIV status, sensitivity increased to 67% (Table 1). The lowest sensitivity was found among illiterate participants (49%) and oldest ages (33% among age 80+ years). Correct report of being HIV positive was more likely among participants 50-59 years old compared to 70+ years old (OR = 1.69, 95% CI: 1.04-2.75) and literate

participants (OR = 1.29, 95% CI: 0.97-1.73). These associations were reduced after adjustment for prior HIV testing, indicating that differences in accuracy of self-report were largely due to differences in testing.

**Conclusions:** The majority of participants were willing to share their HIV status, and false negative reports were largely explained by lack of testing or awareness of status, strongly suggesting that HIV stigma has retreated in this setting. In HIV interventions where testing is not feasible, self-reported status should be considered as a routine alternative to establish status because of the very high PPV and NPV.

Table 1. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of self-reported HIV status compared to dried blood spot (DBS) HIV test results.

Population	N (%)	% HIV+ from DBS	Sensitivity	Specificity	PPV	NPV
<b>Total</b>	3426	21.8%	53%	99%	94%	88%
<b>Report ever had an HIV test</b>	2204 (64.8%)	27.1%	66%	99%	95%	89%
<b>Report knowing their HIV status</b>	2023 (59.4%)	28.4%	67%	99%	95%	88%
<b>Gender</b>						
Male	1455 (42.5%)	20.8%	54%	99%	93%	89%
Female	1971 (57.53%)	22.6%	53%	99%	95%	88%
<b>Age</b>						
40-49 years	501 (14.6%)	38.8%	55%	100%	99%	78%
50-59 years	882 (25.7%)	31.1%	56%	98%	92%	83%
60-69 years	972 (28.4%)	19.8%	51%	99%	93%	89%
70-79 years	665 (19.4%)	11.3%	44%	100%	94%	93%
80+ years	406 (11.9%)	3.0%	33%	100%	80%	98%
<b>Marital status</b>						
Currently married	1714 (50.1%)	15.8%	52%	99%	90%	92%
Not currently married	1710 (49.9%)	27.8%	53%	99%	97%	85%
<b>Able to read and write</b>						
Yes	1639 (47.8%)	24.5%	56%	99%	95%	87%
No	1782 (52.2%)	19.3%	49%	99%	93%	89%
<b>Employment status</b>						
Employed	336 (9.8%)	20.6%	57%	99%	97%	83%
Not employed	3081 (90.2%)	32.7%	52%	99%	94%	89%

**908 Demographics and HIV Care Among New Yorkers Living With HIV by Diagnostic Cohort**

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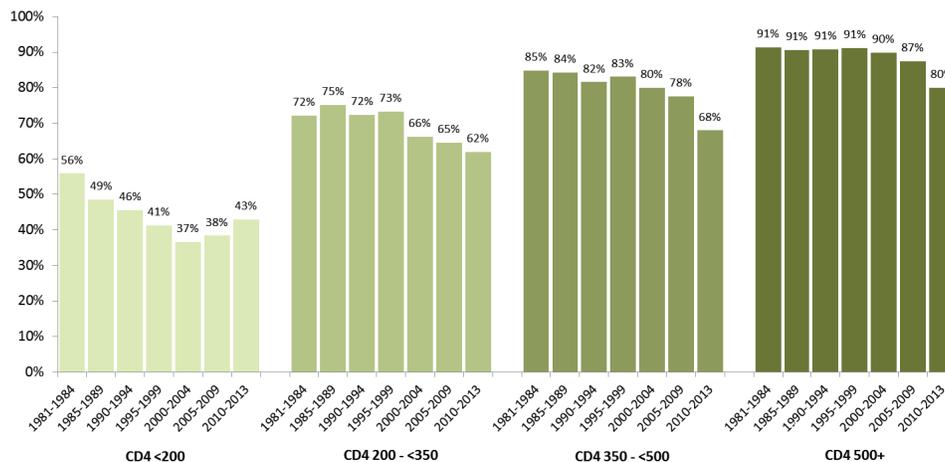
**Background:** While HIV diagnoses in New York City (NYC) have been steadily decreasing over time, the city continues to have a large epidemic with over 2,500 new diagnoses each year. We characterized the demographics and clinical care indicators of all people living with HIV (PLWH) in NYC in 2013 by diagnostic cohort.

**Methods:** Data on all NYC PLWH as of 12/31/2013 were acquired from the NYC HIV Surveillance Registry. PLWH were categorized into the following diagnostic cohorts based on year of HIV diagnosis: 1981-1984, 1985-1989, 1990-1994, 1995-1999, 2000-2004, 2005-2009, and 2010-2013. Demographics and clinical care indicators of these cohorts, including CD4 intervals and viral load (VL) suppression for those in care, were explored. Persons were considered in care if they had at least one HIV VL/CD4 in 2013.

**Results:** There were 100,992 people diagnosed and presumed to be living with HIV in NYC as of 12/31/2013. Of these, 450 were diagnosed in 1981-1984, 7,955 in 1985-1989, 17,792 in 1990-1994, 22,789 in 1995-1999, 21,429 in 2000-2004, 18,699 in 2005-2009, and 11,878 in 2010-2013. The majority of PLWH in each diagnostic cohort were male, younger at diagnosis (20-39 years old), and classified as men who have sex with men. Race/ethnicity distributions differed by cohort. The largest proportions of PLWH from the earlier cohorts were white, while the largest proportions from the more recent cohorts were black. Viral suppression rates for PLWH in care in 2013 ranged from 71-82% across cohorts. Highest suppression was seen for those with a high CD4 count in 2013 (e.g., 80-91% for those with CD4 ≥500 cells/μL vs. 37-56% for those with CD4 <200 cells/μL) (Figure). Within CD4 intervals, there were slightly higher rates of viral suppression in the earlier diagnostic cohorts (e.g., 91% for 1981-1984 diagnoses vs. 80% for 2010-2013 diagnoses with CD4 ≥500 cells/μL) (Figure).

**Conclusions:** Diagnostic cohorts of NYC PLWH in 2013 had similar distributions by sex, age at diagnosis, and transmission risk. They differed by race/ethnicity, though, reflecting the need to address health inequities among people at risk for and newly diagnosed with HIV. Viral suppression rates for PLWH in care were relatively high, regardless of diagnostic cohort. When examined by CD4 interval in 2013, the earlier diagnostic cohorts had higher suppression rates, possibly reflecting a survival advantage or additional time to initiate and remain on treatment. Additional research exploring resilience among earlier diagnostic cohorts is needed.

**Figure: Viral suppression among persons living with HIV as of 12/31/2013 with a VL in 2013 and a CD4 in 2013 by CD4 count and diagnostic cohort, NYC 1981-2013**



**909 The Impact of HIV on Mortality in Nairobi, Kenya**

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**Background:** Declines in HIV prevalence and increases in ART coverage have been documented in Kenya, but mortality associated with HIV has not been directly measured. Adult HIV prevalence in Kenya's capital city of Nairobi is 4.9%. We piloted a surveillance system to monitor HIV-related mortality at two mortuaries in Nairobi, the largest referral hospital and largest county mortuary, which accounted for two-thirds of registered deaths in Nairobi in 2014.

**Methods:** Cadavers from Kenyatta National Hospital (KNH) mortuary and Nairobi City mortuary from Jan 29 – Mar 3, 2015 aged 15 years or older were sampled consecutively for percutaneous cardiac blood collection prior to autopsy. Plasma was screened using the national HIV rapid testing algorithm. Viral load was quantified using the Abbott m2000 Real Time HIV-1 assay. We reviewed medical records for KNH deaths for history of HIV testing. Model-based estimates from Spectrum of people living with HIV (PLHIV) for Nairobi were used to calculate a standardized mortality ratio (SMR) and population-attributable fraction for mortality among the infected vs. uninfected population.

**Results:** Among 610 cadavers tested, the overall HIV prevalence was 19.5% (95% confidence interval [CI] 16.4-22.9%) which differed significantly by sex: 14.6% for males and 29.7% for females ( $p < 0.001$ ), and by mortuary: 12.6% at City and 23.2% at KNH ( $p = 0.002$ ). Of 81 specimens with viral load results, 51.9% (95% CI: 40.5-63.1%) had unsuppressed viral load ( $\geq 1000$  copies/ml). Of 48 HIV-infected KNH deaths with available medical records, 87.5% (95% CI: 74.8-95.3%) had a documented HIV diagnosis. The SMR for HIV infection was 4.12 (95% CI 3.47-4.90), the attributable fraction in PLHIV was 0.753 (95% CI: 0.707-0.792) and the population attributable fraction was 0.148 (95% CI: 0.119-0.177).

**Conclusions:** In spite of a recent reduction in HIV prevalence and 73% of adult PLHIV receiving ART in Nairobi, their risk of death is four-fold greater than in the uninfected, while 14.8% of all adult deaths in the city can be attributed to HIV infection. Higher prevalence at KNH may result from its referral hospital status. Poor viral suppression may indicate many PLHIV who die have not accessed ART or had treatment failure. Routinely estimating the impact of HIV on mortality is feasible and will contribute to understanding the public-health impact of ART.

**910 Access to HIV Care in Health Districts Affected by Ebola Epidemic in Sierra Leone**

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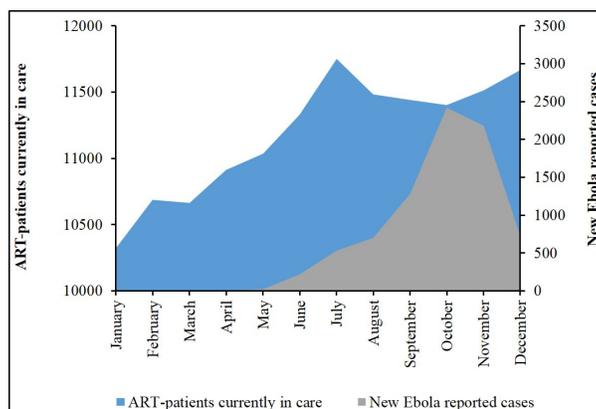
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**Background:** The frequency of deaths caused by the current EVD epidemic among health-care workers severely disrupted supply and quality of routine health activities in West Africa. In addition, cultural beliefs and the fear of being contaminated by the EVD made patients reluctant to seek treatment from health facilities, particularly in areas where EVD cases were diagnosed. In Sierra-Leone, the effect of the current EVD epidemic on the continuity of HIV care has not yet been documented.

**Methods:** The National AIDS Control Programme (NACP) is the specialized programme in charge of monitoring and evaluation of HIV programmes in Sierra Leone. NACP manages a nationwide database including the number of ART patients 'currently on care'. During 2014, 126 HIV facilities reported data to NACP. Missing data for HIV facilities that were still functional during the period was imputed using the multiple imputation method. To assess the impact of the EVD epidemic on the continuity of HIV care during 2014, we first calculated the rate of change (ROC) between successive months of the numbers of ART-patients 'currently in care'. Secondly, we calculated the proportion of the number of months (PNM) in which the ROC was negative during the post-outbreak period. PNM with negative ROC was used to make comparisons between and within health districts. All analysis was made using Stata11.

**Results:** During the year 2014, the number of ART-patients 'currently in care' increased from 10,300 in January to 11,750 in July followed by a slow decline until October (11,400), and a slow increase until December (11,660). The period of July to October corresponds to the peak of the EVD epidemic. The PNM with negative ROC varied within facilities from 0% to 80% and the median PNM with negative ROC was 33% (interquartile range: 17%-50%). The highest PNM with negative ROC was found in 8 HIV facilities located principally in Port Loko, Tonkili, Western rural and Kono, the most affected Ebola health districts. The highest median PNM with negative ROC within health district was at Port Loko health district.

**Conclusions:** Our results support the hypothesis that the decline of ART-patients that were 'currently in care' during 2014 was attributable to the EVD epidemic in Sierra Leone. The impact of Ebola on the continuity of HIV care was variable between and within health districts. This study will contribute to improve future epidemics preparedness.

**911 Effect of Alcohol on All-Cause and Liver-Related Mortality Among Individuals With HIV**

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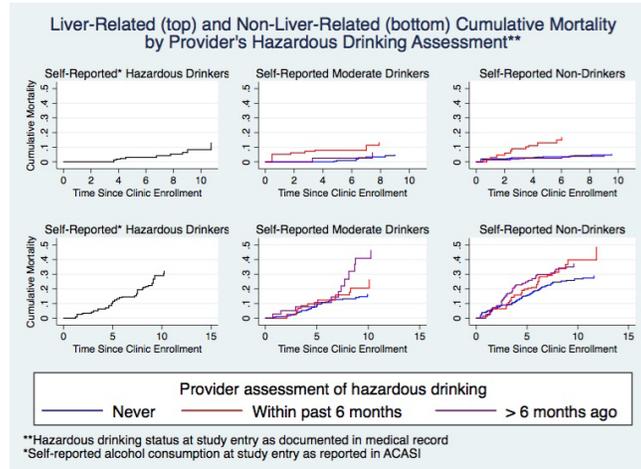
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**Background:** We examined the association between hazardous drinking, all cause and liver-related mortality among HIV-infected individuals.

**Methods:** Study participants included HIV-infected individuals in Baltimore, MD from July 2000 to March 2013. We ascertained alcohol use by two methods: 1) self-report on an Audio Computer Assisted Self Interview (ACASI), and 2) medical record abstraction of a provider documentation of hazardous alcohol use. Self-reported (SR) alcohol consumption was categorized using the NIAAA definition as hazardous (men:  $>4$  drinks/day or  $>14$  drinks/week; women:  $>3$  drinks/day or  $>7$  drinks/week), moderate (any alcohol consumption less than hazardous), and non-drinking. Cause of death was ascertained using national registries. Analyses were conducted using inverse probability weighted survival models. We fit cause-specific regression models and obtained a cumulative incidence of liver-related mortality to account for competing risks. We adjusted analyses for age, sex, race, diabetes, illicit drug use, smoking, HIV transmission risk factor, nadir CD4, ART use, HIV suppression and hepatitis C coinfection.

**Results:** In a study sample of 1855 individuals, all-cause and liver-related mortality rates (MRs) were 43.0 and 4.2 deaths per 1,000 person-years (PY), respectively. All-cause mortality rates were highest among SR non-drinkers with a provider-documented (PD) recent (<6 months) history of hazardous drinking (MR=79.5 deaths per 1,000 PY) and lowest among SR moderate drinkers with no PD history of hazardous drinking (MR=20.5 deaths per 1,000 PY). Cumulative mortality for liver-related and non-liver-related mortality are shown (Figure). Compared to PD never-drinkers, individuals with a PD history of recent hazardous drinking had higher liver-related mortality (HR=6.48, 95% CI 2.82-14.93 and HR=2.82, 95% CI 1.04-7.66 for SR current nondrinkers and moderate drinkers, respectively). However, SR nondrinkers and moderate drinkers with a PD hazardous drinking history of greater than six months ago showed similar rates of liver-related mortality compared to PD never-drinkers (HR=1.23, 95% CI 0.48-3.17 and HR=1.61, 95% CI 0.36-7.19, respectively).

**Conclusions:** Any hazardous alcohol consumption is associated with all-cause mortality among HIV-infected individuals, while only hazardous consumption within the past six-months is associated with liver-related mortality. Helping individuals with hazardous drinking behavior to stop drinking could improve liver-related outcomes for high-risk individuals.



**912 Alcohol Use and Unprotected Sex in HIV-Positive Female Sex Workers in Mombasa**

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**Background:** High levels of alcohol use have been reported in people living with HIV (PLHIV). In addition to adverse health effects, alcohol use could facilitate HIV transmission if it is associated with higher rates of unprotected sex. A number of studies have demonstrated associations between alcohol use and sexual risk behavior, but most have been cross-sectional, relied on self-reported indicators of risk, and used non-standard measures of alcohol use. Few studies have focused on HIV-positive women. We used data from a prospective cohort of HIV-positive female sex workers (FSWs) to test the hypothesis that hazardous or harmful alcohol use is associated with unprotected vaginal sex

**Methods:** Longitudinal data were collected from a cohort of HIV-positive women in Mombasa who reported transactional sex. Hazardous or harmful alcohol use was defined as a score of 7 or higher on the AUDIT, and was measured annually. Unprotected sex was measured by detection of prostate specific antigen (PSA) in vaginal secretions at quarterly examination visits. Generalized estimating equations were used to calculate associations between hazardous or harmful alcohol use in the past year and detection of PSA.

Exploratory analyses examined associations with self-reported indicators of sexual risk and STIs.

**Results:** A total of 2,744 visits with PSA samples were accrued by 402 women over 605 person-years of follow-up. In univariate analysis, hazardous or harmful alcohol use was associated with a 50% higher risk of PSA detection (Table 1). This association was attenuated, and no longer statistically significant, after adjusting for age, work venue, intimate partner violence, depression, and partnership status. In exploratory analyses, hazardous or harmful alcohol use was associated with self-report of unprotected sex after adjusting for potential confounding factors. In addition, low-risk alcohol use was associated with a significantly higher risk of STI acquisition.

**Conclusions:** In this longitudinal cohort of HIV-positive FSWs, hazardous or harmful alcohol use was associated with detection of PSA, but this association was confounded by age and other demographic and social covariates. However, reporting any alcohol use was strongly associated with STI acquisition in multivariable analysis. Randomized trials of interventions that successfully reduce alcohol intake are needed to determine whether lowering alcohol use reduces STI incidence and HIV transmission risk.

**Table 1: Univariate and multivariable GEE regression estimates for associations between hazardous or harmful alcohol use in the past year and sexual risk outcomes among 402 HIV-positive female sex workers in Mombasa, Kenya**

	Univariate regression estimates		Multivariable regression estimates <sup>a</sup>	
	Risk ratio (95% CI)	p-value	Risk ratio (95% CI)	p-value
<b>Outcome: PSA detection</b>				
No alcohol use	REFERENCE		REFERENCE	
Low risk alcohol use	1.20 (0.92, 1.58)	0.2	1.08 (0.83, 1.41)	0.6
Hazardous or harmful alcohol use	1.49 (1.11, 2.00)	0.008	1.12 (0.81, 1.55)	0.5
<b>Outcome: Self-reported unprotected sex</b>				
No alcohol use	REFERENCE		REFERENCE	
Low risk alcohol use	1.67 (1.08, 2.59)	0.02	1.46 (0.95, 2.25)	0.08
Hazardous or harmful alcohol use	2.59 (1.71, 3.93)	<0.001	1.62 (1.04, 2.51)	0.03
<b>Outcome: Sexually transmitted infection</b>				
No alcohol use	REFERENCE		REFERENCE	
Low risk alcohol use	2.61 (1.83, 3.73)	<0.001	2.30 (1.56, 3.38)	<0.001
Hazardous or harmful alcohol use	2.02 (1.34, 3.05)	0.001	1.56 (0.96, 2.55)	0.08

<sup>a</sup>Adjusted for priori confounders (age, venue of work, ever experience of IPV, and depression) plus partnership status; statistically significant ( $\alpha=0.05$ ) p-values are bolded.

**913 Chemsex and High-Risk Sexual Behaviours in HIV-Positive Men Who Have Sex With Men**

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**Background:** Over the past decade in the UK, HIV incidence has been increasing among men who have sex with men (MSM). One proposed reason is a recent rise in “chemsex”: the use of drugs to increase sexual disinhibition and arousal. This has the potential to increase the risk of transmission of HIV and other sexually transmitted infections (STIs). We used a nationally-representative survey to determine the prevalence of, and risk factors for, chemsex in HIV-positive MSM in the UK.

**Methods:** Data were collected as part of the Positive Voices study, a cross-sectional survey, conducted in people attending 30 UK HIV clinics from May - November 2014. Responses were linked to clinical data from national surveillance records and weighted to be representative of the national HIV population accessing care. Analyses were restricted to sexually active MSM. Using multivariable analysis, we assessed factors associated with chemsex, and associations between chemsex and unprotected anal intercourse (UAI), UAI with partners of negative or unknown HIV status (sdUAI), multiple casual partnerships, and diagnosis with an STI or hepatitis C.

**Results:** Of a total 526 MSM responses (39% response rate), 387 (74%) had been sexually active in the previous year. Of these, 29% (95% CI: 21-39%) had engaged in chemsex in the previous year. The most commonly reported chemsex drugs were MCAT (23%; 95% CI: 15-32%), GHB/GBL (20%; 95% CI: 14-27%), and crystal meth (15%; 95% CI: 11-22%). Chemsex was independently associated with being aged 35 to 54, living in London, smoking, diagnosed depression/anxiety, and drug use outside of sex (Table 1). MSM who practiced chemsex had significantly higher odds of engaging in UAI (91% vs. 62%; AOR: 5.3; 95% CI: 1.9-15) and sdUAI (44% vs. 27%; AOR: 2.0; 95% CI: 1.04-3.9) in the previous year, of diagnosis with an STI (71% vs. 39%; AOR: 3.4; 95%CI: 1.7-6.8) and hepatitis C (23% vs. 3.8%; AOR: 6.1; 95% CI: 2.0-18), and had more casual partners in the past year (mean: 33 vs. 8.3; difference: +17; 95% CI: 12-22).

**Conclusions:** A quarter of HIV-positive MSM reported chemsex, which was associated with increased risky sexual behaviours and STI and hepatitis C diagnosis. This is the first nationally representative study to show this association and highlights the need for funding of interventions to address the risk of HIV and STI transmission among MSM combining drugs with sex.

Table 1. Risk profile for chemsex among HIV-positive MSM in the UK

	N	Chemsex		
		%	AOR <sup>†</sup>	
Age group	18-34	62	20.1%	1
	35-44	96	33.3%	1.98 (1.15-3.41)*
	45-54	144	35.1%	2.15 (1.30-3.55)**
	≥50	85	18.9%	0.92 (0.46-1.84)
Region of Birth	UK	292	28.1%	1
	Europe	53	38.7%	1.62 (0.86-3.06)
	Africa	8	5.3%	0.14 (0.02-1.05)
	Other	34	28.8%	1.03 (0.53-2.02)
Education	Up to GCSE (or equivalent qualification at 16)	85	25.7%	1
	A-levels (or equivalent qualification at 18)	74	31.9%	1.30 (0.81-2.09)
	Undergraduate	117	28.9%	1.20 (0.68-2.12)
	Postgraduate	94	28.9%	1.12 (0.69-1.83)
	Other	12	35.8%	1.63 (0.33-7.94)
Employment status	Employed full time	230	27.8%	1
	Employed part time	41	43.7%	2.02 (0.97-4.19)
	Student	7	37.3%	2.13 (0.38-12.0)
	Retired	34	16.1%	0.78 (0.18-3.37)
	Sick/disabled/carer	47	24.2%	0.84 (0.46-1.54)
	Unemployed	21	34.8%	1.08 (0.50-2.36)
	Other	7	22.8%	0.61 (0.04-9.66)
Housing	Own property	196	23.4%	1
	Renting (private landlord)	104	38.2%	1.87 (0.83-4.18)
	Renting (council or housing association)	68	23.5%	0.62 (0.26-1.45)
	Homeless	0	N/A	N/A
	Other	19	41.4%	3.73 (0.95-14.7)
Living in London	No	197	17.3%	1
	Yes	190	37.2%	2.92 (1.26-6.76)*
Has a main partner	No	136	29.5%	1
	Yes	243	28.3%	0.87 (0.58-1.31)
ART status	Not on ART	36	34.2%	1
	On ART	350	28.5%	0.61 (0.24-1.53)
Diagnosed with depression or anxiety	No	252	24.0%	1
	Yes	135	38.4%	2.30 (1.15-4.60)*
Current smoker	No	262	23.6%	1
	Yes	122	38.8%	1.61 (1.04-2.49)*
Hazardous drinking (as defined on the AUDIT-C scale)	No	170	31.8%	1
	Yes	182	27.7%	0.82 (0.41-1.62)
Non-sexual illicit drug use	No	184	12.7%	1
	Yes	203	43.7%	5.03 (2.74-9.24)***

\*p <0.05, \*\*p<0.01, \*\*\*p<0.001

<sup>†</sup>AOR: Adjusted odds ratio controlling for other variables in table as appropriate (based on causal diagram)

## 914 Testosterone Use and HIV Serostatus Among Men Who Have Sex With Men in the MACS

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**Background:** Testosterone therapy (TTh) use has increased markedly in the U.S over the past decade, with current prevalence of about 3% among men >40 years. Given the possible cardiovascular risks associated with TTh use and high burden of cardiovascular disease (CVD) in HIV-infected (HIV+) men, data on the levels and reasons for TTh use in this population are needed. We describe the incidence, prevalence, and characteristics of TTh use among HIV+ and HIV-uninfected (HIV-) men who have sex with men (MSM) in the Multicenter AIDS Cohort Study (MACS).

**Methods:** Data on self-reported testosterone use was collected semiannually since 2012. Our analytic sample included 2319 (1223 HIV+/1096 HIV-) men in Baltimore, Chicago, Pittsburgh, and Los Angeles who completed one or more study visits from 2012-2014. We calculated incident and prevalent TTh use and compared reasons for TTh use by HIV serostatus using Chi-square tests. We used multivariable Poisson regression models with robust variance to estimate prevalence ratios (PR) for TTh use by HIV serostatus and demographic factors.

**Results:** Compared to the HIV- men, HIV+ men were older (median and intraquartile range 59 years (53-66) v 54 years (46-60)) and more likely to be non-white (47% v 27%). TTh prevalence at the most recent visit among HIV+ compared to HIV- men was nearly 4-fold higher in men aged 60 and older (26% v 7%, p<0.001), and almost 3-fold higher among men younger than 60 (18% v 7%, p<0.001 among 50-59 years; 6% v 2%, p=0.03 among <50 years). The TTh initiation rate from 2012-2014 was 21.4/1000 person years. Among the 266 men (197 HIV+/69 HIV-) on TTh, the major self-reported reason for use was low testosterone (88%). HIV+ men were more likely than HIV- men to use TTh to improve strength or energy (36% v 22%, p=0.04), build muscle (26% v 7%, p=0.001), or combat wasting (15% v 1%, p<0.01). In multivariable analysis, the prevalence of TTh was 3.8 times greater in HIV+ compared to HIV- men (p<0.001), and was significantly less prevalent among non-white men (Table 1). We observed strong geographic differences, with the prevalence of TTh use 2.7 and 1.7 times higher among LA and Baltimore men, respectively, compared to Pittsburgh men.

**Conclusions:** MSM in the MACS reported very high rates of TTh use, particularly among older HIV+ men. Given the high TTh use and CVD burden among HIV+ men, the benefits and risks of TTh use should be carefully examined in future studies and closely monitored in clinical practice.

Table 1: Demographic factors associated with testosterone therapy use among 2,318 HIV-infected and HIV-uninfected men in the MACS, 2012-2014

	N	Percent on TTh	Prevalence Rate Ratio	95% Confidence Intervals
HIV-infected	1,222	16.1%	3.82 <sup>****</sup>	[2.93, 4.99]
Age (per 10 years)			1.16 <sup>*</sup>	[1.01, 1.34]
Race				
White	1,445	14.1%	1 (ref)	
Hispanic	316	9.8%	0.83	[0.56, 1.24]
Black	557	5.6%	0.58 <sup>***</sup>	[0.39, 0.87]
MACS cohort entry				
Before 2001	1,272	16.5%	1 (ref)	
2001 and later	1,046	5.4%	0.39 <sup>****</sup>	[0.26, 0.59]
Site				
Pittsburgh	596	6.0%	1 (ref)	
Baltimore	574	10.5%	1.66 <sup>**</sup>	[1.12, 2.45]
Chicago	422	10.2%	1.47	[0.96, 2.24]
Los Angeles	726	17.5%	2.69 <sup>****</sup>	[1.89, 3.81]

Model adjusted for all listed covariates and was fit using Poisson regression with robust variance. One participant declined to provide racial category and was excluded from the multivariable analysis.

\* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001

## 915 Prevalence of Opioid Prescriptions and Risk Factors for Abuse in HIV-Infected Adults

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**Background:** Prescription of opioid pain relievers (OPR) has been increasing in the United States, leading to increased opportunities for OPR abuse and overdose deaths. OPR abuse has been linked to HIV infection and transmission, as evidenced by a recent HIV outbreak in Indiana. Knowing the percentage and attributes of HIV-infected persons who were prescribed OPR may help to identify opportunities for prevention of OPR abuse and related HIV transmission.

**Methods:** We used 2009–2013 cycle data from the Medical Monitoring Project, a surveillance system designed to provide nationally representative estimates of clinical and behavioral characteristics of HIV-infected persons receiving medical care in the United States. Using interview and medical record data from 23,125 persons, we estimated the weighted prevalence of OPR prescriptions during the 12 months prior to interview. We assessed associations between OPR prescription and several risk factors for opioid abuse – mental illness, drug use, and low income – using Rao-Scott chi-square tests. Mental illness was defined as medically diagnosed depression or anxiety, and drug use was defined as injection or non-injection drug use in the previous 12 months.

**Results:** Overall, 20.8% (95% CI 19.3 – 22.3) of patients had at least one OPR prescription documented in their medical record during the previous 12 months. Patients with anxiety or depression were more likely to be prescribed OPRs than those without either condition (31.6% vs 16.2%; P<0.01). Patients living below the federal poverty level were more likely to be prescribed OPRs than those living above the federal poverty level (23.2% vs 17.8%; P<0.01). Patients who reported injection or non-injection drug use were more likely to be prescribed OPRs than those who did not use drugs (23.4% vs 19.9%; P<0.01). Patients who reported injection drug use were more likely to be prescribed OPRs than those who did not use injection drugs (37.2% vs 20.5%; P<0.01) (Table 1).

**Conclusions:** The recent HIV outbreak in Indiana highlights the connection between opioid abuse and HIV transmission. One fifth of HIV-infected persons receiving medical care in the United States during 2009–2013 were prescribed OPRs in the year before interview, and one-third of HIV-infected persons with anxiety or depression had been prescribed OPRs. Increased counseling and monitoring of HIV-infected persons prescribed OPRs for medical purposes may reduce the potential for OPR abuse and HIV transmission.

**Table 1. Characteristics of HIV-infected Adults in Care Receiving Opioid Prescriptions in the United States: Medical Monitoring Project, 2009-2013**

Characteristic	Opioid prescription (n = 4,858) % (95% CI)	No opioid prescription (n = 18,267) % (95% CI)	P for Rao-Scott chi-square test
<b>Anxiety/Depression<sup>1</sup></b>			
Yes	31.6 (29.3 – 33.9)	68.4 (66.1 – 70.7)	< 0.0001
No	16.2 (15.1 – 17.3)	83.8 (82.7 – 84.9)	
<b>Level of poverty<sup>1</sup></b>			
At or below poverty	23.2 (20.8 – 25.6)	76.8 (74.4 – 79.2)	< 0.0001
Above poverty	17.8 (16.0 – 19.7)	82.2 (80.3 – 84.0)	
<b>Any drug use<sup>1,2</sup></b>			
Yes	23.4 (21.8 – 25.0)	76.6 (75.0 – 78.2)	< 0.0001
No	19.9 (18.3 – 21.5)	80.1 (78.5 – 81.7)	
<b>Injection drug use<sup>1,2</sup></b>			
Yes	37.2 (32.4 – 41.9)	62.8 (58.1 – 67.6)	< 0.0001
No	20.5 (19.0 – 22.0)	79.5 (78.0 – 81.0)	

<sup>1</sup>In the past 12 months

<sup>2</sup>Injection or non-injection drug use for non-medical purposes, including one or more of the following substances: heroin, cocaine, methamphetamines, prescription drugs, hallucinogens, ecstasy, ketamine, GHB, marijuana, amyl nitrate, and steroids or hormones

**916 Risk Factors for HIV in an Outbreak Among Persons Who Inject Drugs, Indiana 2015**

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**Background:** In early 2015, an outbreak of HIV infections among persons who inject drugs (PWID) was identified in Scott County in rural southeastern Indiana. Investigation indicated that by July 31, 2015, a single strain of HIV had infected over 170 persons in a matter of months. To identify risk factors for HIV infection and inform control efforts for this unprecedented event, we conducted a cohort study of HIV-positive and HIV-negative PWID in this community.

**Methods:** We included residents of Scott County who had been named syringe-sharing contacts by an HIV-infected person diagnosed from Oct 1, 2014 to July 31, 2015 and were investigated and HIV tested as part of contact tracing efforts through August 6, 2015. We conducted bivariate analysis of routinely collected self-reported sociodemographic and behavioral data by HIV status. Chi-square and Kruskal-Wallis tests were used to test for differences between categorical and continuous variables, respectively. Variables that differed significantly by HIV test status (p<0.05) were examined as potential risk factors. Log-binomial regression modeling with stepwise backwards elimination was used to estimate adjusted risk ratios (adj-RR) and 95% confidence intervals (95% CIs) for potential risk factors.

**Results:** Of 315 investigated contacts named as syringe-sharing partners, 196 (62%) were both HIV tested and had demographic and behavioral data available: 51% tested HIV-positive. The cohort was 58% male, 98% non-Hispanic white, and had a median age of 33 years. Among cohort members, the annual income was <\$10,000 for 92%, and 54% had been incarcerated in the past 12 months. HIV risk behaviors were highly prevalent (Table 1). Sharing injection equipment, sex with a person known to be HIV-positive, and sex with a PWID were substantially more frequent among persons who tested HIV-positive than among those who did not, but these differences were not statistically significant. Only the number of times that a person had been named by an HIV-infected person as a syringe-sharing contact was significantly associated with testing HIV-positive: adj-RR (per each time named as syringe-sharing partner): 1.90, 95% CI: 1.49-2.41.

**Conclusions:** In HIV outbreaks linked to injection drug use, investigation and control efforts should prioritize persons for investigation and HIV testing according to their extent of syringe sharing as indicated by the frequency with which they are named syringe-sharing contacts by HIV-infected persons.

Table 1. Reported behaviors among PWID by HIV status, Indiana Outbreak 2015 (n=196)

	HIV-positive (n=100)	HIV-negative (n=96)	p-value <sup>c</sup>	adj-RR (95% CI) <sup>d</sup>
Annual income <\$10,000 <sup>a</sup>	85 (93)	73 (90)	0.43	--
Incarcerated in last year <sup>a</sup>	52 (53)	52 (56)	0.64	--
Shared drug injection equipment	75 (75)	63 (67)	0.15	--
Sex with HIV-positive person	28 (28)	16 (17)	0.06	--
Sex with a PWID	68 (68)	57 (59)	0.21	--
Number of times named as syringe-sharing partner <sup>b</sup>	4 (2-6)	1 (1-2)	<0.001	1.90 (1.49-2.41)
Sex without a condom	86 (86)	81 (84)	0.75	--
Sex with someone who exchanges sex for money/drugs	11 (11)	7 (7)	0.37	--
Sex for money or drugs	9 (9)	8 (8)	0.87	--

<sup>a</sup> Missing (n, %): Annual income (24, 12%); Incarcerated (4, 2%); <sup>b</sup> Presented as median (interquartile range); distribution for all other behaviors presented as n (%); <sup>c</sup> p-value for difference; <sup>d</sup> risk ratio per each time named as syringe-sharing partner, adjusted for sharing drug injection equipment and sex with HIV-positive person

**917 Cross-Sectional vs Longitudinal HIV Incidence Estimates in People Who Inject Drugs**

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**Background:** HIV incidence can be estimated in cross-sectional surveys and longitudinal cohorts. We compared HIV incidence at enrollment (baseline) and during follow-up in a cohort of people who inject drugs (PWID) in Baltimore, MD. We explored potential explanations for the any difference incidence (differential loss to follow-up or behavioral modification).

**Methods:** Samples were obtained from the AIDS Link to Intravenous Experiences (ALIVE) cohort: 2,938 PWID at baseline (1988-89; 89% Black, 81% male) and 1,772 individuals at the first follow-up visit (4-12 months post enrollment, 92% Black, 79% male). All participants were asked questions about demographics, injection drug use and sexual practices. Blood was drawn at each time point for HIV screening. HIV incidence was determined cross-sectionally at baseline using a multi assay algorithm and longitudinally at follow-up. Differential lost to follow-up and behavioral characteristics were analyzed between baseline and the 1<sup>st</sup> follow-up visit (occurred 4-12 months past baseline).

**Results:** Of the 2,938 individuals, 673 were HIV positive at baseline. One hundred and six appeared recently infected resulting in an HIV incidence estimate of 12.9/100 person-years (95% CI: 10.1-15.7) at baseline. Among the 1,722 individuals who had a follow-up time point between 4-12 months post enrollment, 1,197 were initially HIV negative and 40 seroconverted by their 1<sup>st</sup> visit (longitudinal HIV incidence of 5.4/100 person-years [95% CI: 3.9-7.4]). At baseline, 82% (87/106) of the recent group and 35% (14/40) in the seroconverter group were male. Additionally, those <30 years of age had the highest HIV incidence, 18.5/100 person-years (95% CI: 12.7-24.3) at baseline, and at 1<sup>st</sup> follow-up (9.2 [5.2-15.2]). Drug use practices (injecting cocaine, using speed ball, injecting at a shooting gallery, sharing needles) decreased 50% between baseline and follow-up.

**Conclusions:** Annual HIV incidence in this cohort of PWID was much higher in the months before enrollment than in the first year of the study (12.9% vs. 5.4%). At baseline, most of those with recent infection were men; in contrast, the seroconversion rate was higher in women than men after enrollment. The difference in HIV incidence at vs. after enrollment was not explained by differential loss-to-follow-up, but may have been explained by a decline in drug use practices after study enrollment.

**918 Gender-Specific Factors Related to HIV Risks Among People Who Inject Drugs in India**

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**Background:** People who inject drugs (PWIDs) in India are at high risk for HIV infection and transmission, with women being at elevated risk. Research is needed to examine gender differences in factors contributing to HIV risk. Using a socio-ecological framework, this study identified factors at individual, relationship, and community/institutional levels that were differentially or similarly associated with HIV risk behaviors in men and women PWIDs.

**Methods:** 6453 PWIDs (5677 men and 797 women) from 7 cities in North-east India were recruited using a respondent-driven sampling. Participants completed a survey and point-of-care HIV testing. We used multi-level logistic regression models to assess factors associated with 2 injection-related (daily use and sharing needles/syringes) and 3 sex-related (multiple partners, exchange sex and unprotected sex) HIV risk behaviors separately in men and women.

**Results:** Women (52.8%) were more likely to be HIV positive than men (18.4%). Women were significantly more likely to report multiple recent sex partners, whereas men were significantly more likely to report recent exchange sex. Among women, factors associated with HIV risks included: younger age (*aOR*=0.76 per 5 years older, *CI*=0.61-0.93), earlier initiation of injection drug use (*aOR*=0.70 per 5 years older, *CI*=0.57-0.87), low education (*aOR*=2.05, *CI*=1.09-3.84), marital status (*aOR*=1.87, *CI*=1.13-3.09), and frequent financial stress (*aOR*=2.32, *CI*=1.46-3.69). In contrast, for men, factors associated with HIV risks were older age (*aOR*=1.22 per 5 years older, *CI*=1.03-1.45), later initiation of injection drug use (*aOR*=1.24 per 5 years older, *CI*=1.10-1.38), and higher levels of internalized stigma (*aOR*=1.42, *CI*=1.09-1.84) and enacted stigma (*aOR*=2.56, *CI*=1.77-3.69).

**Conclusions:** The findings highlight the need for gender-specific contextually-integrated HIV prevention and intervention efforts among PWIDs. For example, programs that include economic empowerment, and build skills in addressing gender power dynamics in safe sex negotiation may be effective in preventing HIV among women. Stigma free services and programs that include coping skills training and mental health counseling to address the negative attitudes and behaviors resulting from stigma, may reduce HIV risk, particularly among men.

**919 Depression and Social Isolation Mediate Effect of HIV Stigma on Women’s ART Adherence**

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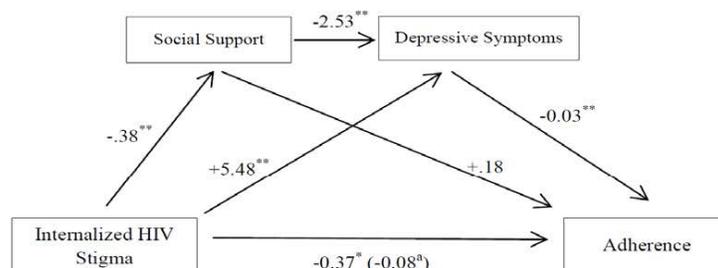
**Background:** Internalization of HIV-related stigma may inhibit a person’s ability to manage HIV disease through adherence to treatment regimens. Studies, mainly with white men, have suggested an association between internalized HIV-related stigma and suboptimal antiretroviral therapy (ART) adherence. However, there is a scarcity of research with women of different racial/ethnic backgrounds and on mediating mechanisms in the association between internalized HIV-related stigma and ART adherence.

**Methods:** The Women’s Interagency HIV Study (WIHS) is a multi-center cohort study. Women living with HIV complete interviewer-administered questionnaires semi-annually. Cross-sectional analyses for the current article included 1168 women on ART for whom data on medication adherence were available from their last study visit between April 2013 and March 2014, when the measure of internalized stigma was initially introduced.

**Results:** The association between internalized HIV stigma and self-reported sub-optimal ART adherence was significant for those in racial/ethnic minority groups (*AOR* = 0.689, *p* = .009, *CI* [0.521,0.911]), but not for non-Hispanic whites (*AOR* = 2.150, *p* = .188, *CI* [0.687,6.725]). Depressive symptoms, loneliness, and low perceived social support mediated the association between internalized stigma and non-adherence in the whole sample, as well as in the subsample of minority participants. In serial mediation models, internalized stigma predicted less perceived social support (or higher loneliness), which in turn predicted more depressive symptoms, which in turn predicted sub-optimal medication adherence (see Figure 1).

**Conclusions:** These findings suggest that interconnected psychosocial mechanisms affect ART adherence, and that improvements in adherence may require multi-faceted interventions addressing both mental health and interpersonal factors, especially for minority women.

Figure 1. Serial mediation model in the association between HIV-related internalized stigma and worse medication adherence for racial/ethnic minority groups (i.e., non-whites). n = 1029.



Note. Associations are presented as path coefficients (unstandardized).

<sup>a</sup> When social support and depressive symptoms are in the model.

\* *p* < .05; \*\* *p* < .01

**920 Depression Increases the Risk of Mortality in a Large Cohort of HIV-Infected Adults**

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**Background:** Depression affects 20-30% of HIV-infected adults and contributes to suboptimal antiretroviral therapy (ART) adherence and ability to maintain viral suppression. Reduced ART adherence and viral suppression associated with depression may lead to an increased risk of mortality. Depression may also independently predict an increased risk of mortality. However, the relationship between depression and mortality remains understudied in large, representative clinical cohorts of adults in HIV care.

**Methods:** We estimated the association between depression and all-cause mortality using data from CNICS, a cohort of over 30,000 HIV-infected adults engaged in HIV care across 8 sites in the United States. CNICS collects comprehensive HIV clinical information on ART use, adherence, HIV-related labs and clinical events and vital status. Depression information in CNICS is collected by self-report using the Patient Health Questionnaire-9 (PHQ-9) at 7 of the 8 sites. Participants were included in the present analysis if they entered care between October 2004 and November 2014 and had a PHQ-9 measure within 1 year. Vital status was ascertained for all individuals. Participants were followed until death or administrative censoring (May-November 2014, depending on site), whichever date came first. Depression was defined as a PHQ-9 ≥ 10. We used Cox proportional hazards models to estimate the association between depression within 1 year of entering care and time to all-cause mortality.

**Results:** 4,029 adults were included for a total follow-up of 10,242 person years. Nearly a third of adults (n=1,246, 31%) met the definition for depression during their first year after enrollment. Overall, 122 (3%) of participants died during the follow-up period. Depression increased the hazard of mortality by 68% (HR 1.68; 95% CI 1.09, 2.59), after adjustment for site, gender, race/ethnicity, HIV acquisition risk group and age, alcohol dependence, panic disorder, ART status and adherence, viral load, and CD4 count at enrollment.

**Conclusions:** Depression increased the hazard of mortality among HIV-infected adults in care even after adjustment for adherence, CD4 count, viral suppression at enrollment and a number of other clinical and demographic factors. Interventions to improve depression treatment and reduce depression are urgently needed to reduce the risk of mortality among HIV-infected persons.

**921 HIV Prevalence and Risk Factors in Men Who Have Sex With Men in Bamako, Mali**

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**Background:** HIV prevalence in Mali is 1.1% and highest in Bamako where it is 1.6% among men. While the epidemic is concentrated in key populations, no data were available on HIV prevalence and risk-behaviors of men who have sex with men (MSM).

**Methods:** Using respondent driven sampling, we conducted a cross-sectional survey among MSM in Bamako between October 2014 and February 2015. Eligibility criteria included being ≥18 years old, residence in Bamako or its suburbs, and having had sex with another man in the last 6 months. Participants underwent a face-to-face interview and then were counseled and tested for HIV. Weighted data analysis was conducted with RDSAT and SAS. Survey logistic procedures were used to identify factors associated with HIV in multivariate analyses, controlling for age and education.

**Results:** We enrolled 552 MSM of which 550 (99.6%) consented to be tested for HIV. MSM in Bamako were young (65.6% were ≤24 years old) and the majority were educated (47.3% had secondary and 16.4% had university education). One-fourth (24.0%) had unprotected anal intercourse with last sexual partner and 47.1% had tested for HIV in the past 12 months. HIV prevalence among MSM in Bamako was 13.7%. Of the HIV-positive MSM, only 13.3% were already aware of their HIV status. One-third (30.4%) of the newly diagnosed HIV+ thought it was not possible for them to be HIV-positive. Of the 15 participants who already knew they were HIV-positive, 11 had enrolled in care and 9 were on treatment. Factors independently associated with HIV infection included: working as an unskilled laborer (vs. not working; aOR 10.8, 95%CI: 1.6-72.7), age of first male sexual partner >25 years vs. 15-24 years (aOR 10.1, 95%CI:3.8-27.2), being the receptive partner (aOR 48.5, 95%CI:10.6-221.0) or being both receptive and insertive (aOR 7.1, 95%CI:1.8-27.4) with the last sexual partner, having STD symptoms in the past 12 months (aOR 4.2, 95%CI:1.6-10.9), knowing other HIV-positive MSM (aOR 5.2, 95%CI:1.7-15.9), and inability to access condoms when needed in the past 6 months (aOR 3.5, 95%CI:1.4-8.9). Identifying as female or transgender was protective against HIV infection (aOR 0.3, 95%CI:0.1-1.0).

**Conclusions:** HIV prevalence among MSM in Bamako is 9 times higher than men in general population. Testing and awareness of HIV infection are very low. The results suggest the need for enhanced HIV services targeted for MSM in Bamako to increase HIV testing and expand the availability of free condoms.

Table 1. RDS weighted associations between HIV infection and selected variables among MSM in Bamako (N=550)

	aOR	(95% CI)	
Employed as an unskilled laborer (vs. not working)	10.8	1.6	72.7
Identifies as female/transgender (vs. male)	0.3	0.1	1.0
First male sexual partner was 25 years or older (vs. 15-24 years old)	10.1	3.8	27.2
Was receptive partner (vs. only insertive) during most recent sexual encounter	48.5	10.6	221.0
Was both insertive and receptive partner (vs. only insertive) during most recent sexual encounter	7.1	1.8	27.4
Reported having STD symptoms in the past 12 months	4.2	1.6	10.9
Reported knowing other HIV+ MSM	5.2	1.7	15.9
Reported being unable to get condoms when needed in past 6 months	3.5	1.4	8.9

**922 Stigma, Access to Care, and HIV Among Men Who Sell Sex in Nigeria**

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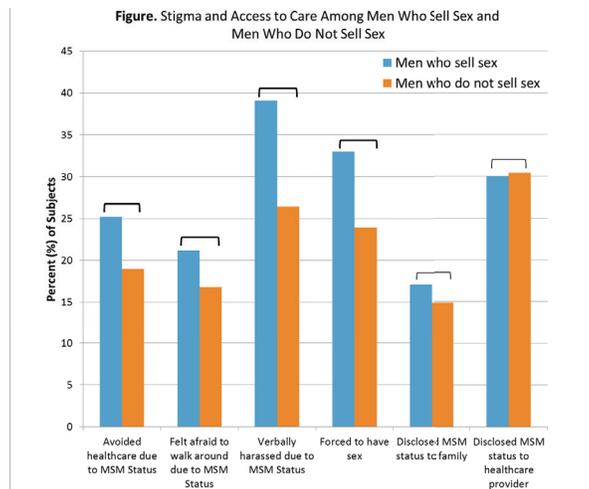
<sup>1</sup>US Military HIV Rsr Prog, Walter Reed Army Inst of Rsr, Silver Spring, MD, USA; <sup>2</sup>Walter Reed Prog - Nigeria, Abuja, Nigeria; <sup>3</sup>Johns Hopkins Bloomberg Sch of PH, Baltimore, MD, USA; <sup>4</sup>Inst of Human Virology, Baltimore, MD, USA; <sup>5</sup>Pop Council Nigeria, Abuja, Nigeria

**Background:** Among men who have sex with men (MSM), men who sell sex to other men (MSS) may be subject to intersectional or compounded stigma that may affect access to healthcare and risk of HIV. The objectives of this study were to characterize stigma, access to care, and prevalence of sexually-transmitted infections (STIs) among MSS in Nigeria.

**Methods:** TRUST/RV368 is a cohort of MSM recruited in Abuja and Lagos, Nigeria, using respondent-driven sampling. Participants are screened for HIV and other STIs and undergo a structured interview to collect detailed data on behaviors, experiences, healthcare engagement and other factors. For this analysis, participants are categorized based on self-report of having received goods or money in exchange for sex with other men in the preceding year. Comparisons are made using Pearson's chi-squared test, t-test, and logistic regression.

**Results:** From March 2013–August 2015, 1374 men (908 in Abuja; 430 in Lagos) answered baseline questionnaires about transactional sex and 660 (48.0%) reported selling sex in the preceding year with a median of 3 (range 1–350) paying partners. Compared to men who had not sold sex, men who sold sex tended to be younger (median 22.7 vs. 25.3 years,  $p < 0.001$ ), more likely to self-identify as gay/homosexual (42.9% vs. 30.4%,  $p < 0.001$ ), and less likely to have progressed beyond secondary education (19.2% vs. 38.5%,  $p < 0.001$ ). Men who sold sex were more likely to have avoided healthcare ( $p = 0.007$ ), felt afraid in public ( $p = 0.040$ ), been verbally harassed ( $p < 0.001$ ), and been forced to have sex ( $p < 0.001$ , Figure). No differences were noted in rates of disclosing MSM status to family ( $p = 0.270$ ) or healthcare providers ( $p = 0.690$ ). Among 937 men with HIV test results, 455 (48.6%) were positive, including 45.8% of men who sold sex and 51.3% of men who had not sold sex ( $p = 0.096$ ). The age-adjusted odds ratio of HIV infection among men who sold sex compared to men who had not sold sex was 0.91 (95% confidence interval 0.70–1.18). No differences were observed in the prevalence or age-adjusted odds of chlamydia or gonorrhea.

**Conclusions:** There appears to be intersectional stigma related to both the selling of sex and same-sex practices among these MSS that may compound the effects of stigma and limit uptake of health care as compared to other MSM. Given that these men appear to be distinct from other MSM, specific interventions may be needed to improve access to HIV treatment and retention in care among this key population in Nigeria.



### 923 Incidence of STIs Among MSM Engaged in Treatment as Prevention in Nigeria

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**Background:** Concurrent sexually transmitted infections (STIs) diminish the effectiveness of the preventative effects of antiretroviral therapy (ART). In a Nigeria-based men who have sex with men (MSM) cohort (TRUST/RV368) we investigated whether clients who achieved effective HIV viral control also demonstrate parallel adherence to beneficial behavior change and whether this change impacts STI incidence.

**Methods:** MSM were recruited in Abuja and Lagos, Nigeria by respondent driven sampling. ART use, viral loads, STIs, and condom use were assessed at enrollment and every 3 months. HIV + clients were categorized into three groups: 1) <200 copies/ml; ART<200, 2) >200 copies/ml; ART>200 and 3) non-ART. Condom use, prevalence and incidence of rectal and urethral chlamydia (CT) and gonorrhea (GC) were analyzed by treatment group using chi-squared statistics and Poisson regression models.

**Results:** From March 2013–August 2015, HIV-infected individuals (135 ART <200; 80 ART >200, 30 non-ART) contributed 245 baseline and 758 follow-up visits. Condom use during receptive sex increased during follow-up (FU) for ART users [(ART <200 (baseline, FU: 3.6, 4.3;  $p < 0.01$ ) (ART >200 (3.4, 4.2;  $p < 0.01$ ), but not for non-ART (3.9, 3.9;  $p = 0.71$ ). Similarly, condom use with insertive sex increased for ART users (<200, >200) as compared to non-ART during follow-up ( $p < 0.01$ ,  $p < 0.01$ ,  $p = 0.70$ ).

The combined prevalence of rectal CT and GC at baseline was 26.9% in ART <200, 40% in ART>200, and 50% in non-ART ( $p = 0.02$ ). The prevalence of urethral STIs was 5.3%, 8.8%, and 13.3% ( $p = 0.27$ ). The crude incidence of rectal STIs was 46.2 per 100 person-years (PY) among ART <200, 43.2/100 PY among ART >200, and 32.7/100 PY among non-ART ( $p = 0.73$ ,  $p = 0.28$ ).

The crude incidence of urethral STIs was 5.8/100 PY, 5.4/100 PY, and 12.2, respectively ( $p = 0.90$ ,  $p = 0.22$ ). In a stratified analysis, incidence of rectal STIs remained high regardless of consistent condom use with receptive sex during follow-up (always vs. < always; 39.5/100 PY vs. 47.4/100 PY,  $p = 0.42$ ). Similarly, incidence of urethral STIs did not differ by consistent condom use with insertive sex during follow-up ( $p = 0.60$ ).

**Conclusions:** Adherence to ART translates into increased condom use but in the absence of 100% condom use STI incidence remains high.

### 924 Online Sex-Seeking Among MSM in Nigeria: Implications for Online Intervention

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**Background:** The TRUST project was undertaken to apply innovative strategies to engage Nigerian MSM into HIV care. Respondent Driven Sampling (RDS) was found to be effective in reaching marginalized MSM especially in later waves of recruitment. In this analysis we evaluate characteristics of Nigerian MSM who use the Internet for sex-seeking.

**Methods:** We analyzed data collected from the TRUST study, a cohort of 1,370 MSM recruited using RDS from March 2013 to August 2015 in Abuja and Lagos, Nigeria. Participants were administered face-to-face interviews and HIV/sexually transmitted infection (STI) testing over the course of 7 study visits for up to 15 follow-up months. Logistic regression models were used to measure associations of MSM social/sexual networks, stigma, and HIV with online sex-seeking at baseline. Generalized estimating equation models were used to assess associations of online sex-seeking with STIs and HIV treatment-related variables across study visits.

**Results:** Most (61.5%) participants reported meeting male sex partners online at baseline, primarily using websites/mobile-apps such as 2GO (22%), Facebook (20%), and WhatsApp (12%). Grindr, a popular sex-seeking mobile-app among MSM in the US, was reported by 1.5%. Online sex-seeking was positively associated with reporting participation

in MSM community activities, larger networks of MSM, participation in an earlier RDS accrual wave, greater numbers of male anal sex partners, and higher levels of sexual identity stigma (Table 1). After adjusting for age, sexual orientation, education, marital status, religion, and study site, online sex-seeking was associated with testing positive for HIV at a follow-up visit (Adjusted Odds Ratio [aOR]=1.79, 95% Confidence Interval [CI]=1.17, 2.74) among those who were unaware of or not living with HIV at baseline. Online sex-seekers were marginally more likely to test positive for chlamydia/gonorrhea (aOR=1.28, 95% CI=1.00, 1.65). Among those living with HIV, online sex-seekers were not more likely to be taking antiretroviral medication (aOR=1.33, 95% CI=0.93, 1.90) or demonstrating suppressed viral load (aOR=0.74, 95% CI=0.48, 1.13).

**Conclusions:** Online sex-seekers in Nigeria are at increased risk for HIV/STIs but are not benefiting from Internet-based risk reduction opportunities. Furthermore, MSM less engaged with the MSM or gay community are not connected to the Internet. The potential for applying Internet-based interventions for risk reduction requires further study in the African context.

**Table 1. Bivariate associations of MSM social/sexual networks and sexual identity stigma with online sex-seeking, among MSM in Nigeria TRUST study at baseline (N=1,370)**

Explanatory variable	Odds Ratio	95% Confidence Interval
<i>MSM social and sexual networks</i>		
MSM network size (per 15 MSM)	1.02	1.01, 1.03**
Participates in MSM community activities	1.81	1.36, 2.41***
RDS accrual wave number	0.93	0.92, 0.95***
Number of male anal sex partners within past 12 months (per 5 MSM)	1.05	1.00, 1.11*
<i>Sexual identity-related stigma (ever experienced/perceived)</i>		
Did not feel protected by police	2.42	1.75, 3.36***
Felt scared to walk around in public	1.41	1.06, 1.88*
Verbally harassed	1.71	1.35, 2.18***
Blackmailed	2.39	1.80, 3.18***
Physically hurt	2.51	1.85, 3.42**

\*p<0.05; \*\*p<0.01; \*\*\*p<0.001

**925 Stigma and Openness About Sexual Identity Among MSM: A Latent Class Analysis**

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**Background:** Men who have sex with men (MSM) in Sub-Saharan Africa are subjected to high levels of sexual identity-related stigma, which may affect mental health and sexual risk behaviors. MSM who are open about their sexual identity appear to be most affected by stigma. Characterizing the mechanism of action of stigma in potentiating HIV-risks among MSM is important to support the development of interventions.

**Methods:** MSM were recruited across 5 cities/towns in Swaziland through snowball sampling ending in December 2014. Participants (N=532) completed a survey that included questions about demographics, stigma, and mental and sexual health. Latent class analysis was used to identify classes based on self-reported measures of sexual identity stigma and whether the sexual identity of the participant was known to his family or healthcare workers. Logistic regression was used to identify demographic characteristics, sexual risk behaviors, and mental health characteristics (i.e., depression – PHQ9) associated with latent class membership.

**Results:** A three-latent-class model was selected. The first class consisted of MSM who demonstrated overall low probabilities of sexual identity stigma (55%). MSM in the second class exhibited high probabilities of physical violence and fear/avoidance of healthcare, and were less likely to have their sexual identities known (10%). Members of the third class demonstrated high probabilities of verbal harassment, stigma from family and friends, and were more likely to have their sexual identities known (34%). Relative to the “low stigma” class, participants who were sampled from an urban area (Adjusted Odds Ratio [aOR]=2.78, 95% Confidence Interval [CI]=1.53, 5.07) and who engaged in condomless anal sex (aOR=1.85, 95% CI=1.17, 2.91) were more likely to belong to the “high stigma, out” class. In contrast, participants who had a concurrent partner (including female partners) were more likely to belong to the “high stigma, not out” class (aOR=2.73, 95% CI=1.05, 7.07). Depression was associated with membership in both high-stigma classes (aOR=2.42, 95% CI=1.51, 3.87 “out” and aOR=3.14, 95% CI=1.50, 6.55 not “out”).

**Conclusions:** Community-level sexual identity stigma is associated with individual-level risk behaviors among MSM and these associations vary by level of sexual identity openness. Comprehensive HIV interventions should aim to reduce stigma and encourage community-level support.

**Table 1. Item-response probabilities conditional on latent class membership, among MSM in Swaziland (N=502)**

Item	Low stigma	High stigma, not "out"	High stigma, "out"
Felt excluded at family gatherings	0.06	0.02	<b>0.62</b>
Felt that family members made discriminatory remarks or gossiped	0.16	0.00	<b>0.70</b>
Felt rejected by friends	0.09	0.00	<b>0.48</b>
Felt police refused to protect you	0.05	0.15	<b>0.28</b>
Felt scared to walk around in public places	0.24	0.01	<b>0.73</b>
Verbally harassed	0.25	0.00	<b>0.87</b>
Blackmailed	0.12	0.04	<b>0.44</b>
Physically hurt	0.03	<b>0.62</b>	0.33
Tortured	0.03	<b>0.67</b>	0.25
Felt not treated well in a health center	0.00	<b>0.51</b>	0.15
Heard healthcare providers gossiping	0.02	<b>0.55</b>	0.21
Felt afraid to go to healthcare services	0.07	<b>0.85</b>	0.72
Avoided going to healthcare services	0.05	<b>0.79</b>	0.72
Told any family member or any family member knows he has sex with men	0.38	0.11	<b>0.63</b>
Told any healthcare provider or any healthcare provider knows he has sex with men	0.15	0.05	<b>0.34</b>

**926 Group Sex: A Cross-Sectional Online Survey Among Men Who Have Sex With Men in China**Songyuan Tang<sup>1</sup>; Weiming Tang<sup>1</sup>; Joseph D. Tucker<sup>2</sup>; Bin Yang<sup>3</sup>; Ye Zhang<sup>1</sup>; Wei Zhang<sup>1</sup>; Shujie Huang<sup>3</sup>; Ligang Yang<sup>3</sup>; Chongyi Wei<sup>4</sup>; Chuncheng Liu<sup>5</sup>; Yilu Qin<sup>1</sup><sup>1</sup>Univ of North Carolina Proj China, Guangzhou, China; <sup>2</sup>Univ of North Carolina at Chapel Hill Inst for Global Hlth & Infectious Diseases, Chapel Hill, NC, USA; <sup>3</sup>Guangdong Provincial Cntr for Skin Diseases and STI Control, Guangzhou, China; <sup>4</sup>Univ of California San Francisco, San Francisco, CA, USA; <sup>5</sup>SESH Global, Guangzhou, China**Background:** Group sex is relatively common among men who have sex with men (MSM) and plays an important role in the transmission of HIV and other sexually transmitted infections (STIs). However, little attention has focused on this high risk behavior, particularly among Chinese MSM. This study was therefore aimed to determine the frequency and correlates of group sex among Chinese MSM.**Methods:** MSM participants were recruited from three online portals popular with gay men in China. An online survey was conducted from September–October 2014, collecting data on socio-demographics, sexual behaviors, gay app use and group sex among Chinese MSM. We defined group sex as sex at the same time among three or more people. Participants who reported any use of gay apps to find sex partners in the last six months were categorized as gay app users. Univariate and multivariate logistic regressions were used to determine the factors associated with group sex.**Results:** Of the 1,424 surveyed MSM, the majority were under 30 years old (77.5%), single (83.8%), with at least a college education (74.0%) and used gay apps (57.9%). Overall, 9.9% (141) of participants reported that they engaged in group sex in the last 12 months. Multivariate analyses showed that HIV-positive MSM were more likely to engage in group sex (adjusted OR=3.74, 95% CI 1.92, 7.28). Individuals with riskier sexual behaviors, such as condomless anal intercourse with men in the last three months (adjusted OR=2.88, 95% CI 2.00–4.16) were also more likely to engage in group sex. In addition, we observed a trend towards Chinese MSM gay app users having more group sex (adjusted OR=1.46; 95% CI: 1.00–2.13).**Conclusions:** Our data suggest that MSM group sex is more common among HIV-infected individuals and those with high risk behaviors. Our data are consistent with an emerging literature suggesting that mobile app-facilitated sex parties among HIV-infected individuals may be facilitating HIV transmission. Future interventions, surveillance programs and research should give consideration to this population as a distinct risk group.**927 Risk Factors for Acute and Early HIV Infection Among MSM, Bangkok, Thailand 2010-2015**Warunee Thienkrua<sup>1</sup>; Marcel Curlin<sup>2</sup>; Eileen Dunne<sup>3</sup>; Kanokpan Pancharoen<sup>1</sup>; Boonyos Raengsakulrach<sup>1</sup>; Wanee Chonwattana<sup>1</sup>; Wanna Leelawit<sup>1</sup>; Philip A. Mock<sup>1</sup>; Anupong Chitwarakorn<sup>1</sup>; Timothy H. Holtz<sup>3</sup><sup>1</sup>Thailand Ministry of PH US CDC Collab, Nonthaburi, Thailand; <sup>2</sup>Oregon Hlth and Scis Univ, Portland, OR, USA; <sup>3</sup>CDC, Atlanta, GA, USA; <sup>4</sup>Ministry of PH, Muang Nonthaburi, Thailand**Background:** Identifying and treating acute and early HIV infection (AEHI) may result in reductions in HIV transmission. We evaluate factors associated with AEHI among HIV-uninfected men who have sex with men (MSM), and transgender women (TGW), in the Bangkok MSM Cohort Study (BMCS).**Methods:** From 2006 to 2010, we enrolled Thai MSM and TGW aged  $\geq 18$  years into the BMCS with 3–5 years of follow-up from date of enrollment. Participants provided socio-demographic and behavioral information by audio computer-assisted self-interview at each 4-month visit. MSM who had an HIV-1 non-reactive oral fluid test at baseline or at any 4-month follow-up with serologic confirmation were evaluated for AEHI. AEHI was defined as having non-reactive result by oral fluid anti-HIV rapid test, detectable HIV-1 RNA by nucleic acid amplification testing (NAAT), or reactive HIV Ag/Ab by 4th generation enzyme immunoassay (EIA). We calculated the overall prevalence of AEHI (number of acute and early infections per 100 tests), and factors associated with AEHI using generalized estimating equations logistic regression modeling.**Results:** From February 2010 to June 2015, there were a total of 977 participants with 7383 visits with a non-reactive anti-HIV rapid test. Of these, 6826 visits (92.5%) had testing for AEHI. Overall we detected 52 (5.3%) participants with AEHI at baseline or at follow-up (0.76 per 100 tests, 95% Confidence Interval (CI) 0.56–0.99). By age (years), AEHI occurred in 1.34 per 100 tests (13/967, 95% CI 0.72–2.30) among those 18–21, 0.69 per 100 tests (24/3436, 95% CI 0.45–1.04) among those 22–29, and 0.62 per 100 tests (15/2423, 95% CI 0.34–1.02) among those  $\geq 30$  years. Independent factors associated with AEHI at the time of the visit included young age (age 18–21 years, adjusted Odds Ratio (aOR) 2.6, 95% CI 1.1–6.1), inconsistent condom use with a steady male partner in the past 4 months (aOR 3.8, 95% CI 1.8–8.3), positive rectal *Neisseria gonorrhoea* (aOR 7.5, 95% CI 1.4–39.1), positive hepatitis A antibody (aOR 5.6, 95% CI 1.5–20.8) and positive hepatitis B core antibody (aOR 14.1, 95% CI 5.6–35.4).**Conclusions:** We detected AEHI in over 5% of participants in our cohort study, and AEHI was most common in the young as well as those with a history of STIs, inconsistent condom use, and hepatitis A immunity. Given the expense of NAATs and fourth generation EIAs, factors associated with AEHI could be used to target testing to men at greatest risk for early and acute HIV and facilitate early treatment.**928 High Prevalence of HIV Among Wives of Married Men Who Have Sex With Men in India**Aylur K. Srikrishnan<sup>1</sup>; Shruti H. Mehta<sup>2</sup>; Cecilia Tomori<sup>2</sup>; Santhanam Anand<sup>1</sup>; Pachamuthu Balakrishnan<sup>3</sup>; David Celentano<sup>2</sup>; Gregory M. Lucas<sup>2</sup>; Sunil S. Solomon<sup>2</sup><sup>1</sup>YRG Cntr for AIDS Rsr and Educ, Taramani, India; <sup>2</sup>Johns Hopkins Univ, Baltimore, MD, USA; <sup>3</sup>YRG Cntr for AIDS Rsr and Educ, Chennai, India**Background:** Despite global declines in HIV prevalence, control in vulnerable populations such as men who have sex with men (MSM) in low- and middle-income countries (LMICs) remains a challenge. MSM in LMICs continue to have high HIV prevalence and serve as a potential bridge to the general population because a significant proportion marry women (>50% in some settings) to satisfy socio-cultural norms. HIV prevalence among married MSM tends to be higher, likely related to a need to conceal behavior. However, to-date, no data exists on HIV prevalence among wives of MSM.**Methods:** From March 2015–present, 129 married MSM couples have been recruited across 3 Indian cities – Bengaluru, Hyderabad and New Delhi (target 50 couples/city). To be included, men had to have disclosed their MSM behavior to their wives. Both members of the couple also needed to consent independently and be  $\geq 18$  years of age. An additional 149 married MSM who had not disclosed their MSM behavior to their wives were included. All completed a survey and were tested for HIV; men and their wives were interviewed separately. Logistic regression was used to identify factors associated with HIV infection in wives.**Results:** The median age of MSM and their wives whose data were available at the time of analysis (n=129 couples) was 36 and 31 years, respectively. They were married for a median 15 years. HIV prevalence among disclosed MSM was 47% (95% confidence interval [CI]: 38, 56%) compared to 31% in undisclosed MSM (95% CI: 24, 39%). HIV prevalence among the wives of disclosed men was 28% (95% CI: 20, 37%). Only 6 of 36 HIV positive women had an HIV negative husband. The strongest predictor of HIV infection in wives was husband's HIV status (Adjusted Odds Ratio: 13.7; 95% CI: 4.41, 42.7). Only 11 (9%) women reported >1 lifetime sex partner (4 had >2 partners). 87% of the wives of HIV positive MSM were aware of their husband's HIV status and 89% were also aware of their own HIV status. Of women aware of their status, 97% had been linked to care and 80% were on ART.**Conclusions:** We observed an alarmingly high prevalence of HIV among married MSM and their wives in India. It was encouraging that most wives were aware of their HIV status and engaged in care. However, these couples may represent a select group where MSM have disclosed their MSM behavior, potentially because of their HIV diagnosis. Innovative interventions are needed to reach wives of married MSM and engage them in HIV prevention prior to transmission of HIV.**929 Subsequent HIV Disease Risk Following Syphilis Diagnosis in a Southern MSM Population**David Sweat<sup>1</sup>; Sulaiman Aizezi

Shelby County Hlth Dept, Memphis, TN, USA

**Background:** Pathela et al in New York City reported the risk of subsequent HIV infection diagnosis within 30 months among MSM was 1:20, or a 5% overall risk for MSM diagnosed with either primary or secondary syphilis (Clinical Infectious Diseases<sup>®</sup> 2015;61(2):281–7). We applied the methods described in the Pathela study to reported disease registries for Shelby County, Tennessee, to determine whether our experience was similar to, better than, or worse than the experience reported in New York City. Shelby County is

an urban southern county that includes the City of Memphis, the largest metropolitan area in Tennessee. Shelby County has a population of 940,000+ residents, 52% of which are non-Hispanic Black residents.

**Methods:** The initial study population included 1,184 people diagnosed with either Primary or Secondary Syphilis from 2005-2014 inclusive. After eliminating duplicate reports, 1,020 unique Syphilis patients were matched against the Enhanced HIV/AIDS Reporting System (eHARS) used to track HIV Disease and AIDS diagnoses in the United States, maintained by the Centers for Disease Control and Prevention and State Health Departments. Data and statistical analysis was conducted in SAS, including descriptive epidemiology, HIV Free survival estimation, adjusted hazard ratio analysis, odds ratio analysis and assessment of interaction effects. Data were represented graphically and in tabular form with estimate values and calculated 95% confidence intervals.

**Results:** Overall, 54 of 1,021 or 5% of P&S patients were subsequently diagnosed HIV+ in the study population, findings that initially appeared consistent with the New York City experience. For specific subpopulations, however, the risk of developing subsequent HIV disease was greater. For MSM, the overall risk of subsequent HIV infection was 1:16 or 6.25%, and for Black MSM the risk of subsequent HIV Disease diagnosis was 1:8 or 12.5% within 30 months. 50% of male P&S Syphilis patients who later tested HIV+ did so within 24 months. HIV Free survival times were less for P&S patients  $\geq 35$  years old at the age of Syphilis diagnosis (1.3 years compared with 2.1 years HIV Free for < 35 year old patients).

**Conclusions:** HIV negative patients diagnosed with P&S Syphilis are at extremely high risk to subsequently be diagnosed with HIV Disease. Particularly for Black MSM, the risk may exceed 10% that they will become infected within 24 months. HIV-, Syphilis + MSM must be offered pre-exposure prophylaxis and other interventions to lower their risk of HIV.

### 930 Behavioral Differences Between Young and Older Black Men Who Have Sex With Men

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**Background:** In the United States, black men who have sex with men (BMSM) are disproportionately affected by HIV. Among BMSM, HIV incidence is highest and increasing in those younger than 25 years old. The aim of this study was to identify behavioral and service utilization differences between young and older BMSM to help explain the higher and increasing incidence among the younger group.

**Methods:** We used data from the 2011 National HIV Behavioral Surveillance System MSM cycle. The analysis was limited to BMSM aged 18-44 years. We compared selected characteristics between young BMSM (aged 18-24 years) and older BMSM (aged 25-44 years) using chi-square tests, stratified by self-reported (SR) HIV status. Variables associated with age in bivariate analyses were analyzed as outcomes using separate log-linked Poisson regression models with generalized estimating equations to estimate adjusted prevalence ratios (aPR) and 95% confidence intervals. Models were adjusted for potential confounders and clustered on recruitment event.

**Results:** Of 1,876 BMSM interviewed, 43% (n=802) were 18-24 years old and 57% (n=1,074) were older. HIV prevalence was 21% among young men and 32% among older men ( $p < 0.01$ ). The percent with HIV who were unaware of their infection was 56% among young men and 47% among older men ( $p = 0.04$ ). Among SR HIV-negative BMSM (including some who were infected), young men were more likely to know their last sex partner for <1 year (aPR 1.14, 1.07-1.22) and have a black partner (aPR 1.14, 1.07-1.22) or report receptive sex (aPR 1.21, 1.08-1.36) at last sex with a man. Few SR HIV-negative BMSM had taken pre-exposure prophylaxis (PrEP) (1%, n=13) and many did not know their last partner's HIV status (42%, n=671); these factors were not associated with age. Among SR HIV-positive BMSM, young men were less likely to be on antiretroviral therapy (ART) (aPR 0.67, 0.51-0.89) and more likely to have a black partner at last sex with a man (aPR 1.16, 1.03-1.29).

**Conclusions:** Among HIV-negative BMSM, younger men are more likely than older men to engage in sexual behaviors with higher risks for infection and have black sex partners, a partner pool with potentially high HIV prevalence. Among HIV-positive BMSM, young men are less likely to be on ART. These differences may contribute to increasing HIV incidence among young BMSM. Increasing awareness of acquisition risks associated with different sexual behaviors and of effective interventions like ART and PrEP may help reduce HIV incidence in young BMSM.

### 931 Association Between Family Environment and HIV-Related Risk Behavior Among Young MSM

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**Background:** HIV incidence estimates suggest that young men who have sex with men (YMSM) are increasingly affected by HIV. Given that high levels of social support have been associated with safer behaviors, we evaluated the association between measures of family environment and HIV-related sexual risk behaviors among YMSM 13 to 18 years of age.

**Methods:** We examined data from the National HIV Behavioral Surveillance System among YMSM in three cities (Chicago; New York City; Philadelphia). YMSM ages 13 to 18 years (y) were recruited for interview and HIV testing. We used separate GEE models with a robust error variance procedure to estimate adjusted prevalence ratios (aPR) and 95% confidence intervals (CI) for the associations between measures of family environment (currently living with parents vs. other living arrangements, ever kicked out of the house or run away, perceived family support scale, out to mother, out to father) and engagement in each of three sexual risk behaviors as outcomes: condomless anal sex in past 12 months (CAS),  $\geq 4$  male oral or anal sex partners in past 12 months, and vaginal or anal sex before 13y. Models were adjusted for age, race/ethnicity, and city.

**Results:** Of 278 YMSM, 79% ever had oral or anal sex with a male, 41% had CAS, 32% reported  $\geq 4$  partners, and 23% had vaginal or anal sex before 13y. Multivariable analyses revealed that positive family environment factors were associated with a lower prevalence of all three risk behaviors. YMSM who lived with parents were less likely to report CAS (aPR 0.64, CI 0.47-0.86),  $\geq 4$  partners (0.45, 0.32-0.64), and sex before 13y (0.60, 0.41-0.89). Those who had never been kicked out were less likely to report CAS (0.69, 0.53-0.90),  $\geq 4$  partners (0.49, 0.35-0.69), and sex before 13y (0.69, CI 0.49-0.97). Higher perceived family support was associated with a lower likelihood of reporting sex before 13y (0.92, 0.88-0.96). Being out to one's mother or father was associated with a lower likelihood of CAS [(0.59, 0.38-0.90) / (0.72, 0.53-0.98)] and  $\geq 4$  partners [(0.35, CI 0.18-0.66) / (0.61, CI 0.42-0.89)].

**Conclusions:** These findings suggests that for YMSM, a stable and supportive family environment may serve as a protective factor against engaging in HIV-related risk behaviors. Interventions that build upon these protective factors and promote acceptance within the families of YMSM may play an important role in comprehensive HIV prevention efforts for this population.

### 932 STIs and Predictive Sexual Risk Behaviors Among HIV+ Military Cohort Members

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**Background:** HIV+ individuals often continue to engage in behaviors that put them at risk for STIs. We have shown that STI incidence occurs within the U.S. Military HIV Natural History Study (NHS), a cohort of HIV+ active duty members and beneficiaries. Prior to the repeal of Don't Ask Don't Tell (DADT), very little sexual risk behavior data were available from military populations. A risk behavior survey was recently implemented to begin to identify individual risk to ultimately target for prevention. We were interested in identifying associations between sexual behaviors and STIs.

**Methods:** All NHS participants are screened for STIs every six months. From 9/2014-2/2015, participants were administered a tablet-based CASI survey on sexual risk behaviors, with the option to respond "prefer not to answer" (PNA) on each question. Incident STIs (chlamydia, gonorrhea, HBV, HSV2, and syphilis) were defined as a negative followed by a positive test within one year of their survey.

**Results:** 1349 NHS participants completed the risk survey, 93.62% male, 39% white, 44% African-American, 17% Hispanic/Other, 37% active duty, 32% married, median 11 yrs from HIV (IQR 3.8-21), CD4 656 cells/mm3, VL 1.3 log10 at survey. 76% of men reported ever engaging in MSM behavior (6% PNA), 88% at last sexual encounter (5.6% PNA).

247 (18%) had an incident STI. Being single, African American, active duty, carrying a condom, inconsistent condom use, and having a male or non-steady partner at last sexual encounter were significant on univariate analyses, while alcohol use and alcohol consumption were not. In multivariate analyses, STI risk increased with number of sex partners ( $p=0.001$ ), history of sex with a man (OR 4.3 and 6.0 for PNA,  $p<0.01$ ) and the odds of getting an STI increased as self-perceived risk increased (low, med/high, unsure) compared to no risk (ORs: 3.4, 4.4, 8.9, respectively, all  $p<0.001$ ). Age and race remained significant. In a separate model, condom use, number of new sex partners and non-steady last sex partner were all independently associated with perceived risk.

**Conclusions:** MSM behavior among those with HIV is higher than previously reported in a military setting. While we know that STIs continue to occur in our population (18%), not all risk behaviors were predictive of incident STIs. Further research to understand correlates beyond individual risk such as social networks are warranted. STI prevention efforts should focus on condom use and new partners as opposed to perceived risk.

**933 Intentional HIV Seroadaptive Behavior Patterns Among Seattle MSM, 2008-2014**

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**Background:** Seroadaptive behaviors are an HIV risk reduction strategy practiced by men who have sex with men (MSM) and include serosorting and modifying sexual behavior on the basis of viral load among HIV-infected MSM. Most previous estimates have measured serosorting behaviors without assessing whether they are a product of intentionality.

**Methods:** We evaluated intentional seroadaptive behaviors among MSM using serial cross-sectional data from the 2008, 2011, and 2014 MSM cycles of the Seattle-area National HIV Behavioral Surveillance system (N=1,242). MSM were recruited using venue-based sampling. Local questions asked about intentional seroadaptive behaviors in the last year including condomless anal sex (CAS) due to knowledge of concordance with a partner's HIV status (intentional serosorting), HIV-concordant CAS at last sexual encounter (serosorting proxy), and CAS due to undetectable viral load among HIV-infected MSM (intentional viral load sorting). We also asked about a decision to refrain from CAS due to knowledge of a partner's HIV status. We stratified findings by self-reported HIV status and assessed differences in behaviors over time. We used multivariable logistic regression to identify factors differentiating MSM reporting seroadaptive behaviors from other MSM.

**Results:** Self-reported HIV prevalence was 17%. Intentional serosorting in the last year was reported by 64% of HIV-infected MSM and 48% of HIV-uninfected MSM. Among HIV-infected MSM, STD diagnosis in the last year was associated with intentional serosorting (aOR=3.9, 95% CI: 1.3-12.0). Not having sex due to knowledge of a partner's HIV status was reported by 21% of HIV-uninfected and 24% of HIV-infected MSM. There was no evidence of differences in intentional concordant CAS over time (Table). There was a significant increase in concordant CAS at last sex among HIV-uninfected MSM only. There was no significant change in intentional viral load sorting. Among HIV-infected MSM, 27% engaged in intentional viral load sorting in the last year, and this was associated with age (aOR=0.94 per year, 95% CI: 0.89-1.00), ≥10 male sex partners (aOR=4.2, 95% CI: 1.5-11.5), and methamphetamine use (aOR=0.3, 95% CI: 0.1-1.0).

**Conclusions:** Intentional seroadaptive behaviors among Seattle MSM were common and stable from 2008-2014, and among younger MSM, viral load sorting is an emerging seroadaptive behavior. Assessing intentionality of serosorting resulted in a different temporal pattern than a measure based solely on reported behavior.

**Table.** Trends in seroadaptive behaviors with male partners by self-reported HIV status and viral load among MSM in the Seattle-area National HIV Behavioral Surveillance survey, 2008-2014.

	n	2008	2011	2014	$\chi^2$ test p-value
<b>Intentional serosorting: Intentional concordant CAS, last year</b>					
HIV-infected MSM	194	63.5%	69.2%	59.7%	0.50
HIV-uninfected MSM	921	44.8%	49.8%	48.0%	0.51
<b>Serosorting proxy: Concordant CAS with last male partner, last year</b>					
HIV-infected MSM	198	34.0%	42.4%	30.4%	0.31
HIV-uninfected MSM	900	26.1%	30.0%	36.1%	0.02
<b>Intentional viral load sorting: Intentional CAS due to participants' undetectable viral load, last year</b>					
HIV-infected MSM	139	NA	20.3%	32.0%	0.12

Abbreviations: MSM, men who have sex with men; CAS, condomless anal sex.

**934 Determinants of HIV Transmission Risk Among HIV-Infected Persons Engaged in Care**

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**Background:** HIV incidence in the US has not decreased over the past decade likely due to the inability of prevention efforts to reach those at risk of HIV transmission and acquisition. HIV-infected persons in medical care represent an accessible group in whom preventive efforts can have maximal success. We sought to identify factors associated with HIV transmission among HIV-infected persons engaged in care, including younger age, alcohol or illicit drug use, depression, low socioeconomic status, less education, and men who have sex with men (MSM).

**Methods:** We used data from the University of Pennsylvania Center for AIDS Research Clinical Core Cohort to examine factors associated with HIV transmission risk. For each visit, HIV transmission risk was based on the presence of both HIV viremia >1500 copies/mL and reported unprotected sexual activity. We calculated the proportion at risk for HIV transmission and explored the incidence of changing HIV transmission risk within person over time. We used multivariable mixed effects logistic regression models to estimate the association between HIV transmission risk and our hypothesized risk factors, with random intercepts to account for the correlation between multiple study visits per person.

**Results:** From 2/28/2007 to 5/28/2015, 1,883 HIV-infected persons contributed a median (IQR) of 3 study visits (2 – 5) for a total of 4,582 visits. Median time between consecutive visits was 11.6 months (7.3 – 17.8). 174 (9.2%) individuals were at risk for HIV transmission during at least once during the period, with 27 (1.4%) having multiple at risk intervals. In addition, 114 (6.0%) individuals changed HIV transmission risk state over time, of which 23 (20%) underwent multiple shifts. Factors associated with HIV transmission risk included younger age (OR [95% CI] per 10-year decrease=2.06 [1.65, 2.47]), illicit drug use (OR=2.40 [1.56, 3.68]), depression (OR=1.99 [1.29, 3.06]), and education < 12<sup>th</sup> grade (OR=1.87 [1.08, 3.25]). Alcohol use, sexual orientation and gender were not predictive of HIV transmission risk.

**Conclusions:** Nearly 1 in 10 HIV-infected individuals engaged in care were at risk of transmitting HIV during the study period due to concomitant uncontrolled HIV infection and high risk sexual behavior. Behavioral interventions to decrease HIV transmission need to focus on patients in care who are younger, less educated, depressed, and actively using illicit drugs.

**935 Food Insecurity Is Associated With HIV Serostatus and Sexually Transmitted Infections**

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**Background:** Food insecurity is associated with HIV risk behavior in multiple studies, suggesting a link to HIV acquisition. Yet no population-level studies have examined the association between food insecurity, HIV risk factors, and prevalent HIV among men, the group representing the majority of HIV diagnoses in the United States. We hypothesized that food insecurity would be associated with HIV seropositivity and increased prevalence of HIV risk factors including STIs and illicit drug use in this population.

**Methods:** We conducted cross-sectional analyses using 14 years of data from the National Health and Nutrition Examination Survey (NHANES) 1999-2012, a nationally representative survey of the civilian non-institutionalized population of the United States. Household food security was measured using the validated 18-item Household Food Security Survey module. The primary outcome was HIV serostatus. Secondary outcomes were measures of HIV risk, including herpes simplex virus 2 (HSV-2) serostatus, self-reported STIs (genital warts, gonorrhea or chlamydia), and recent illicit drug use. HIV and HSV-2 serostatus were based on blood tests conducted as part of NHANES. Analyses were adjusted for age, race/ethnicity, relationship status, household size, poverty-income ratio, education, smoking, and heavy drinking, and utilized appropriate design weights and complex survey commands to estimate nationally-representative associations.

**Results:** We analyzed data for 9150 men representing 61 million individuals in the US. Unadjusted prevalence of HIV was 1.5% among food insecure men, compared to 0.4% among food secure men (p<0.001). In adjusted models, food insecure men had over 2 times higher odds of HIV infection compared to food secure men (adjusted odds ratio (AOR)=2.09; 95% CI 1.05-4.13; p<0.05). Food insecurity was also significantly associated with 1.29 higher adjusted odds of HSV-2 seropositivity (95% CI 1.05 - 1.59; p<0.05), 1.55 higher adjusted odds of self-reported STIs (95% CI 1.08 - 2.21; p<0.05), and 1.63 higher adjusted odds of illicit drug use (95% CI 1.19 - 2.24; p<0.01). Results were robust to sensitivity analyses restricting analyses to lower income men.

**Conclusions:** Food insecurity is associated with prevalent HIV, STIs and illicit drug use among men in the United States, and should be addressed as part of structural approaches to HIV prevention among men. Further research is needed to establish whether and through what mechanisms improved food security may help prevent new HIV infections.

**Table: Association between food insecurity and HIV risk outcomes among men age 20-49 in the United States, NHANES 1999-2012**

Outcome:	HIV-1 seropositivity	HSV-2 seropositivity	History of gonorrhea, chlamydia and/or genital warts	Illicit substance use, past year <sup>b</sup>
	AOR (95% CI)	AOR (95% CI)	AOR (95% CI)	AOR (95% CI)
<b>Food insecure</b>	2.09* (1.05 - 4.13)	1.29* (1.05 - 1.59)	1.55* (1.08 - 2.21)	1.63** (1.19 - 2.24)
<b>Age</b>				
20-29	Ref	Ref	Ref	Ref
30-39	2.35* (1.01 - 5.48)	2.86*** (2.22 - 3.67)	1.22 (0.85 - 1.75)	0.91 (0.66 - 1.25)
40-49	2.56* (1.10 - 5.99)	4.07*** (3.17 - 5.21)	1.03 (0.71 - 1.49)	0.76 (0.53 - 1.08)
<b>Poverty-income ratio</b>	0.85 (0.68 - 1.04)	0.95 (0.90 - 1.02)	1.13* (1.02 - 1.26)	0.92 (0.83 - 1.01)
<b>High school education</b>	1.68 (0.71 - 3.98)	0.80* (0.64 - 0.99)	1.37 (0.94 - 2.01)	0.74 (0.53 - 1.04)
<b>Race/ethnicity</b>				
Non-Hispanic white/other	Ref	Ref	Ref	Ref
Hispanic	3.58** (1.58 - 8.13)	1.29* (1.03 - 1.61)	0.64* (0.45 - 0.92)	1.22 (0.90 - 1.66)
Non-Hispanic black	5.36*** (2.60 - 11.08)	5.22*** (4.34 - 6.28)	1.26 (0.93 - 1.72)	0.65* (0.46 - 0.92)
<b>In a committed relationship</b>	0.43* (0.20 - 0.96)	1.07 (0.88 - 1.31)	0.75 (0.55 - 1.03)	0.40*** (0.29 - 0.54)
<b>Household size</b>	0.68** (0.52 - 0.89)	0.94 (0.89 - 1.00)	0.90 (0.82 - 1.00)	1.02 (0.94 - 1.12)
<b>Smoking</b>	2.29** (1.22 - 4.30)	1.32** (1.09 - 1.59)	1.10 (0.80 - 1.51)	4.67*** (3.43 - 6.35)
<b>Heavy drinking in past year</b>	0.68 (0.33 - 1.38)	1.11 (0.91 - 1.36)	1.11 (0.79 - 1.55)	0.54*** (0.40 - 0.73)
<b>History of substance use</b>	0.90 (0.44 - 1.83)	1.90*** (1.56 - 2.32)	2.60*** (1.90 - 3.57)	--
Observations	7,246	7,098	6,935	5,313

Notes: \*\*\* p<0.001; \*\*p<0.01; \*p<0.05. \* Estimate has a relative standard error (RSE) of 35%. RSEs over 30% may not be statistically reliable per NHANES analytic guidelines. <sup>b</sup> Illicit substance use in the last year only available for survey years 2005-2012. <sup>c</sup> Analysis: Analyses used 14-year analytic weights constructed for analysis according to NHANES guidelines and adjustments for sampling design.

**936 Impact of Male Partners and Schooling on HIV Risk Among South African Girls: HPTN 068**

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**Background:** Despite the high HIV risk faced by young women in Sub-Saharan Africa, there is limited data examining factors associated with HIV incidence in this population, something that is necessary for effective prevention programming.

**Methods:** HPTN 068 was a phase III individually randomized trial to assess the impact of a conditional cash transfer on the acquisition of HIV among young South African women. The intervention was conducted from April 2011 to March 2015. Participants enrolled in the study were aged 13-20, in high school, not married or pregnant, and resident in the Agincourt Health and Socio-Demographic Surveillance System (AHDSS) site, in rural Mpumalanga. Participants were seen at baseline, then annually for up to three follow-up visits, where HIV and HSV-2 testing were conducted and an interview was completed using Audio Computer Assisted Self Interviewing (ACASI). Participants were tested for HIV infection using two HIV rapid tests with Western blot confirmation. The proportional hazards regression model with time varying explanatory variable was used to examine the effect of specified exposures on HIV incidence (SAS version 9.4).

**Results:** 2328 HIV negative young women at baseline who had at least one follow up visit comprised the main analytic sample. 107 incident HIV infections were identified during the study resulting in an incidence of 1.8%. After adjusting for age at baseline, attending school <80% of school days/month, having a male partner who was 5 or more years older, and engaging in transactional sex were significantly associated with HIV incidence (See Table 1). Permanent school drop out and reporting more than one sex partner in the past 12 months were marginally significant with regard to increased risk of HIV acquisition. Experiencing intimate partner violence, unprotected sex in the past 3 months and becoming pregnant in the past 12 months were not associated with HIV incidence.

**Conclusions:** Older partners, engagement in transactional sex and <80% school attendance/month significantly increased the risk of HIV acquisition among this cohort of young South African women. These findings confirm the importance of keeping girls in secondary school but also focusing on reducing transactional sex and older partners to prevent new HIV infections.

Table 1. Behavioral and Socio-demographic factors associated with HIV incidence among adolescent women in Agincourt, South Africa HPTN 068

Factor	Unadjusted RR	Adjusted RR *
School attendance: <80% vs. >=80%	3.05 [1.81, 5.13] (p<0.0001)	1.88 [1.08, 3.27] (p=0.0256)
permanent dropout : yes vs. no	3.21 [1.81, 5.71] (p<0.0001)	1.77 [0.95, 3.28] (p=0.0709)
Any IPV during the last 12 months: yes vs no	0.98 [0.65, 1.47] (p=0.9251)	0.83 [0.55, 1.25] (p=0.3696)
Older male partner – having any male sex partner 5 or more years older: yes vs no	3.26 [2.06, 5.15] (p<0.0001)	2.63 [1.65, 4.17] (p<0.0001)
Number of sex partners in past 12 months **		
>1 vs 0	2.67 [1.05, 6.8] (p=0.0399)	2.42 [0.95, 6.18] (p=0.0646)
1 vs 0	2.10 [0.92, 4.79] (p=0.0792)	1.93 [0.84, 4.41] (p=0.121)
Unprotected sex in the past 3 months- any unprotected act in the past 3 months**		
>1 vs 0	1.09 [0.51, 2.36] (p=0.8211)	1.01 [0.47, 2.19] (p=0.9735)
1 vs 0	1.58 [0.66, 3.76] (p=0.3043)	1.56 [0.65, 3.72] (p=0.3161)
Pregnant in last 12 months: Yes vs no	1.55 [0.9, 2.68] (p=0.1175)	1.14 [0.65, 1.98] (p=0.6437)
Transactional sex	2.50 [1.55, 4.03] (p=0.0002)	1.92 [1.18, 3.13] (p=0.009)

\*adjusted for age at enrollment \*\* Among young women who reported that they had ever had vaginal sex at baseline

937 **Correlates of HIV in Large Population Surveys: A Comprehensive HIV Association Study**

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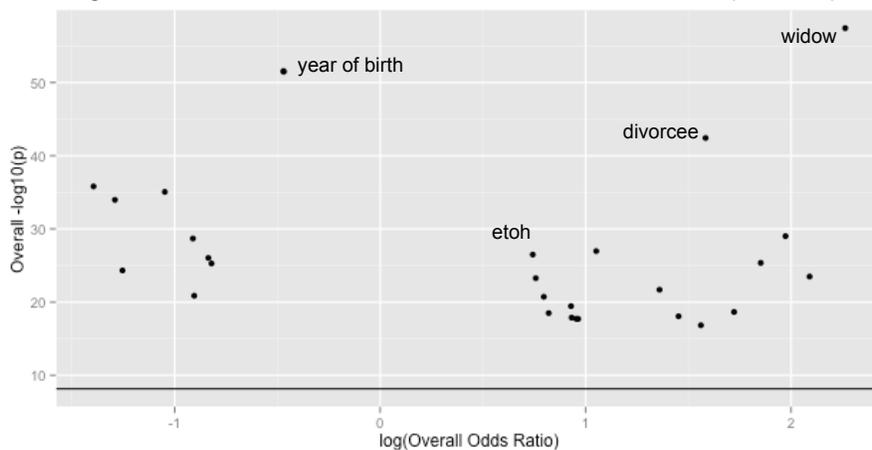
**Background:** Identification of at-risk individuals is a major challenge for global HIV public health goals such as “90-90-90” (90% testing, 90% on treatment, and 90% virally suppressed). We develop a new method for identifying HIV risk factors based on big data approaches, and show an application using nationally representative HIV survey in Zambia.

**Methods:** We develop generalizable methods for simultaneously exploring the association of very many correlates of HIV infection status. We use Zambia’s 2013-2014 Demographic and Health Survey, a nationally representative survey that included an HIV test, as the main source of data. Each individual’s HIV status was tested against 699 indicators derived from the surveys. All indicators with at least 90% completion rate were used. We split the data into a training and testing datasets, and accepted variables with Bonferroni-adjusted association either dataset ( $p < 7 \times 10^{-9}$  in our data) and  $p < 0.05$  in the other dataset. We then tested the explanatory power of the novel models to estimate the discrimination between true positive and false positive cases.

**Results:** We analyzed data on 14,719 women between 15 and 49 years old with HIV prevalence of 13.1%. After survey weighting and Bonferroni adjustments, we identified 26 indicators that were strongly associated with HIV infection. In addition to familiar risk factors such as residence in rural areas and age, self-identifying as widowed (OR 9.6,  $p < 1 \times 10^{-47}$ ) or divorced (OR 3.9,  $p < 1 \times 10^{-22}$ ), working in a service job (OR 3.4,  $p < 1 \times 10^{-10}$ ), and drinking alcohol (OR 2.0,  $p < 1 \times 10^{-15}$ ) were strong positive risk factors. Protective associations included currently breastfeeding (OR=0.4,  $p < 1 \times 10^{-27}$ ), and living in households with larger number of residents (OR 0.7 for each additional person,  $p < 1 \times 10^{-15}$ ). In a predictive model, the identified associations were more likely to yield true positive rather than false positive predictions.

**Conclusions:** We develop a new approach for high-dimensional association studies of HIV risk in household surveys. We identify under-recognized risk factors for HIV in Zambia, including being a widow and drinking alcohol. This approach could be expanded to improve risk identification throughout sub-Saharan Africa.

Figure 1: Associated HIV risk factors above the Bonferroni threshold (out of 699)



**938 When to Start Antiretroviral Therapy in HIV-Positive Persons Aged Over 50 Years**

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**Background:** The START randomized controlled trial has shown that early initiation of combined antiretroviral therapy (cART) reduces the risk of serious AIDS and non-AIDS events in HIV-positive individuals. However, since the individuals included in START were relatively young and the number of deaths was low, it is unknown whether early initiation reduces mortality in older HIV-positive individuals. While earlier cART initiation may help decrease mortality associated with non-AIDS comorbidities, it might complicate clinical management because of polypharmacy and drug interactions. Our objective is to compare the risk of mortality in ART-naïve and AIDS-free individuals aged ≥50 years under i) immediate initiation and ii) initiation at AIDS or CD4 count <350 cells/mm<sup>3</sup>.

**Methods:** We used data from the HIV-CAUSAL Collaboration of 12 HIV cohorts from Europe and the US. We included ART-naïve and AIDS-free individuals who were enrolled after 1999 with age ≥50 years. Follow-up started at enrolment and ended at the earliest of death, 12 months with no laboratory measurement, or administrative censoring. We applied the parametric g-formula, a generalisation of standardisation, to estimate the 7-year risk of all-cause and non AIDS-related mortality under each cART initiation strategy. Estimates were adjusted for time-varying CD4 count, HIV-RNA and AIDS, and for baseline characteristics (calendar period, risk group, gender, geographical origin, and cohort). To interpret these results, the same analyses were conducted on individuals in age groups 18-34 and 35-49 years at enrolment. Standard errors were computed using 200 bootstrap repetitions.

**Results:** Of 9,837 eligible individuals with age ≥50 at enrolment (84% men), 73% initiated cART during follow-up. Median [interquartile range (IQR)] age at baseline was 54 [51,59] years. Median [IQR] CD4 count at baseline and at cART initiation were 365 [209,532] and 258 [164,350] cells/mm<sup>3</sup>, respectively. During a median [IQR] follow-up of 37 [18,68] months, we identified 1085 deaths of which 468 were non AIDS-defining. The 7-year risk of all-cause and non AIDS-defining mortality for initiation with CD4 <350 cells/mm<sup>3</sup> was lower compared with immediate initiation in individuals with age ≥50 years (Table).

There was a trend for increase benefit of early initiation with older age. **Conclusions:** Earlier initiation might be effective in reducing all-cause and non AIDS-related mortality in AIDS-free patients with age ≥50 years. Earlier initiation should be prioritised in older patients.

**Table.** Seven-year mortality risk and risk ratios under two cART initiation strategies in AIDS-free patients aged ≥50, 35-49 and 18-34 years.

cART initiation strategy	All-cause mortality		Non-AIDS-related mortality	
	7-year risk (%)	Risk ratio (95% CI)	7-year risk (%)	Risk ratio (95% CI)
<i>Age ≥50 years (N=9,857)</i>				
Immediate treatment	13.8 (13.0,15.1)	1(ref)	6.4 (5.8,7.1)	1 (ref)
CD4<350 cells/mm <sup>3</sup>	15.2 (14.4,16.7)	1.10 (1.07,1.11)	7.0 (6.5,7.5)	1.09(1.06,1.12)
<i>Age 35-49 years (N=29,700)</i>				
Immediate treatment	5.49 (4.91,5.89)	1(ref)	2.25 (1.95,2.55)	1 (ref)
CD4<350 cells/mm <sup>3</sup>	5.80 (5.22,6.17)	1.06 (1.04,1.08)	2.38 (2.08,2.66)	1.06 (1.01,1.11)
<i>Age 18-34 years (N=28,940)</i>				
Immediate treatment	1.56 (1.36,1.85)	1(ref)	0.64 (0.49,0.85)	1 (ref)
CD4<350 cells/mm <sup>3</sup>	1.65 (1.47,1.89)	1.05 (0.97,1.14)	0.63 (0.50,0.79)	0.98 (0.85,1.12)

**939 Changes in Markers of T-Cell Senescence and Exhaustion With HIV Therapy**

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**Background:** Residual immune activation and immunosenescence may contribute to chronic comorbidities in treated HIV-1 infection. It is unclear whether the integrase inhibitor, raltegravir (RAL), which has increased penetration into the gastrointestinal and lymphoid tissues, affects immunosenescence and T cell exhaustion to a greater extent than ritonavir boosted protease inhibitors (PIs).

**Methods:** A5260s is a substudy of a large prospective, randomized multicenter clinical trial that included HIV-1 infected treatment-naïve participants randomized to receive tenofovir disoproxil fumarate-emtricitabine (TDF/FTC) plus: atazanavir/ritonavir (ATV/r), darunavir/ritonavir (DRV/r), or RAL. Analyses include 234 participants (71% of all A5260s participants) who achieved and maintained plasma HIV-1 RNA <50 copies/ml by week 24. Blood cellular markers of immunosenescence and exhaustion of both CD4+ and CD8+ T cell subsets included: %CD28-CD57+, %CD28-CD57+ PD1+ and % PD1+. Changes from baseline were examined at earlier (24 weeks) and later (96 weeks) time points as fold change and 95% confidence intervals. Pairwise treatment group comparisons used Wilcoxon rank-sum tests with p-values adjusted with false discovery rate control.

**Results:** Sustained declines over time were evident in all treatment groups for all CD4+ T cell markers of immunosenescence and exhaustion, with no apparent differences between treatment groups. Markers of CD8+ T cell exhaustion (but not immunosenescence) declined over time in all groups, without major differences between groups.

**Conclusions:** In this prospective randomized clinical trial of initially ART-naïve individuals initiating successful ART regimens of TDF/FTC with RAL, ATV/r or DRV/r, we did not find between groups differences in measured markers of T cell senescence or exhaustion after 96 weeks of ART. Despite successful ART therapy, markers of CD8+ T cell immunosenescence did not decrease by 96 weeks. These data support further the accumulating evidence of incomplete reversal of immune activation and senescence in the setting of current effective ART compared to ART naïve HIV-1 infected

Biomarkers	Mean fold change (95% CI) from baseline over time by treatment group	ATV/r		RAL		DRV/r	
	Baseline Median (Q1, Q3)	Week 24 (n=58)	Week 96 (n=61)	Week 24 (n=65)	Week 96 (n=70)	Week 24 (n=73)	Week 96 (n=72)
<b>Immunosenescence</b>							
%CD8+CD28-CD57+	24.35 (17.8, 30.75)	1.04 (0.91,1.19)	0.96 (0.85, 1.09)	1.15 (1.07, 1.23)	1.07 (0.98, 1.17)	1.13 (1.07, 1.20)	0.99 (0.89, 1.10)
%CD4+CD28-CD57+	5.01 (2.24, 9.97)	0.73 (0.60, 0.90)	0.66 (0.52, 0.85)	0.85 (0.72, 1.00)	0.76 (0.62, 0.93)	0.85 (0.74, 0.97)	0.65 (0.54, 0.79)
<b>Cell exhaustion</b>							
%CD8+PD1+	2.33 (1.48, 3.87)	0.43 (0.34, 0.55)	0.42 (0.33, 0.54)	0.42 (0.36, 0.49)	0.33 (0.27, 0.40)	0.35 (0.30, 0.42)	0.32 (0.24, 0.43)
%CD4+PD1+	4.37 (4.08, 5.01)	0.58 (0.46, 0.70)	0.38 (0.30, 0.47)	0.60 (0.50, 0.70)	0.44 (0.37, 0.52)	0.50 (0.44, 0.58)	0.39 (0.32, 0.48)
<b>Immunosenescence and exhaustion</b>							
%CD8+CD28-CD57+PD1+	0.08 (0.05, 0.14)	0.41 (0.33, 0.51)	0.43 (0.34, 0.56)	0.47 (0.32, 0.69)	0.42 (0.30, 0.58)	0.39 (0.30, 0.51)	0.34 (0.25, 0.45)
%CD4+CD28-CD57+PD1+	0.03 (0.02, 0.06)	0.35 (0.26, 0.48)	0.26 (0.18, 0.37)	0.43 (0.31, 0.61)	0.30 (0.19, 0.46)	0.32 (0.22, 0.46)	0.26 (0.18, 0.38)

**940 Should NNRTIs Remain As the First-Line Therapy Choice in Resource-Limited Settings?**

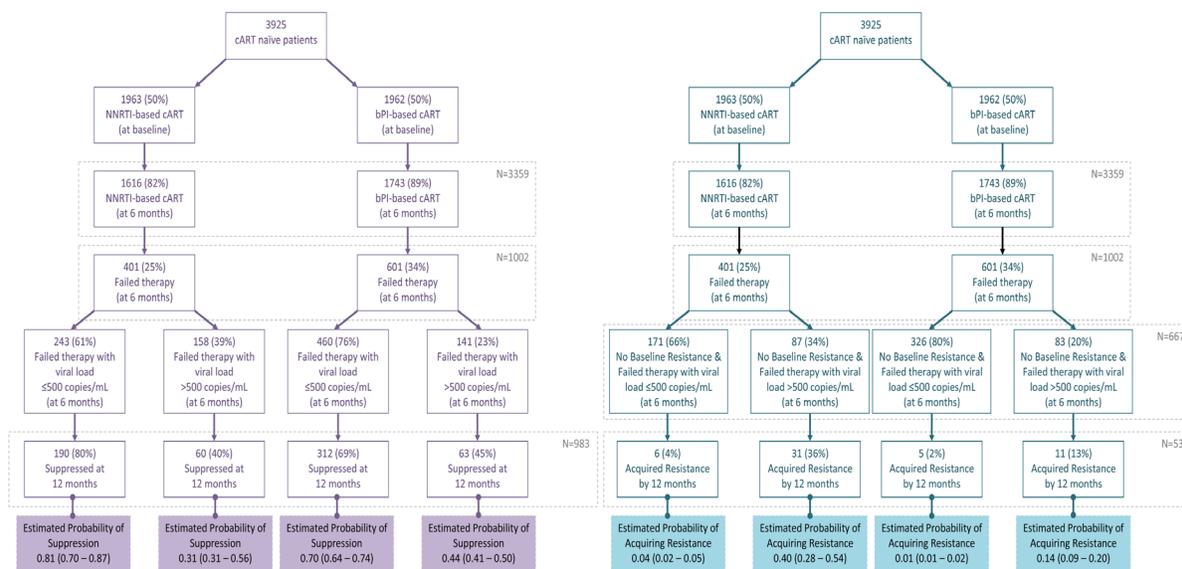
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**Background:** In many resource-limited settings, treatment failure is usually diagnosed clinically or immunologically. As such, there is a high likelihood that patients will stay on a failing regimen for a long period of time, with increased risk for resistance. In this study, we compared the long-term effectiveness of non-nucleoside reverse transcriptase inhibitors (NNRTIs) and boosted protease inhibitors (bPIs) among patients remaining on their initial failing regimens.

**Methods:** We followed prospectively 3925 ART-naïve patients who started on regimens containing either NNRTIs (N=1963, 50%) or boosted protease inhibitors (bPIs) (N=1962; 50%) from January 1, 2000 until June 30, 2013 in British Columbia, Canada (Figure). At six months, we assessed whether patients virologically failed therapy (a viral load >50 copies/mL), and we classified these patients as those who failed with a viral load ≤500 versus >500 copies/mL. We then followed these patients for 12 months and calculated their probability of achieving subsequent viral suppression (two consecutive viral loads <50 copies/mL) and of developing drug resistance to any ART class. These probabilities were adjusted for fixed and time-varying factors, including ART adherence. Patients did not change regimen during the study period.

**Results:** At six months, the virologic failure rate of those on NNRTI and bPI was, respectively, 15.5 and 23.0 cases per 100 person-years. Patients on NNRTI-based regimens who failed therapy with a viral load ≤500 copies/mL had a 16% higher probability of achieving subsequent suppression at 12 months than those on bPI (0.81 (Q1-Q3 0.70-0.87) versus 0.70 (0.64-0.74)). However, if patients on NNRTI-based regimens failed therapy with a viral load >500 copies/mL, they had a 30% lower probability of suppressing at 12 months than those on bPI (0.31 (0.31-0.56) versus 0.44 (0.41-0.50)). In terms of resistance, those who failed on NNRTI performed worse than bPI in all scenarios, especially if they failed with a viral load >500 copies/mL.

**Conclusions:** Our results showed that patients who failed with a high viral load and continued on their first-line NNRTI regimen, in comparison to those on bPI, had a lower probability of subsequently achieving viral suppression. These patients had a higher chance of acquiring drug resistance in all scenarios. Thus, if NNRTI continues to be the preferred choice for first-line therapy, these results highlight the importance of improving access to regular virologic monitoring of these patients.



**941 Lower Mortality Among Patients Starting ART From 2008 to 2010 Than Earlier in the ART Era**

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**Background:** Antiretroviral drugs available at the beginning of the ART era were inferior (in terms of both toxicity and efficacy) compared with those recommended in current guidelines. Other aspects of health care for HIV-1 positive patients have also improved since 1996. The implications of these changes for survival after starting ART are unclear.

**Methods:** We analysed all-cause and cause-specific mortality in previously ART-naïve adults from 19 European and North America cohorts contributing to the ART Cohort Collaboration (ART-CC) who started triple ART between 1996 and 2010 and had at least 3 years potential follow up. We used Cox models, stratified by cohort, to estimate adjusted (for age, sex, AIDS diagnosis, IDU risk group, CD4 count and HIV-1 RNA at start of ART) hazard ratios (HR) for calendar period of starting ART (2000-03, 2004-07, 2008-10 compared with 1996-99) during (1) the 1st year and (2) the 2nd and 3rd years of ART.

**Results:** Of 88,504 eligible patients, 2,106 died during the 1st year and 2,302 during the 2nd and 3rd years of ART. One-year all-cause mortality was notably lower among patients starting ART in 2008-10 than in previous years [adjusted HR (aHR) 0.70 (95% CI 0.60-0.82) compared with 1996-9] (see table). Declines in 2 and 3 year all-cause mortality were even more marked [aHR 0.47 (95% CI 0.40-0.55)]. Improvements were similar in men and women and consistent across Europe and North America. Trends in 2 and 3 year mortality were consistent across most subgroups and were partially explained by response to ART [HR for 2008-10 vs 1996-9 additionally adjusting for 12 month CD4 count and viral load 0.75 (0.63, 0.90)]. Rates of AIDS [aHR 0.63 (95% CI 0.49, 0.82) for 2008-10 vs 1996-9], suicides, accidents and overdoses [aHR 0.47 (95% CI 0.22, 0.99)] and cardiovascular [aHR 0.21 (95% CI 0.06, 0.73)] mortality were lower during the 1st year of ART. Rates of all specific causes of death examined were lower in the 2nd and 3rd years of follow-up for those starting 2008-10 vs 1996-99. Overall life-expectancy at age 35 improved by nearly 10 years between 1996-99 and 2008-10.

**Conclusions:** Marked improvements in survival during the first 3 years of ART likely reflect availability of better antiretrovirals, more options for patients who develop resistance, and improvements in health care for HIV-positive patients. Prognostic models should be updated to account for these improvements.

**Table:** Hazard ratios (95% CI) (adjusted for age, sex, CD4, viral load, AIDS and IDU, stratified by cohort) for all-cause and cause-specific mortality by calendar period (vs. 1996-99), during the 1<sup>st</sup> year and the 2<sup>nd</sup> and 3<sup>rd</sup> years after starting ART.

Cause of death	First year of ART			2nd and 3rd years of ART		
	2000-3	2004-7	2008-10	2000-3	2004-7	2008-10
All causes	0.98 (0.88 1.09)	0.95 (0.85 1.07)	0.70 (0.60 0.82)	0.82 (0.75 0.91)	0.65 (0.58 0.73)	0.47 (0.40 0.55)
AIDS	1.06 (0.89 1.26)	0.93 (0.77 1.11)	0.63 (0.49 0.82)	0.77 (0.64 0.93)	0.48 (0.39 0.60)	0.20 (0.14 0.30)
Non-AIDS infection	1.49 (0.89 2.51)	1.49 (0.87 2.55)	0.51 (0.22 1.20)	1.28 (0.84 1.94)	0.78 (0.47 1.28)	0.23 (0.09 0.59)
Non-AIDS, non-liver cancer	0.93 (0.55 1.59)	1.48 (0.90 2.44)	0.67 (0.34 1.31)	0.66 (0.46 0.96)	0.92 (0.65 1.31)	0.36 (0.21 0.63)
Liver-related	1.04 (0.58 1.89)	1.02 (0.54 1.91)	0.43 (0.16 1.15)	1.05 (0.69 1.59)	0.47 (0.27 0.82)	0.18 (0.06 0.51)
Cardiovascular	1.06 (0.57 1.97)	0.76 (0.38 1.51)	0.21 (0.06 0.73)	1.36 (0.83 2.24)	1.01 (0.58 1.76)	0.29 (0.11 0.76)

**942 Impact of Each Month Delay in Initiation on the Effect of 1 Year of ART on CD4 Count**

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**Background:** START results demonstrate that earlier antiretroviral treatment (ART) benefits HIV-infected individuals. We assess the impact of time from infection to starting ART on one-year ART effects using data from the observational prospective Acute Infection Early Disease Research Program (AIEDRP). Challenges arise from confounding by indication (e.g., time from infection to initiation of ART may depend on disease state).

**Methods:** AIEDRP data, which provide well-characterized estimated dates of infection (EDI), permit estimation of the dependence of change in CD4 counts after one year of ART on the time from EDI to initiation. We considered treatment initiation times within one year of EDI and investigated the effect of a year of ART in two ways: ignoring ART interruptions, and censoring patients who interrupted ART for more than 21 days. We used doubly robust estimation of Coarse Structural Nested Mean Models to adjust for baseline and time-varying confounders to eliminate selection bias likely to be present in a longitudinal observational study.

**Results:** We included 1696 patients; 36.7% and 24.5% initiated ART during the acute and early phases respectively, and 38.8% did not initiate ART during follow-up. We modeled the expected CD4 count increase due to one-year ART as a linear function of time from EDI to ART initiation, given pre-treatment covariates. The results were presented in Table 1. These findings show a benefit of earlier ART initiation—particularly if there is no interruption in the first year of therapy; for example, starting 6 months earlier would lead to an expected added improvement in CD4 counts of 45.6 cells/μl after a year of therapy. These findings support those from Le et al (NEJM 2013), based on the same data but analyzed using different methods.

**Conclusions:** Earlier initiation of ART during acute and early infection improves the CD4 count gain associated with one year of uninterrupted ART, emphasizing the importance of early detection of HIV infection and subsequent therapy. The new causal methods permit estimation of quantities that may be clinically relevant—the cost of each month of delay in ART initiation on CD4 response to therapy—that require sophisticated methods for adjustment for confounding by indication.

	Estimate	95% Confidence Interval
Effect of one-year of uninterrupted ART		
Effect if started at EDI	317	(262, 359)
Decrease for each month of delay after EI (per month)	-7.6	(-14, -0.4)
Effect of one-year of ART (some with interruption, 243 of 1038 on treatment, or 23%)		
Effect if started at EDI	311	(273, 351)
Decrease for each month of delay after EI (per month)	-5.0	(-12, 0.5)

**943 Outcomes on cART in France According to Geographic Origin, Sex, and Transmission Group**

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**Background:** Cohort studies on outcomes after cART initiation according to geographic origin (GO) show conflicting results. We thus aimed to compare biological and clinical outcomes on cART in HIV-1-infected individuals in France, according to GO, sex and transmission group.

**Methods:** Antiretroviral-naïve HIV-1-infected adults enrolled in the FHDH-ANRS CO4 cohort, originating either from France (FRA) or from sub-Saharan Africa or non-French West Indies (SSA/NFW), and who started cART between 2006 and 2011 were included. Women initiating cART because of pregnancy were excluded. We assessed 2-year Kaplan-Meier (KM) probabilities and adjusted hazard ratios (aHRs) for undetectability (2 consecutive plasma viral loads (pVL) <50 copies/mL) and CD4 cell recovery (2 consecutive CD4 >500/μL), and 5-year cumulative incidences (CI) and adjusted subdistribution-hazard ratios (aSHRs) for clinical outcome (AIDS-event, serious non-AIDS-event (SNAE) or death) using competing risk models taking into account loss to follow-up, according to GO, sex and transmission group. Models were adjusted for demographic, immunovirological data and therapeutics at cART initiation (table), plus time-updated undetectability in immunological analysis or plus time-updated undetectability and CD4 increase >50/μL in clinical analyses.

**Results:** Among 9746 individuals, 7297 (74.9%) were native from FRA and 2449 (25.1%) were migrants from SSA/NFW, of whom 1552 (21.3%) and 1350 (55.1%) were women respectively. A higher proportion of patients from SSA/NFW (38.1%) than from FRA (27.5%) initiated cART with CD4 <200 cells/μL (p<0.0001). After cART initiation and compared to FRA men who have sex with men (MSM), aHRs for undetectability were lower in men whatever their origin and slightly lower in FRA women; aHRs for CD4 cell recovery were lower in all groups of patients except for FRA non homosexual men; aSHRs of clinical outcomes (359 individuals with a new-AIDS event mainly in the first 6 months of cART, 1366 with SNAE, 49 deaths) were higher in men whatever their origin and borderline significant higher in SSA/NFW women. AIDS status, older age and lower CD4 cell count at cART initiation had the highest impact on the change between the crude and adjusted SHRs of clinical outcomes.

**Conclusions:** Although migrants initiated cART at lower CD4 count and had the lowest likelihood of CD4 cell recovery compared to FRA MSM, male sex in non homosexuals whatever the geographic origin had a negative impact on undetectability and clinical outcome.

**Table: Two-year KM probabilities (95%CI) and adjusted HRs [95%CI] for biological response, and 5-year % (95% CI) and adjusted SHR [95%CI] of clinical outcome**

GO, sex and transmission group	Undetectability		CD4 cell recovery		Clinical outcome	
	2-year %	Adjusted HR*	2-year %	Adjusted HR*	5-year CI	Adjusted SHR*
FRA MSM	87.5(86.4-88.5)	1	53.6(51.9-55.3)	1	20.6(19.3-21.9)	1
FRA Non homosexual men	80.9(79.2-82.6)	0.82[0.77-0.87]	37.7(35.5-39.9)	0.92[0.84-1.02]	30.0(28.0-32.0)	1.18[1.03-1.34]
FRA Women	83.9(82.0-85.7)	0.92[0.86-0.99]	41.0(38.4-43.5)	0.89[0.80-0.98]	22.0(20.0-24.1)	1.03[0.88-1.20]
SSA/NFW Men	80.0(77.7-82.4)	0.84[0.77-0.91]	28.4(25.7-31.1)	0.67[0.58-0.77]	26.6(24.0-29.2)	1.18[1.01-1.39]
SSA/NFW Women	86.1(84.3-88.0)	1.04[0.97-1.12]	35.9(33.3-38.5)	0.73[0.65-0.82]	22.3(20.0-24.5)	1.16[0.99-1.36]

\*Adjusted for age, region, period, CD4, pVL, AIDS status, HBV/HCV, primary infection, time since diagnosis, treatment at cART initiation

#### 944 CD4 Cell Response to First-Line cART by HIV Type in European Cohort Collaborations

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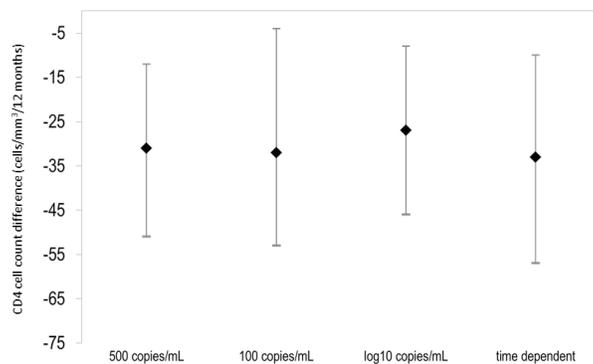
**Background:** CD4 cell recovery following first line combination antiretroviral treatment (cART) is known to be poorer in HIV-2 than in HIV-1 infected patients (Matheron et al AIDS 2006; Drylewicz et al AIDS 2008) but has not been studied yet in large datasets while adjusting for plasma viral load (pVL) and other characteristics at cART initiation.

**Methods:** Adult patients from two European multi-cohort collaborations, COHERE (HIV-1) and ACHIEV2e (HIV-2) were included, if they were treatment-naïve and started first line cART (NNRTI-containing regimens excluded) from 1997 to 2011, had  $\geq 1$  CD4 cell measure before and after start of cART, and a pretreatment pVL. Evolution of CD4 cell count was studied using linear mixed models adjusting for pre-treatment pVL, age, sex, geographic origin, HIV transmission route, previous AIDS diagnosis, cART regimen, period of cART initiation, and pre-treatment CD4 cell counts. We did several sensitivity analyses considering pVL as a binary variable (with different cut-offs), as a continuous and as a time dependent covariable.

**Results:** We included 159 HIV-2 and 42,735 HIV-1 infected patients with a median age of 46 (IQR: 36; 52) and 37 (IQR: 32; 44) years, respectively. Median pre-treatment CD4 cell counts/mm<sup>3</sup> were 182 (IQR: 83-285) and 224 (IQR: 100-352) in HIV-2 and HIV-1 infected patients, respectively. The median observed CD4 cell counts at 12 months were 276 (171; 416) in HIV-2 and 382 (244; 550) in HIV-1 infected patients. Adjusted estimated mean CD4 cell increases within the first year of cART were overall significantly lower in HIV-2 compared to HIV-1 infected patients: 31 CD4 cells/mm<sup>3</sup>/12 months less (95% CI: 12; 51;  $P=0.002$ ).

In sensitivity analyses (Figure) similar differences between HIV-2 and HIV-1 have been found. In stratified analysis, differences in CD4 cell increases for HIV type were not modified by the initial cART regimen (interaction test:  $P=0.93$ ). Patients receiving a boosted lopinavir or darunavir containing cART HIV-2 infected patients had a slower CD4 cell increase than HIV-1 infected patients (33 cells/mm<sup>3</sup>/12 months less (95% CI: 3-62;  $P=0.03$ )).

**Conclusions:** Poorer CD4 cell increase following first-line cART in HIV-2 infected patients was consistent in all analyses and independent of pre-treatment pVL, as well as of age, cART regimen and other covariables adjusted for. Our results are in favour of early treatment of HIV-2 infection and the need to identify the most potent drugs against HIV-2.



**Figure : Adjusted estimated mean CD4 cell difference 12 months after cART initiation between HIV-2 versus HIV-1 from adjusted linear mixed models, main and sensitivity analyses.** Legend: Vertical bars indicate 95% confidence intervals. The negative difference indicates a slower increase in CD4 cell counts in HIV-2 versus HIV-1 infected patients. Plasma viral load was considered in the linear mixed model as a categorical variable with a cut-off of 500 copies/mL (main analyses), 100 copies/mL, as a continuous co-variable ( $\log_{10}$  copies/mL), and as a time dependent co-variable allowing plasma viral load values to change over time.

#### 945 Efficiency (Cost-Effectiveness) of EFV/FTC/TDF vs FTC/RPV/TDF in Naïve Patients

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**Background:** FTC/RPV/TDF (EPA) has advantages compared with EFV/FTC/TDF (ATR) in patients with baseline viral load of less than 10<sup>5</sup> copies/ml. Yet, a formal cost-effectiveness (efficiency) analysis had not been performed so far.

**Methods:** All HIV antiretroviral naïve patients of a single center who initiated ATR or EPA and with a minimum of 48 weeks potential follow-up were eligible and followed up to censoring date (Dec 2014). Data were prospectively collected in a predefined database. Effectiveness was measured as percentage of patients < 50 copies/ml. at 48 weeks by ITT

(Missing or NC=Failure). Costs included the direct cost of antiretrovirals (Spanish official prizes) plus those related with outpatient visits, hospital admissions and resistance tests (local prizes). Efficiency or cost-effectiveness was the ratio between costs and effectiveness for the base case scenario and for the most (higher 95% CI for effectiveness and lower 95% CI for cost) and less favorable (lower 95% CI for effectiveness and higher 95% CI for cost) scenarios as a sensitivity analysis.

**Results:** 501 patients (329 ATR and 172 EPA) were included. Median age was 35 years; 92% were males, 8% were co-infected with HCV, 17% had a CD4+ cell count < 200 and 23% had viral load >100,000 copies/ml (34% in the ATR group vs 1% in the EPA group;  $p < 0.0001$ ). Median duration of assigned regimen was 3.7 and 1.6 years for the ATR and EPA respectively due to late enrollment of EPA patients. Almost all patients (99% and 100% in the ATR and EPA arms respectively) completed at least one year of follow-up. Response rate at 48 weeks were 77% and 88% for ATP and EPA (difference of 9.2%; 95% CI 5.9% to 12.5%;  $p < 0.0042$ ) respectively. Virological failure and interruptions for tolerance problems were 3% and 19% the ATR arm and 2% and 9% in the EPA arm respectively ( $p < 0.001$ ). In the case base scenario cost per responder at 48 weeks (cost-effectiveness or efficiency) was 12,177 euros in the ATR arm and 9,366 euros in the EPA arm (ratio of 0.85 or 15% lower). Similar trends were observed in the less and most favorable scenarios.

**Conclusions:** The cost per responder at 48 weeks (cost-effectiveness or efficiency) of EPA in naïve patients was 15% lower than ATR mainly driven by a better tolerance. Our data supports the recommendation of EPA as opposed to ATR when baseline viral load is below  $10^5$  HIV RNA copies/ml of plasma.

#### 946 Correlations of Pre-ART HIV-DNA With Outcome in First-Line Treated ART Patients

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**Background:** Quantification of HIV-1 DNA reflects the size of viral burden in HIV-1 infected patients (pts). By using a commercial assay, we aimed to investigate the correlation of pre-ART (baseline, BL) HIV DNA with the BL viro-immuno-clinical status and on the viro-immuno-clinical response to ART in pts starting their first regimen.

**Methods:** HIV+ pts of the ICONA cohort, starting 1st-line ART, for whom PBMC or blood sample was stored at BL were analysed. Total HIV-DNA was quantified by using a modified version of the Cobas HIV-1 test (Surdo et al 2015). Results were normalized by CD4+ cells nr. Associations between BL DNA levels and pts BL characteristics were evaluated using chi-square/signed-rank test and Pearson correlation. In a subset of pts starting "modern" ART (after 2004), standard survival analysis was used to examine the association between BL HIV-DNA and pre-defined time to event endpoints: viral load (VL)  $\leq 50$  cp/mL, virological rebound defined by a confirmed VL >50 cp/mL after VL  $\leq 50$  cp/mL, gain in CD4 count >200 cells/mm<sup>3</sup> after ART and AIDS diagnosis or serious non-AIDS or death. Kaplan-Meier curves and Cox regression models were also used.

**Results:** We included 607 pts (23% female, 38 yrs median age, median [IQR] CD4 = 288 [144-401], who started ART on median [IQR] = 2010 [2002-2011]. BL median [IQR] HIV-DNA and HIV-RNA was 10574 [3208-38218] cp/10<sup>6</sup> CD4+ and 4.83 [4.31-5.33] log cp/mL, respectively. According to BL HIV-DNA levels (divided in 3 groups: 10-1000 cp/10<sup>6</sup> CD4+, n=69, 1000-10000 cp/10<sup>6</sup> CD4+, n=224, and >10000 cp/10<sup>6</sup> CD4+, n=314), a strong significant correlation ( $p \leq 0.001$ ) was observed with BL HIV-RNA (Pearson rho = +0.41), CD4 (-0.48) and CD4/CD8 ratio (-0.40) and less strong ( $p \leq 0.03$ ) with IL-6 (+0.15), sCD14 (+0.11) and CD8 (-0.09). By wk 48, 393/607 (65%) achieved HIV-RNA  $\leq 50$  cp/mL (290/395, 73% in patients starting ART after 2004). Within this subset, median (95% CI) times to a HIV-RNA  $\leq 50$  cp/mL were 7 (4-11) mo in people with 10-1000 HIV-DNA cp/10<sup>6</sup> CD4+, 8 (5-11) mo in those with 1000-10000 cp/10<sup>6</sup> CD4+ vs. 10 (7-15) mo in those with >10000 cp/10<sup>6</sup> CD4+ ( $p = 0.0008$ ). Unadjusted and adjusted hazard ratios of the pre-defined outcomes from fitting the Cox models are shown in Table.

**Conclusions:** Pre-ART HIV-DNA content in CD4+ cells strongly correlated with BL viro-immunologic parameters and inflammation markers. BL HIV-DNA was also found to predict virological and clinical outcome of 1st-line ART, although not independently of plasma VL and CD4 count.

Table hazard ratios of various outcomes per log10 10<sup>6</sup>/CD4 HIV-DNA higher levels in patients starting 1<sup>st</sup> line ART after 2004

Outcomes	Hazard Ratio (95% CI) p-value			
	Unadjusted	Adjusted (a)	Adjusted (b)	Adjusted (c)
Viral load $\leq 50$ cp/mL	0.71 (0.62, 0.82) P<.001	0.73 (0.63, 0.85) P<.001	0.86 (0.72, 1.02) P=0.074	0.89 (0.74, 1.06) P=0.197
CD4 count gain >200 cells/mm <sup>3</sup> above pre-ART	1.01 (0.88, 1.16) P=0.896	1.00 (0.86, 1.16) P=0.994	0.88 (0.74, 1.05) P=0.156	0.87 (0.73, 1.04) P=0.133
Viral rebound >50 cp/mL	2.14 (1.41, 3.25) P<.001	1.99 (1.29, 3.09) P=0.002	1.45 (0.88, 2.39) P=0.142	1.23 (0.71, 2.12) P=0.459
AIDS, serious non-AIDS and death	2.14 (1.13, 4.05) P=0.019	2.08 (1.00, 4.33) P=0.051	1.49 (0.69, 3.20) P=0.309	1.53 (0.59, 3.95) P=0.380

(a) Adjusted for calendar year of ART initiation and type of regimen started

(b) Adjusted for viral load and CD4 count at ART initiation

(c) Adjusted for all factors in footnotes a-b, + age, smoking, HCV status, IL-6 and sCD14.

#### 947 Comparable Viral Decay in Dual and Triple Dolutegravir-Based Antiretroviral Therapy

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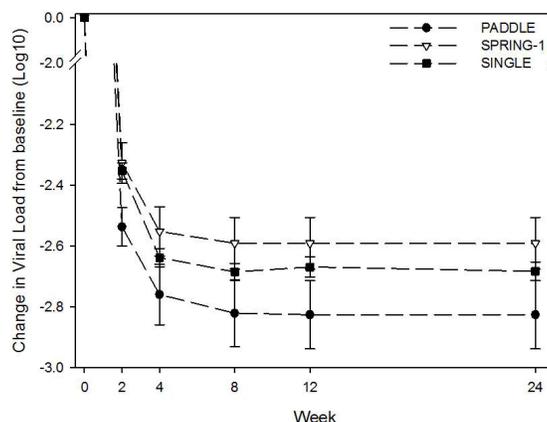
**Background:** Drug-sparing strategies have been explored lately, aiming to improve tolerability and adherence, reduce toxicity, and costs. Two studies have shown non inferiority of dual therapy in ARV-naïve HIV-1 infected individuals (NEAT and GARDEL). The PADDLE study showed that a dual therapy regimen based on dolutegravir plus lamivudine (DTG/3TC) induced a rapid viral decay in treatment naïve patients with screening pVL <100K (EACS 2015). Our objective is to compare differences between plasma viral load (pVL) change at each time point with a dual therapy, DTG/3TC, to triple therapy regimens used in the SPRING-1 (DTG 50 mg +2NRTIs) and SINGLE study (DTG plus abacavir/lamivudine), in patients with baseline (BL) pVL < 100,000 copies/mL.

**Methods:** In PADDLE (n=20), pVL was tested at BL, days 2, 4, 7, 10, weeks 2, 4, 8, 12, 24 and thereafter. In SINGLE (n=280) it was measured at BL, wk 2, 4, 8, 12, 16, 24 and thereafter. In SPRING-1 (n=39) pVL was measured at BL, wk 1, 2, 4, 8, 12, 16, 20, 24 and thereafter. Change in pVL vs. baseline was calculated only for time points with data for the three studies (Weeks 2, 4, 8, 12 and 24). Effects of time and treatment, was analyzed by two-way ANOVA, followed by Tukey-HSD post-hoc test.

**Results:** BL pVL (Mean±SD) was 4.43 (0.50), 4.30 (0.45) and 4.31 (0.52) for PADDLE, SINGLE and SPRING-1 respectively. Rapid decline in viral load was observed in the three regimens. Two-way ANOVA revealed significant effects for treatment ( $F_{2,1605}=30.3$   $p<0.001$ ) and time ( $F_{4,1605}=22.8$   $p<0.001$ ), without significant interactions. Average effects of treatment in PADDLE, SPRING-1 and SINGLE were  $-2.75\pm 0.45$  (Mean ±SD),  $-2.53\pm 0.49$  and  $-2.61\pm 0.48$   $\log_{10}$  respectively. Significant differences were observed between PADDLE and SPRING-1 or SINGLE studies ( $p<0.01$  and  $p<0.05$ ).

The figure shows the viral load change at each time point in the studies (Mean ± Standard error of the mean)

**Conclusions:** Viral load change was of similar magnitude after a dual therapy regimen DTG/3TC compared to 2 DTG-based triple therapy regimens. These results, albeit encouraging, should be interpreted with caution, as the analysis is based on a cross-study comparison of mean values and PADDLE is a small pilot study. Full powered, randomized studies are in progress to evaluate DTG/3TC as a valid option for first line therapy.



#### 948 Unexpectedly High Rate of Intolerance for Dolutegravir in Real-Life Setting

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**Background:** Integrase inhibitors are now preferred antiretrovirals in first line cART. Dolutegravir (DGV) is possibly considered as one of the most efficacious, convenient and tolerated INSTI, with hardly any chance for drug-drug interactions. Since we encountered many patients who stopped DGV because of intolerance, we analyzed the experience with DGV in our whole patient population since licensing in the Netherlands.

**Methods:** In our hospital cohort we retrospectively analyzed all patients who started DGV, either as initial therapy or after switching from other antiretrovirals for any reason. Baseline characteristics at the moment of DGV start were recorded. We calculated the proportion of patients who stopped DGV, analyzed the reason for interruption and evaluated potential risk factors. We used the Chi-squared test to check for significant differences between groups.

**Results:** In our cohort of almost 3000 hiv infected patients (97,6% on cART), 388 patients started DGV from August 2014 for a median period of 219 days (range 5-376) and at a median age of 48 years (range 23-77); 46 were female. In total 65 started as naives (median CD4 495/mm<sup>3</sup> (range 70-1610)). One patient died from progressive prostate carcinoma and was excluded from the analysis.

DGV treatment was stopped in 62/387 (16,0%) patients after a median of 78 days (range 5-327). Of the naives 9/65 (13,8%) stopped DGV compared to 53/322 (16,4%) of non-naives ( $p=ns$ ). Of the women 5/46 (10,9%) stopped, compared to 57/341 men (16,7%) ( $p=ns$ ). Of those who used DGV-only tablets 24/158 (15,6%) stopped, compared to 38/230 (16,5%) using combination-tablet (with ABC/3TC) ( $p=ns$ ). Main reason for DGV interruption was intolerance in 55/62 (88,7%) patients: 19/55 (34,5%) sleeping problems, 18/55 (32,7%) gastrointestinal problems, 12/55 (21,8%) psychiatric problems, 7/55 (12,7%) headache, 6/55 (10,9%) musculoskeletal problems and 6/55 (10,9%). Some patients reported more than one toxicity. There were no virological failures.

**Conclusions:** In a real life setting a substantial proportion of patients (16%) unexpectedly interrupted DGV treatment for reasons of intolerance, in particular sleeping-, gastrointestinal- and psychiatric problems. This was much higher than reported in clinical trials, where discontinuation of DGV due to adverse reactions is reported to be less than 3%.

#### 949 Association of Antiretroviral Use and Abnormal Uterine Bleeding in Women With HIV

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**Background:** Abnormal uterine bleeding is thought to be more common in women with HIV. Menstrual irregularity is a key health outcome that leads to anemia and poor quality of life. Factors associated with menstrual cycle disruption in HIV-positive women have not been well delineated. We measured the prevalence of abnormal uterine bleeding among women with HIV, and the association with current use of antiretroviral therapy (ART) and other covariates.

**Methods:** We used cross-sectional questionnaire data from the Canadian HIV Women's Sexual and Reproductive Health Cohort Study (CHIWOS) enrolling women with HIV (self-identified, aged  $\geq 16$  years) from British Columbia, Ontario and Québec. Eligible participants for this analysis were aged 16 to 45 years, and responded to questions on menstruation and ART use. Participants were excluded if they reported: a history of gynecologic cancer; amenorrhea attributed to spontaneous, surgery-induced, chemotherapy or radiation therapy-related menopause; premature ovarian failure; current pregnancy or hormonal contraception at or within 6 months prior to interview. Our primary outcome was presence of abnormal uterine bleeding, which included amenorrhea, oligomenorrhea, and/or intermenstrual bleeding. Multivariable logistic regression analysis examined independent correlates of abnormal uterine bleeding, including current use of ART.

**Results:** Of 1335 women enrolled in CHIWOS, 493 (37%) met the eligibility criteria. Overall, 71% reported abnormal uterine bleeding. In adjusted analyses, African, Caribbean and Black Canadian women and women who identify as "Other" or with multiple ethnicities had increased odds of abnormal uterine bleeding (AOR 5.82, 95%CI:2.99-11.30) compared to Caucasian women (and Indigenous women). Lower odds of abnormal uterine bleeding was found in women with no history of recreational drug use versus current users (AOR 0.06, 95%CI:0.02-0.15) as well as women who were treatment naïve (AOR 0.26, 95%CI:0.12-0.53) compared to women currently on ART.

**Conclusions:** Abnormal uterine bleeding was commonly reported by women with HIV participating in CHIWOS (71%) as compared to rates in the general population (30%). Correlates of abnormal uterine bleeding included current ART use, reporting an ethnicity other than Caucasian or Indigenous and recreational drug use. The mechanisms for abnormal uterine bleeding with regards to the identified correlates warrants further research in order to identify solutions.

**950 No Selection of X4 Viruses by Maraviroc in Cell Reservoirs in R5X4 HIV Infections**

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**Background:** The CCR5 antagonist maraviroc, used in combination with antiretroviral drugs in HIV-infected patients, is only active against CCR5-using viruses. However, some patients were given maraviroc although they were infected by R5X4 dual-mixed viruses. This was to assess the immunological benefit of maraviroc therapy. In the MARIMUNO study, patients with undetectable plasma HIV RNA received a 24-week maraviroc supplement to an efficient antiretroviral therapy. Since the positive selection of CXCR4-using viruses in cell reservoirs may influence any response to later treatment, we investigated how the frequency of CXCR4-using variants in R5X4 dual-mixed virus populations responded to maraviroc selection pressure using ultra-deep sequencing (UDS).

**Methods:** We explored 22 patients from the MARIMUNO study infected with R5X4 dual-mixed viruses according to the recombinant virus assay Toulouse Tropism Test. The frequency of CXCR4-using variants was determined in peripheral blood mononuclear cells (PBMCs) before maraviroc intensification (week 0) and after 24 weeks of maraviroc (week 24); both samples were tested in the same run. UDS was performed on a 454 GS Junior system. The sequence reads of the V3 *env* regions were analysed with PyroVir software (Inserm-Transfert) developed to provide a fast and automated position-specific process for inferring HIV-1 tropism from V3 *env* 454 ultra-deep pyrosequencing data.

**Results:** The mean total HIV-1 DNA before maraviroc intensification was 2.4 log copies/10<sup>6</sup> cells; it was 2.5 log copies/10<sup>6</sup> cells 24 weeks later (Wilcoxon rank test, P=0.3). UDS with the PyroVir genotypic algorithm detected CXCR4-using viruses in the 22 R5X4 infected patients at week 0 with a mean frequency of 59% (range: 3-100%). CXCR4-using viruses were detected in 21/22 patients at week 24 with a mean frequency of 52% (range: 10-92%). We found no correlation between the HIV DNA concentration in PBMCs and the number of CXCR4-using variants or their frequency. The frequency of CXCR4-using variants did not increase between weeks 0 and 24 except in patient 4 whose increase was 32%. The mean numbers of unique amino acid variants and X4 amino acid variants were similar at both stages.

**Conclusions:** A 24-week course of a CCR5 antagonist does not select CXCR4-using viruses in the PBMCs of patients on suppressive therapy infected with R5X4 dual-mixed viruses. These results indicate little or no residual HIV replication that could be subjected to selection pressure.

**951 Failure Rate in NRTI-Free Treatment Is Higher in 2 Compared to 3 Class Regimens**

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**Background:** Little knowledge exists about the optimal drug combination in nucleoside/tide (NRTI)-free antiretroviral treatment (ART). We studied the effectiveness of NRTI-free ART containing two compared to three drug classes.

**Methods:** We studied time to virological failure (VF) in 503 NRTI-free treatment episodes from 342 patients participating in the Swiss HIV Cohort Study (ART start after 01/01/2007). VF was defined as two consecutive viral loads (VL) >50 copies/mL or 1 VL >50 copies/mL followed by a treatment change or interruption after 180 days of continuous treatment or previous viral suppression. Cox proportional hazards models with robust standard errors to account for intra-patient correlation were performed. The following variables were considered as potential confounders: VL before start of NRTI-free ART, CD4 cell count, gender, risk group, age, previously reported non-adherence, previously experienced VF, year of first ART initiation, genotypic sensitivity score of NRTI-free ART (Stanford algorithm version 7.0) and presence of major drug resistance mutations (IAS-USA list 2014). In the multivariable model, all variables with a p-value <0.2 were included.

**Results:** A comparable number of NRTI-free episodes included two and three drug classes, 252 (50.1%) and 251 (49.9%), respectively. Most patients were ART-experienced (99%) and in 65% of patients VL was <50 copies/mL when starting the NRTI-free episode. Overall, 7% of patients experienced a treatment failure. Patients treated with two drug classes had a higher risk for ART failure, the multivariable hazard ratio (mHR) was 3.0 (95% CI: 1.4-6.6, p=0.007). Another important risk factor for VF was the VL level before NRTI-free ART initiation. The mHR was 3.2 (95% CI: 1.1-9.1, p=0.032) and 6.7 (95% CI: 2.6-17.7, p<0.001) for VL 50-10,000 and >10,000 copies/mL, respectively (reference <50 copies/mL). In addition, we found that male participants were more likely to fail NRTI-free ART (mHR: 3.0, 95% CI: 1.1-8.0). No other factor was significantly associated with VF.

**Conclusions:** NRTI-free ART with three drug classes was more effective compared to two drug classes. Thus, NRTI-free ART with two classes should be prescribed very cautiously, if at all, especially for patients with a detectable VL before starting NRTI-free treatment.

**952 The Impact of Medication Adherence on Virologic Failure in A5202**

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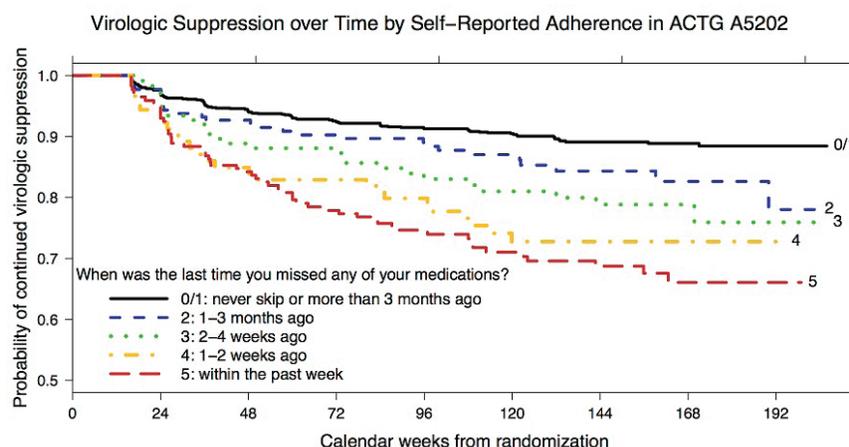
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**Background:** Poor adherence is widely recognized to decrease the efficacy of antiretroviral therapy (ART). This effect may vary depending on regimen. To quantify the impact of self-reported adherence on virologic failure (VF) in ART-naïve participants, we evaluated data from ACTG A5202. We also assessed the impact of adherence on the previously reported increased rate of VF in the abacavir-lamivudine (ABC-3TC) group compared to tenofovir-emtricitabine (TDF-FTC) in participants with screening HIV RNA ≥100,000 copies/mL.

**Methods:** We limited our analysis to the 1,798 (of 1,857 eligible; 97%) participants with adherence data. There were 11,303 self-reports, assessed at follow-up weeks 8, 24, and every 24 weeks thereafter, of when ART was most recently missed using a 6-point scale ranging from "never" to "within the last week". We used adherence as a time-varying covariate in a Cox proportional hazards model of time to VF in the entire cohort to estimate the hazard ratio (HR) with reduced adherence. We did additional analyses incorporating the randomized NRTI assignment as a fixed covariate in the 761 participants (of 798; 95%) with high screening viral load (HI-VL) and adherence data.

**Results:** Overall, 15% had study-defined VF. Adherence was a strong predictor of time to VF (Figure). With the "never skipped medications" and missed "more than 3 months ago" (rarely) reports pooled *post hoc* as the reference group, the HR increased monotonically from 1.6 (95%CI: 1.04-2.5) for the "1-3 months ago" group to 3.4 (95% CI: 2.4-4.8) for the "within the last week" group. To illustrate this impact, at 96-weeks 20% (95% CI: 12-29%) of participants who consistently missed pills "1 to 2 weeks ago" would be expected to have failed, compared to 9% (95%: 7-11%) of participants who rarely or never missed pills. In the analysis of participants with HI-VL, those randomized to ABC-3TC had a significantly increased HR of VF compared to TDF-FTC (1.70; 95% CI: 1.19-2.41, P=0.003). Adjusting for self-reported adherence in the period prior to VF partially accounts for this effect (HR: 1.56; 95% CI: 1.09-2.23, P=0.014).

**Conclusions:** Recent self-reported reduced adherence more than tripled the risk of VF in A5202. Although lower adherence partially accounted for the increased risk of VF in participants starting initial treatment with a HI-VL receiving ABC-3TC compared to TDF-FTC, significantly elevated risk of VF remained. Continued efforts to improve adherence will increase rates of virologic suppression with these regimens.



Legend: The figure shows the probability that virologic failure (VF) has not been detected (which we term "continued virologic suppression") in participants based on the most recent self-reported adherence. Unlike a standard Kaplan-Meier curve, the participants in each group change over time. Thus, for a participant who reported "missed within the last week" (code 5) at a visit in week 26, following a previous report of "no missed pills" (code 0) at a visit in week 8, their risk time would be included in the 0/1 group from weeks 0 to 8, and in the code 5 group from weeks 8 to 26. There is no VF for the first 16 weeks because, by study definition, VF can only occur at or after 16 weeks.

**953 INSTI In-Class Switching on Continued Viral Suppression in the OPERA Cohort**

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**Background:** The US HIV guidelines include within-class switches to simpler and less frequent regimens as a valid reason for changing a regimen. With its low risk of cross-resistance and once daily dosing, clinicians may consider substituting dolutegravir (DTG) for raltegravir (RAL) in an effort to improve adherence and durability. Clinical trials have evaluated INSTI intra-class switching, however, real-world assessments of stable RAL to DTG switching is currently lacking. This study tested the hypothesis that patients stably suppressed on RAL and switched to DTG would not significantly differ in risk of virologic failure from patients who continued on RAL.

**Methods:** Using the OPERA longitudinal database, individuals who initiated RAL after their first prospectively-collected visit were identified. Additionally, they were required to have RAL as their first INSTI regimen and achieve stable suppression (2 consecutive VL<75 copies/mL at least 90 days but not more than 365 days apart). Patients on RAL were followed from the time they first achieved stable suppression with a subset of these being followed after a switch to DTG while still stable. The primary outcome was time to virologic failure (VL>200 copies/mL). DTG-switch to RAL-continuation was compared by estimating hazard ratios (HR) with propensity score adjusted Cox proportional hazards models.

**Results:** Out of 64,759 HIV+ individuals in OPERA, RAL had been prescribed to 9,677 patients. Of 2,755 eligible stable-suppressed patients taking RAL, 229 (8%) switched to DTG, after a median (IQR) of 1,202 (693, 1763) days on RAL. Those continuing RAL and switching to DTG were followed for a median (IQR) of 616 (275,1164) and 337 (145, 564) days, respectively. No difference in risk of virologic failure was observed for patients switching to DTG compared to those continuing on RAL (weighted HR: 0.74, 95% CI: 0.38, 1.42). In sensitivity analyses, findings were robust to alternative definitions of stable-suppression.

**Conclusions:** Within-class switching from RAL to DTG was found to be equally successful at maintaining stable viral suppression as compared to continuing on RAL in both crude and adjusted models as well as in sensitivity analyses.

Table 1: Summary of Cox Proportional Hazards Models

	Crude HR (95% CI)	Weighted HR (95% CI)
Primary analysis: controlling for suppressed (most recent viral load <75 copies/mL)	0.55 (0.32, 0.97)	0.74 (0.38, 1.42)
Sensitivity analyses: Suppressed if VL<200 copies/mL	0.60 (0.36, 1.02)	0.71 (0.37, 1.37)
Controlling for suppressed (2 consecutive VLs <75 copies/mL)	0.50 (0.27, 0.93)	0.72 (0.37, 1.38)

**954 The Validity of Self-Reported ART Use in Persons Living With HIV in Rakai, Uganda**

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**Background:** Despite massive rollout of antiretroviral therapy (ART) across Sub-Saharan Africa, little is known about the validity of self-reported ART use in the general population outside of clinical trial settings.

**Methods:** We compared self-reported ART use and non-use to a validated lab assay in 558 participants (n=337 women, 221 men) in the Rakai Community Cohort Study, who were surveyed between September 2011 and December 2011 in rural Rakai District, Uganda. The study population included 302 participants (54%) from an HIV endemic (>40% prevalence) fishing community on Lake Victoria. ART use was assessed using liquid chromatography-tandem mass spectrometry (API 4000 Triple Quadruple Mass Analyzer) which

detects 20 antiretroviral (ARV) drugs. Individuals who had two or more detectable ARVs consistent with a known drug regimen were considered to be using ART, and individuals with a plasma HIV viral load <400 copies/ml were considered virologically suppressed. Associations between demographic and behavioral factors and inaccurate self-reported ART use were estimated as adjusted prevalence risk ratios (adjPRR) using modified multivariable Poisson regression.

**Results:** There were 153 (27%) participants who self-reported ART use and of these individuals 148 (97%) had detectable ARVs included in common ART regimens in Uganda. We also detected ARVs in 11% (n=46/405) of individuals who did not self-report ART use. Overall the specificity of self-reported ART use was 99% and the sensitivity was 76%. Positive and negative predictive values were 96.7% and 89.1%, respectively. Underreporting of ART use decreased with older age (adjPRR=0.96; 95%CI: 0.92-1.00) and was higher with trading occupations (adjPRR=2.81; 95%CI: 1.17-7.48), but not with residence in a fishing community. Among 284 individuals with HIV plasma viral load data, 12% (n=10/81) of individuals with two or more ARVs had a detectable HIV viral load compared to 85% (n=173/213) of those who had one or no ARVs detected. Among those with detectable ARVs, levels of viral suppression were similar irrespective of self-reported ART use.

**Conclusions:** Individuals who self-report ART use are most likely to be using ART; however, there is under-reporting of ART use among younger persons and those involved in trading. Further research on barriers to self-reported ART use, the source of these drugs, and the potential for ART drug resistance is critically needed in African populations.

**955 Hospital-Based ART Delivery and Patient Outcomes: A Prospective Open Cohort in China**

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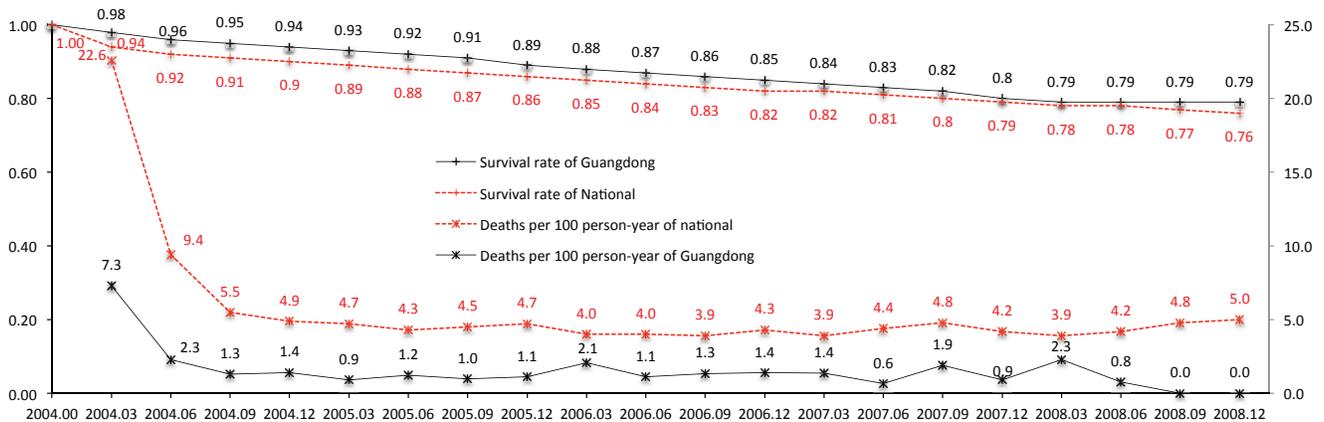
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**Background:** Enormous efforts have been made to develop the capability of treating large numbers of people across wide geographic areas, increased link to care in Center for Disease Control or developed thousands of countryside hospitals network spread all over China in every village. To obtain insights into the long-term outcome of hospital based ART delivery system in China. We report 10-year sentinel hospital-based outcomes of combined ART in HIV/AIDS in Guangdong Province of China.

**Methods:** Post-hoc analysis of a prospectively open cohort from collected, observational database from Guangdong. All patients in the outpatient health care facilities providing HIV medical care from Jan 2004 to June 2014. Patients were excluded if they had not started triple therapy, had missing treatment regimen information, or used ART outside of national guidelines. Mortality rate and survivor rate, according to World Health Organization criteria were calculated. Data were used to homochromous compare to previous published mortality and survivor rates of national cART program in 2004-2008.

**Results:** Of 18,921 infections, 16,757 (88.6%) were link to care in 24 HIV/AIDS sentinel hospitals, among 14,885 were initialed cART were included in analysis. Mean age were 38.6 years, 73.2% were men, 55.5% were infected through heterosexual transmission, and 17.9% were injection drug use and 19.0% were man sex with man. Mortality was greatest during the first three months of treatment (15.9 deaths per 100 person-years) but decreased to a steady average rate of 3.1 deaths per 100 person-years after 6 months. The average median baseline CD4 was 164 cells/ml and increased to 204 cells/ml in 2014 in hospital. In an adjusted Cox regression analysis, the strongest risk factors for death were having a low CD4 and multiple baseline symptom categories at treatment initiation. Those with CD4 less than 50 cells/ml had an adjusted HR of 6.3 (95% CI, 2.2 to 20.0) compared with those with CD4 cell counts of 350 cells/ml or greater. The average virological suppressive rate was 92% from 2005-2014. National ART program mortalities were significant difference in 3-4 folds than hospital based ART delivery system in Guangdong 2004-2008, P<0.05 (Figure 1).

**Conclusions:** The hospital-based Guangdong model of HIV service delivery was associated more access to medical and better outcomes. Scaled up hospital model can continece enhance follow-up management on infected persons and patients expand treatment coverage and improve nadir CD4 on baseline.



Compare to HAART in Guangdong Province and national among 2004-2008

Guangdong survival table per 3-month interval among 2004-2008

	2004.00	2004.03	2004.06	2004.09	2004.12	2005.03	2005.06	2005.09	2005.12	2006.03	2006.06	2006.09	2006.12	2007.03	2007.06	2007.09	2007.12	2008.03	2008.06	2008.09	2008.12
<b>Entered</b>	2372	1991	1727	1511	1324	1113	956	826	691	572	468	389	331	285	242	193	154	111	68	34	34
<b>Censored</b>	331	240	193	174	196	147	120	119	112	97	73	53	44	38	47	35	42	43	34	34	34
<b>Death</b>	173	50	24	23	13	15	10	10	16	7	7	6	5	2	5	2	4	1	0	0	0

National survival table per 3-month interval among 2004-2008

	2004.00	2004.03	2004.06	2004.09	2004.12	2005.03	2005.06	2005.09	2005.12	2006.03	2006.06	2006.09	2006.12	2007.03	2007.06	2007.09	2007.12	2008.03	2008.06	2008.09	2008.12
<b>Entered</b>	48785	39403	35378	32227	29175	26278	23891	21805	19802	17789	16136	14351	12693	10810	9294	7763	5394	4119	3020	2178	2178
<b>Censored</b>	6887	3150	2684	2677	2570	2118	1832	1760	1824	1483	1635	1513	1768	1406	1932	1803	1229	1062	811	957	957
<b>Death</b>	2495	875	467	375	327	269	254	243	189	170	150	145	115	110	99	66	46	37	31	21	21

956 **Virologic Failure Is Uncommon After Treatment Is Initiated During Acute HIV Infection**

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**Background:** When initiated during chronic HIV infection, antiretroviral therapy (ART) generally produces a swift decrease in viral load (VL) and suppression within 24 weeks. We investigated viral dynamics and common criteria for treatment success after ART initiation during acute HIV infection (AHI).

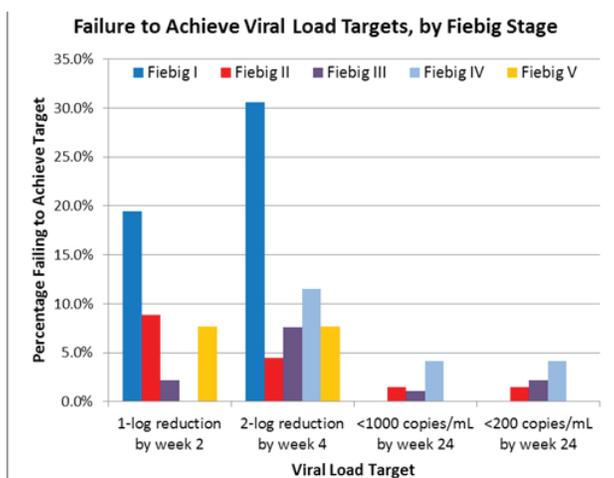
**Methods:** Subjects were prospectively enrolled and offered ART during AHI from May 2009–February 2015 in Bangkok, Thailand. Regimens included tenofovir, lamivudine, and efavirenz with or without raltegravir and maraviroc. Subjects were monitored for the following VL endpoints: (1) 1-log reduction by week 2; (2) 2-log reduction by week 4; (3) <1000 copies/mL by week 24; and (4) <200 copies/mL by week 24. Associated factors were explored using the chi-squared test and t-test. The log-rank test was used to evaluate associations with time to VL <200 copies/mL.

**Results:** From 130,704 samples screened for HIV, 237 Thai subjects were enrolled and initiated ART during AHI. Their median age was 27 years and 95% were male. ART was initiated during Fiebig I in 15%, Fiebig II 29%, Fiebig III 40%, Fiebig IV 11%, and Fiebig V 5%. ART included raltegravir and maraviroc for 84 subjects (35%) and regimens did not differ by Fiebig stage ( $p=0.382$ ).

Only 16 of 236 (6.8%) subjects with VL data at week 2 did not achieve a 1-log reduction in VL. At 4 weeks, 25 of 234 (10.1%) had not achieved a 2-log reduction in viral load. At 24 weeks, 3 of 230 (1.3%) had not reached VL <1000 copies/mL and 4 (1.7%) had not reached VL <200 copies/mL.

Subjects treated during Fiebig I had lower VL at ART initiation (mean [SD] 4.31 [0.69] log<sub>10</sub> copies/mL vs. 6.0 [0.88] in all other subjects,  $p<0.0001$ ) and were the least likely to achieve early reductions in VL (Figure). At baseline, nine (25%) had VL <5000 copies/mL and none had VL <500 copies/mL. All subjects who initiated ART during Fiebig I achieved VL <200 by week 24 and the median time to viral suppression in this group was only 4 (IQR 2–8) weeks compared to a median 8 (IQR 4–12) weeks for all other Fiebig stages ( $p=0.002$ ). Subjects who received raltegravir and maraviroc had a median time to suppression of 4 (IQR 4–8) weeks compared to a median 8 (IQR 8–12) in subjects who did not ( $p<0.0001$ ).

**Conclusions:** Subjects who begin ART during Fiebig I have a low VL and do not demonstrate the same magnitude of early VL decline as is seen when ART is started later. Treatment success can be readily assessed after 24 weeks of ART initiated during AHI. Virologic failure was uncommon in this cohort.

957 **A Meta-Analysis Estimating Early Mortality ART in HIV-Positive Adults on ART in LMIC**

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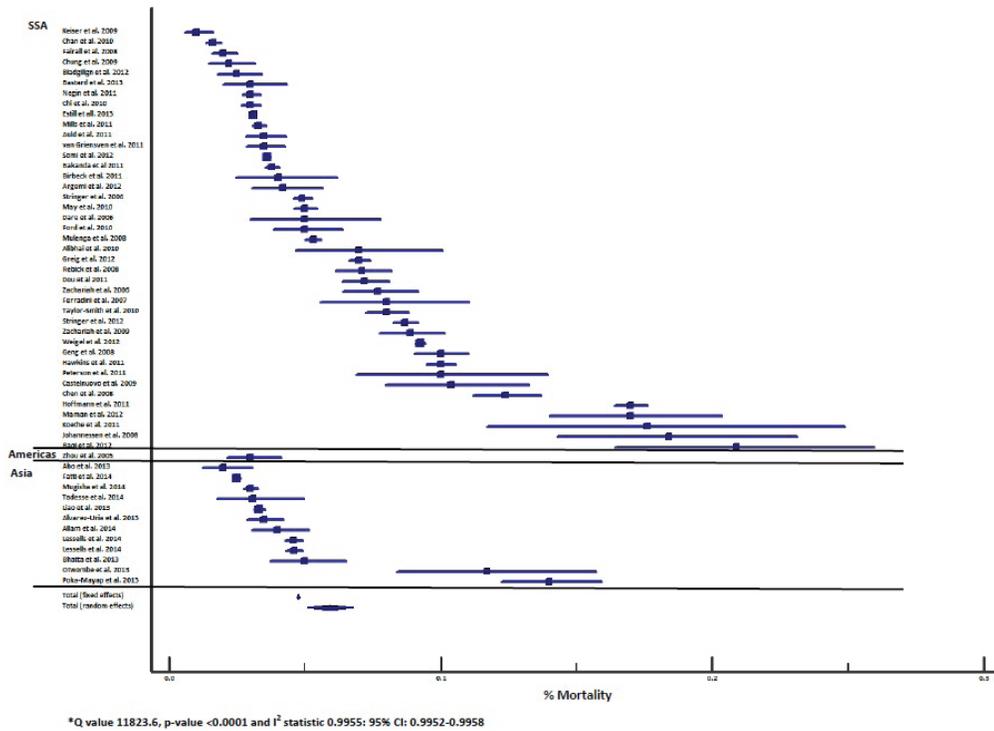
**Background:** While scale-up of ART in low- to middle-income countries (LMIC) has decreased mortality amongst HIV patients, it is unclear whether changes in ART guidelines to make more people eligible has decreased early mortality on ART. Previous meta-analyses reported mortality estimates of 12-months post-ART initiation; however, 40–60% of deaths occur in the first 3-months on ART, which is a more sensitive measure of averted deaths through early ART initiation than 12-month rates. We systematically reviewed studies of mortality in the first 3-months post-ART initiation in Asia, sub-Saharan Africa (SSA), and the Americas.

**Methods:** Studies of mortality within 3-months post-ART initiation published in English from January 2003–October 2014 were searched in PubMed, Web of Science, EMBASE and conference abstracts (IAS and AIDS). Articles were included if they were conducted in a LMIC; in a non-trial setting; participants were  $\geq 15$  and reported 3-month mortality. Using random effects models (high heterogeneity between studies) we assessed 3-month mortality overall and stratified by region, CD4 count at ART initiation and time.

**Results:** 54 studies were included; 43 (78%) from SSA, 10 (19%) from Asia, 1 (2%) from the Americas. Overall 3-month mortality was 5.9% (95%CI:5.1–6.8%). Mortality for SSA, the Americas and Asia was 5.9% (95%CI:5.0–7.0%), 7.1% (95%CI:6.1–8.1%) and 5.4% (95%CI:4.0–7.1%), respectively (Figure). Studies with a median CD4  $\geq 200$  cells/mm<sup>3</sup> at ART initiation had lower mortality (4.4%; 95%CI:3.3–5.6%) vs. studies reporting a median of 100–200 cells/mm<sup>3</sup> (6.2%; 95%CI:5.0–7.5%) and <100 cells/mm<sup>3</sup> (8.4%; 95%CI:7.5–10.5%). The overall pooled estimate shows no difference in mortality when comparing studies whose enrollment of patients ended <2010 (5.7%; 95%CI:4.7–6.8%) to  $\geq 2010$  (6.2%; 95%CI:4.8–7.8).

**Conclusions:** Excluding the Americas, as summary estimates were based on one study, our results showed mortality in the first 3-months on ART were comparable in SSA and Asia. As expected, patients with low CD4 count at ART initiation were at higher risk of death. Our results showed no difference in early mortality over time, potentially due to lack of follow-up time in studies to evaluate the impact after the effect of the 2010 WHO guideline changes. As LMIC increase access to care, raise the CD4 eligibility threshold to 500 cells/mm<sup>3</sup> or move towards a test-and-treat model of care, the expectation is mortality in the first 3 months on ART will begin to decline.

Figure. Forest plot of estimates of mortality at 3-months by individual studies and pooled by region.



958 **Response to ART and Mortality in Older HIV Patients in a Latin American Cohort**

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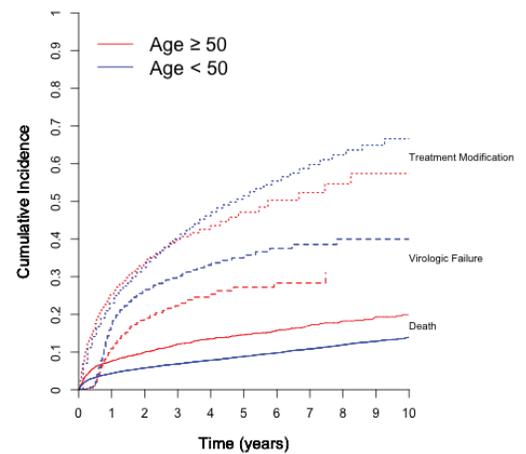
**Background:** With successful highly active antiretroviral therapy (ART), HIV-infected persons are living longer. As these patients age, it is unclear whether comorbidities and their associated therapies, or the aging process itself, alter the response to cART. In this study, we compare HIV treatment outcomes and corresponding risk factors in older ART-treated patients relative to younger patients using data from the Caribbean, Central, and South America Network for HIV Epidemiology (CCASAnet).

**Methods:** HIV-positive adults (≥ 18 years) initiating ART at 7 sites in Argentina, Brazil, Chile, Haiti, Honduras, Mexico, and Peru were included. Patients were classified as older (≥ 50 years) or younger (< 50 years) based on age at ART initiation. ART effectiveness was measured using three outcomes: death, virologic failure, and ART regimen modification. Cox regression models for each outcome compared risk between older and younger patients, adjusting for other covariates (gender, clinical AIDS at baseline, nadir CD4, ART initiation year, ART class, intravenous drug use, time to ART modification and site).

**Results:** Among 21,716 patients initiating ART between 1996 and 2014, 2530 (12%) were ≥ 50 years. Older patients were more likely to have heterosexual sex as their probable route of infection (32% vs. 29%; p<0.001). The majority of patients in both ≥ 50 and < 50 age groups received an NNRTI-based regimen (81% vs. 79%), were male (63%), and did not have AIDS at baseline (60%). Differences between groups in baseline CD4 and log<sub>10</sub> viral load were not significant. Older patients had a higher risk of death (adjusted hazard ratio (AHR) 1.71; 95%CI: 1.52-1.92) and a lower risk of virologic failure (AHR: 0.74; 95%CI: 0.65-0.85); differences in risk of ART regimen modification were not significantly different (AHR: 1.00; 95%CI: 0.94-1.07) (Figure). Risk factors for death, virologic failure, and treatment modification were similar for each group (results not shown).

**Conclusions:** In this study from diverse HIV care sites in Latin America, we found that older patients had a higher risk of death but a lower risk of virologic failure compared to younger patients. Risk factors associated with these outcomes did not differ according to age. Given the complexity of issues related to aging with HIV- such as comorbid conditions, polypharmacy, immune senescence, and chronic inflammation- studies assessing the role of non-traditional HIV biomarkers and risk factors are needed.

Figure: Crude cumulative incidence of mortality, treatment modification, and virologic failure.



959 **Immunovirological Responses to HAART Between HIV-1 Group O- and M-Infected Patients**

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**Background:** The divergent HIV-1 group O strains (HIV-1/O) are endemic in Cameroon and naturally resistant to NNRTI, largely used as first-line therapy in this country. Alternative therapeutic strategies are thus needed. DynaM-O is a prospective open-label study comparing the immuno-virological response to HAART, including two NRTI and one PI in HIV-1/O and HIV-1 group M (HIV-1/M) infected-naïve patients. Secondary objectives are to compare the kinetic of viral load responses and the CD4 restoration.

**Methods:** HAART was initiated in naïve patients according to the national guidelines; HIV-1/O and HIV-1/M patients were matched on sex, age, CD4, Hb level and HBV status with ratio of 1:2. The primary endpoint was the percentage of patients having an undetectable viral load (VL < 60 cp/mL) at W96. Adherence to treatment was also monitored.

**Results:** 47 Cameroonian patients HIV-1/O and 94 HIV-1/M were included; results were available for 128 patients (13 died or were lost-to follow-up). At baseline, VL was significantly lower ( $p < 0.0001$ ) in HIV-1/O with a median at 4.3 log cp/mL versus 5.1 in HIV-1/M. Kinetic of VL response was faster for HIV-1/O infected patients until W24. At W96, 95% of HIV-1/O samples were < 60 cp/mL vs 83% of HIV-1/M in per protocol analysis ( $p=0.09$ ); but no difference was observed at the threshold of 200 cp/mL (97% in both groups). At baseline, median CD4 counts were well balanced between the two groups (227 vs 215, in HIV-1/O and HIV-1/M respectively,  $p=0.68$ ); at W96, a +50% CD4 gain compared to baseline was observed for 78% vs 89% of the HIV-1/O and HIV-1/M patients respectively ( $p=0.27$ ), confirming the differences observed at W24 and W48. Adherence monitoring at W24, W48, and W96 revealed no impact on the differences observed between the two groups.

**Conclusions:** DynaM-O is the unique study analyzing the HAART responses in HIV-1/O infected patients compared to HIV-1/M patients. Data at W96 showed good efficacy of the regimens in both groups, but with a higher rate of achievement of the virological response in HIV-1/O infected patients. In contrast, the CD4 restoration was lower in HIV-1/O than that observed for HIV-1/M patients. These data indicate that group O infected patients should be successfully treated by treatment excluding NNRTI. Moreover, studying the mechanisms underlying these differences in response to HAART between these highly divergent HIV-1 strains are of importance in our understanding of the HIV natural history.

## 960 Clinical Outcomes With Tenofovir Use in ART: Regression Discontinuity Analysis

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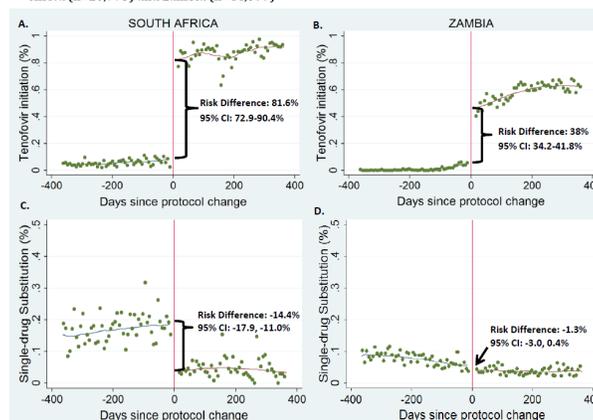
**Background:** Most countries now recommend initiating HIV patients on tenofovir (TDF) as the standard NRTI in first-line therapy to reduce toxicities associated with the NRTI stavudine. Exploiting national guideline changes in South Africa (SA) and Zambia, we assessed the causal impact of a policy to initiate TDF on ART outcomes using regression discontinuity.

**Methods:** Prospective cohort study of ART-naïve, non-pregnant, HIV patients  $\geq 16$  years who initiated first-line ART in SA or Zambia (JeDEA-SA). Patients initiating ART +/-12 months around the national guideline changes were included: SA-1 April 2010 and Zambia-1 July 2007. We implemented a regression discontinuity, a quasi-experimental design, using the timing of national guideline changes as natural experiments. Patients initiating just before/after guideline change are similar but receive different regimens. Comparing those patients, we estimated the intent to treat (ITT) effect of guideline change on single-drug substitution (SDS), death, loss to follow-up (LTFU), CD4 response and virologic failure (VF, SA only) in the first 24-months on ART on a risk difference (RD) scale using local linear regression. We excluded patients initiating +/-14 days of the date of the guideline change in all estimates due to imprecision in the implementation of the guidelines. We then collapsed across country to estimate combined ITT effects.

**Results:** 16,773 South African and 44,399 Zambian patients were eligible. The probability of initiating TDF increased in both countries for patients starting ART after the guideline changes (Figures A and B). ITT estimates showed a significant decrease in the risk of SDS in SA (RD: -14%; 95%CI: -18%, -11%) (Figure C), while we saw no difference in Zambia (Figure D). In both countries we saw no effect on mortality (SA RD: 1.0%; 95%CI: -2.2, 4.0%; Zambia RD: -0.3%; 95%CI: -2.2, 1.5%), LTFU (SA -RD: 2.6%; 95%CI: -6.8, 1.5%; Zambia RD: -1.0%; 95%CI: -3.4, 1.3%), mean CD4 (SA RD: 10.1; 95%CI: -43.5, 23.2; Zambia RD: 5.9; 95%CI: -9.1, 20.9), or VF in SA (RD: 0.0%; 95%CI: -2.1, 41.9%). Combined ITT estimates showed a significant increase in TDF (RD: 37%; 95%CI: 25%, 48%) and no difference in outcomes.

**Conclusions:** Guideline changes led to an impressive increase in tenofovir initiation in SA and Zambia. Initiating patients on TDF led to reductions in SDS in SA, suggesting that a global policy to initiate TDF may have resulted in fewer patient-years spent on sub-optimal therapy and fewer patients experiencing side effects/toxicities. No change was observed in other outcomes.

Figure. Regression discontinuity showing the probability of receiving tenofovir in South Africa (n=16,773) and Zambia (n=44,399)



## 961 24-Week Results of Elvitegravir-Cobicistat-Etricitabine-Tenofovir DF for HIV-2

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**Background:** There is an urgent need for safe and effective antiretroviral therapy (ART) for HIV-2 infection. HIV-2 treatment is complicated by intrinsic resistance to many FDA-approved HIV-1 drugs, and multidrug-resistance is common in individuals failing ART. There are limited options for 1<sup>st</sup>- and 2<sup>nd</sup>-line ART for HIV-2 in resource-limited settings. An increasing body of data suggests that integrase inhibitor-based regimens may be of potential utility for the treatment of HIV-2. We have undertaken the first clinical trial of a once-daily fixed-dose combination pill containing elvitegravir, cobicistat, emtricitabine, tenofovir disoproxil fumarate (EVG/c-FTC-TDF) to assess the effectiveness of this regimen in HIV-2-infected subjects in Senegal, West Africa.

**Methods:** HIV-2-infected, ART-naïve adults with WHO stage 3 or 4 disease or CD4 counts below 500 cells/ul were eligible for this open-label pilot trial (NCT02180438), with planned enrollment of 30 subjects and follow-up for 48 weeks. Enrolled subjects are monitored every four weeks for clinical and immuno-virologic outcomes as well as adverse events. For this 24-week interim analysis, changes in plasma viral load, CD4 counts, adverse events, all-cause mortality and loss to follow-up were analyzed.

**Results:** To date, 18 subjects started ART with EVG/c-FTC-TDF. Twelve subjects have achieved at least 24 weeks of follow-up. The majority are female (83%), with a median age of 47.5 years at enrollment. There were no deaths, no loss to follow up, and no AIDS-associated clinical events. There was one withdrawal. Median baseline CD4 count was 386 cells/ul (IQR: 335-465) and increased to 452 cells/ul (IQR: 354-534) by week 24. All subjects had baseline viral loads of fewer than 50 copies/ml of plasma, including five subjects who had viral loads below the reproducible limit of detection (10 copies/ml). All patients had achieved undetectable plasma viral loads by week 4 and maintained them. EVG/c-FTC-TDF was generally well tolerated and there were no grade 3-4 adverse events. Adherence was good by self-report and pill count.

**Conclusions:** Long-term outcomes of HIV-2 infected patients on ART in West Africa are suboptimal and new therapeutic options are needed. Initial data suggest that EVG/c-FTC-TDF, a once-daily single-tablet regimen, is safe, effective, and well-tolerated in this population. Our findings support the use of integrase inhibitor-based regimens for HIV-2 treatment.

## 962 Severe Neutropenia in HIV-Infected People on Antiretroviral Therapy in West Africa

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**Background:** In Sub-Saharan Africa, antiretroviral therapy (ART) with potential toxicity such as Zidovudine (AZT) is still massively prescribed in HIV-infected patients. Nevertheless, little information is available on adverse drug reactions such as neutropenia in real life conditions. This study aimed at estimating the incidence of severe neutropenia and associated factors after ART initiation in five West African countries.

**Methods:** A retrospective cohort analysis was conducted within the international epidemiologic database to evaluate AIDS (IeDEA) collaboration in West Africa. All HIV-infected adults, starting ART between 2002 and 2014, with a baseline and at least one follow-up absolute neutrophil count (ANC) measurement were eligible. The main outcome was the first episode of severe neutropenia (ANC <750 cells/mm<sup>3</sup>). Incidence was estimated with 95% confidence interval (CI) according to age, gender, clinic, hemoglobin, CD4 count, clinical stage, ART duration. A Cox proportional hazard model identified the factors associated with the outcome and associations were expressed by adjusted hazard ratios (aHR).

**Results:** Between 2002 and 2014, 9,426 HIV-infected adults were enrolled in eight clinics in Benin, Burkina Faso, Cote d'Ivoire, Mali and Senegal at a median age of 37 years [interquartile range (IQR) 31-44] and with a median participation time of 12 months in [IQR 6-19]. Since 2008, more than 60% of the 4,911 patients enrolled initiated an AZT-based ART regimen (Figure 1). The crude incidence of first severe neutropenia episode was 9.1 per 100 person-years (CI: 8.6-9.8). Factors associated with severe neutropenia were the cumulated exposure to AZT <6 months (aHR=2.2; CI: 1.8-2.6), ≥6-12 months (aHR=2.1; CI: 1.6-2.8) and ≥12 months (aHR=1.6; CI: 1.2-2.2) [Ref. no AZT exposure], CD4 count <350 cells/mm<sup>3</sup> (aHR=1.3; CI: 1.1-1.5), advanced clinical stage (aHR=1.2; CI: 1.0-1.4), an ANC ≥750-1000 cells/mm<sup>3</sup> (aHR=2.5; CI: 2.0-3.1) and an ANC ≥1000-1300 cells/mm<sup>3</sup> (aHR=1.5; CI: 1.3-1.8) [Ref. ANC ≥1300 cells/mm<sup>3</sup>] at ART initiation. Of the 555 patients on AZT with incident severe neutropenia, 51 (9%) switched to an alternative ART following severe neutropenia.

**Conclusions:** The incidence of severe neutropenia after ART initiation among HIV-infected adults in West Africa is high and associated with AZT exposure. In a context where cytotoxic drugs such as AZT are still widely prescribed, future efforts are needed to scale-up access to less toxic drugs such as tenofovir according to recent WHO recommendations.

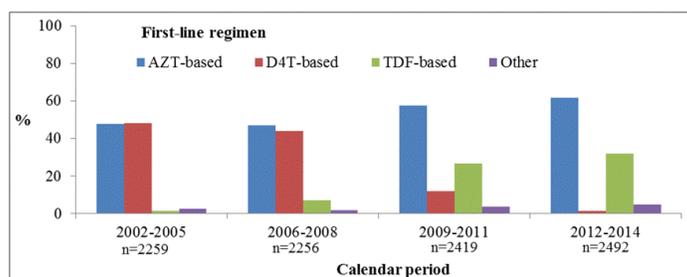


Figure 1. Distribution of first-line antiretroviral regimens prescribed by calendar period, IeDEA West Africa collaboration, 2002-2014 (N=9,426)

## 963 Efavirenz Toxicity Manifesting As Cerebellar Ataxia: Case Series From South Africa

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**Background:** WHO treatment guidelines recommend efavirenz (EFV) as the backbone of first line antiretroviral therapy (ART). Fixed dose combination (FDC), which contain efavirenz 600mg, improve adherence but dose adjustments cannot be made easily. EFV commonly causes early neuro-psychiatric adverse events. We present seven cases with late EFV toxicity causing severe cerebellar ataxia.

**Methods:** Consecutive HIV-infected adults taking EFV-containing combination ART admitted to hospital with cerebellar ataxia were included in this case series. Patients were neurologically examined, and all had lumbar punctures, chest x-rays, and CT scans of the brain. EFV concentrations (therapeutic levels 1-4mg/l), serum vitamin B12 levels, thyroid stimulating hormone (TSH) and syphilis serology were done on all patients to exclude other common causes of ataxia. We did not assess EFV metabolizer genotype.

**Results:** We identified seven women with severe cerebellar signs who were on ART for a median period of 2 years (IQR:1.5-5.5). All had staccato speech, severe truncal and limb ataxia, and were unable to walk or sit unaided, but none had nystagmus. Their median weight was 34kg (IQR:29.7-35.3); median CD4 count 299 (IQR:258-300); all were virologically suppressed at admission and none reported alcohol use. Two were on rifampin-containing TB treatment, and one had epilepsy treated with phenytoin. All chest x-rays were normal. CT scans were either normal or showed generalised atrophy. All patients' TSH, B12, and syphilis serology were normal. Six patients' plasma EFV level was >20 mg/l (the upper limit of detection), and one was 18 mg/l. Ataxia resolved in all patients after withdrawal of EFV at a median time of 2 months (IQR1.25-4) after withdrawal of EFV. The patient taking phenytoin also had concurrent elevated phenytoin levels, which was stopped but the ataxia resolved only after subsequent EFV withdrawal.

**Conclusions:** As far as we are aware, this is the first report of severe cerebellar ataxia caused by toxic EFV dosages likely related to treatment with FDCs occurring after years of therapy. All patients had low weight and were treated with 600 mg EFV in FDC and had extremely high EFV concentrations, suggesting they were genetic slow metabolizers. Clinicians should be aware of this presentation of EFV toxicity. We recommend that patients weighing <40kg receive lower doses of EFV or, in areas where only FDC is available, not receive EFV FDCs.

**964 Effective Disease Intervention Is Key Component to Acute HIV Detection**

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**Background:** North Carolina (NC) performs statewide testing for acute HIV (AHI) with expedited partner services (PCRS) and referral to care. AHI consistently represents 2.4% of new diagnosis and reflects populations most at risk. We sought to assess if use of the HIV Ag/Ab assay led to increased AHI detection.

**Methods:** In 2013, the NC State Laboratory of Public Health (SLPH) implemented HIV Ag/Ab combination testing on all samples from publicly funded sites. As of 2013, HIV and STI results and PCRS data were combined in the NC Electronic Disease Surveillance System (NCEdSS), improving availability of data for Disease Intervention Specialists (DIS) who perform PCRS for HIV, AHI and early syphilis diagnoses in the state. We compared case data on AHI and other HIV cases diagnosed through the SLPH versus other settings since the implementation of HIV Ag/Ab testing by SLPH. We evaluated the yield of PCRS with AHI cases. We compared age, race, and gender of AHI cases, mode of case detection and links between AHI and STI cases on data from NCEdSS.

**Results:** NC had a notable increase in the number of syphilis cases from 2012 to 2014 with 564, 688, and 1,113 cases per year respectively. AHI cases at the SLPH increased from 2013 to 2015, with 23 AHI cases in 2013, 23 cases in 2014 and 27 in only the first six months of 2015 compared to non-SLPH testing with 17, 27 and 23 cases, respectively. All AHI cases at the SLPH were detected the HIV Ag/Ab assay, but accounted for <50% of non-SLPH cases. AHI cases detected at the SLPH vs non-SLPH sites were similar; both were young (70% vs 57%, respectively), Black (75%, 58%) and MSM (79%, 70%). In 2014, co-infection with a STI prior to AHI diagnosis was common (22/50; 44%) and more frequent than established HIV cases (44% vs 20%); p<0.0001. PCRS resulted in AHI detection in 39/140 (28%) cases detected as part of a PCRS-prompted testing. More than half of AHI cases (78/140, 56%) were part of a sexual or social network including others with STIs.

**Conclusions:** In the first half of 2015, AHI detection at the SLPH doubled compared with prior years while cases detected in the community were stable. It remains unclear if the HIV Ag/Ab assay increased case detection rates given the delay from implementation. The focus on rapid interview of new AHI cases allows DIS to interview persons closer to the testing event, possibly increasing yield of new HIV cases from PCRS. HIV testing in persons with recent STIs results in the detection of AHI.

**965 Late HIV Diagnosis and Missed Opportunities for HIV Testing in South Carolina**

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**Background:** Previous studies, prior to routine HIV testing guidelines, showed that 43.4% individuals diagnosed with HIV in South Carolina (SC) between 2001-2005 were late testers, of these 73.4% had visited a SC healthcare (HC) facility in the years preceding their HIV diagnosis, representing missed opportunities for early diagnoses. The current study sought to re-visit the previous investigation to determine if there has been a reduction in missed opportunities for early diagnosis and evaluates predictors of late testing.

**Methods:** The SC enhanced HIV/AIDS Reporting System and a statewide all payer HC data base that includes inpatient (IP), outpatient (OP), and emergency department (ED) visits made to a SC facilities were linked. Analysis includes individuals diagnosed with HIV in SC from 2006-5/2015 and HC visits made from 2006 to date of HIV diagnosis. HC visits were categorized as likely to prompt an HIV test [sexually transmitted infections (STI), acute retroviral syndrome (ARS), HIV related, and intravenous drug use (IDU)] and not likely to prompt an HIV test. Individuals were classified as late testers (AIDS ≤1 year of HIV diagnosis) or early testers. Descriptive statistics and logistic regression analyses were conducted to determine associations with late testing.

**Results:** From 1/2006-5/2015, 7,109 individuals were diagnosed with HIV in SC. Almost one-third were late testers (2,244; 31.6%). Of the total, 4,843 (68.1%) visited a SC HC facility prior to their HIV diagnosis. These 4,843 individuals made 29,969 HC visits prior to the HIV diagnosis, including 24,121 (80.5%) ED, 2,956 (9.9%) IP, and 2,039 (6.8%) OP visits. The mean number of visits was 6.1 (SD 9.1; range: 1-183). Among HC visits, 21,929 (73.2%) were for diagnoses unlikely to prompt an HIV test. The remaining 8,040 (26.8%) visits included diagnoses related to STI [851 (2.8%)], ARS [5,495 (18.3%)], HIV related [2,390 (7.9%)], and IDU [641 (2.1%)]. The Table shows predictors of late HIV testing. Individuals with at least one HC (or missed opportunity for testing) were more likely to be late testers than those with no HC visits. Individuals residing in rural areas were more likely to be late testers. Older individuals were also more likely to be late testers (p-value for trend: <0.0001).

**Conclusions:** Despite the recommendations for routine HIV screening, late testing and missed opportunities for early diagnosis continue to be a problem in SC. Continued emphasis on implementing routine screening in HC facilities statewide is needed.

Predictors of Late Testing		
	Odds Ratio	95% Confidence Interval
<b>Gender</b>		
• Female	1.00	-
• Male	0.91	0.80, 1.02
<b>Race/Ethnicity</b>		
• White	1.00	-
• Black	1.12	0.99, 1.27
• Hispanic	1.86	1.45, 2.38
• Unknown	1.08	0.72, 1.62
<b>Year Diagnosis</b>		
• 2006-2010	1.57	1.41, 1.74
• 2011-2015	1.00	-
<b>Missed Opportunities</b>		
• No	1.00	-
• Yes	1.29	1.15, 1.44
<b>Residence</b>		
• Urban	1.00	-
• Rural	1.22	1.08, 1.38
<b>Age (years)</b>		
• 18-19	1.00	-
• 20-24	1.46	1.01, 2.10
• 25-29	2.18	1.51, 3.15
• 30-39	3.64	2.55, 5.19
• 40-49	4.95	3.48, 7.04
• ≥50	6.85	4.80, 9.79

**966 Missed Opportunities for HIV Testing During Routine Doctor Visits, BRFSS, 2011-2013**

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**Background:** The Centers for Disease Control and Prevention and the United States Preventive Services Task Force (USPSTF) recommend HIV screening in clinical settings for all adolescents and adults regardless of risk. Yet, many people have never been screened for HIV. We estimated the number of adults never tested for HIV who had a recent routine doctor visit (past year) to identify state differences in and characteristics associated with missed opportunities for HIV screening.

**Methods:** We analyzed 2011-2013 Behavioral Risk Factor Surveillance System data to estimate, nationally and by state, the number and percent of persons aged 18-64 years who had never been screened for HIV and who had a recent routine doctor visit (missed opportunities). Logistic regression models assessed trends in the percent never tested and with missed opportunities; year as a continuous variable; p<0.01 considered significant. Persons with missed opportunities for HIV screening were described by sociodemographic and health care characteristics.

**Results:** During 2011-2013, the percent never tested did not change significantly and the percent with missed opportunities increased from 61.8% in 2011 to 63.7% in 2013 (p<0.0001). In 2013, an estimated 96.9 million (95% confidence interval (CI): 96.1-99.7 million; 56.6%; 95% CI: 56.2-56.9%) adults aged 18-64 years had never been tested for HIV; 59.7 million (95% CI: 59.1-60.4 million) of whom had a recent routine doctor visit. By state, the percent never tested ranged from 22.8% in the District of Columbia to 71.7% in Utah. Missed opportunities ranged from 50.5% in Oregon to 75.7% in Rhode Island. The highest percent of persons with missed opportunities were female (52.5%), aged 45-64 years (56.4%), non-Hispanic white (69.8%), and had health insurance coverage (88.6%). In 2012 (most recent year available), 12.4 million (95% CI: 12.1-12.7 million) persons with missed opportunities received a flu vaccine in a clinical setting.

**Conclusions:** During 2011-2013, nearly 100 million US adults had never been tested for HIV; more than half of whom had recent missed opportunities for HIV screening. Missed opportunities increased during 2011-2013, varied by state, and occurred among persons who received other clinical preventive care. States with high prevalence of undiagnosed HIV (e.g., ≥0.1%) especially need to reduce missed opportunities. Education for providers and implementation strategies (e.g., clinical protocols) are needed to reduce missed opportunities for HIV screening.

Persons never tested for HIV who had a routine doctor visit in the past year, BRFSS, 2013						
	Estimated No.	Lower 95% CI	Upper 95% CI	Percent of total	Lower 95% CI	Upper 95% CI
<b>Sex</b>						
Male	28,372,634	27,895,445	28,849,823	47.5	46.9	48.1
Female	31,370,339	30,938,450	31,802,227	52.5	51.9	53.1
<b>Age group (years)</b>						
18-24	10,246,571	9,874,058	10,619,083	17.2	16.6	17.7
25-34	7,091,565	6,843,694	7,339,436	11.9	11.5	12.3
35-44	8,692,944	8,422,092	8,963,795	14.6	14.1	15.0
45-64	33,711,893	33,309,503	34,114,284	56.4	55.8	57.0
<b>Race/ethnicity<sup>1</sup></b>						
White, non-Hispanic	40,941,825	40,530,219	41,353,432	69.8	69.2	70.5
Black, non-Hispanic	4,837,828	4,626,385	5,049,271	8.3	7.9	8.6
Hispanic	7,933,482	7,589,030	8,277,934	13.5	13.0	14.1
Other	4,912,725	4,639,891	5,185,558	8.4	7.9	8.8
<b>Education</b>						
Less than high school	7,103,218	6,772,244	7,434,193	11.9	11.4	12.4
High school degree or GED	17,280,195	16,922,850	17,637,540	29.0	28.5	29.6
At least some college	35,187,540	34,755,766	35,619,315	59.1	58.5	59.7
<b>Has health insurance coverage</b>						
Yes	51,989,415	51,463,098	52,515,733	88.6	88.2	89.1
No	6,669,007	6,402,256	6,935,758	11.4	10.9	11.8
<b>Has at least one primary doctor</b>						
Yes	51,141,312	50,623,107	51,659,516	85.9	85.4	86.4
No	8,416,715	8,104,289	8,729,141	14.1	13.7	14.6
<b>Received flu vaccine in clinic in last year<sup>1</sup></b>						
Yes	12,376,041	12,067,129	12,684,953	22.0	21.5	22.5
No	43,935,819	43,392,527	44,479,112	78.0	77.5	78.5
<b>Reported HIV Risk<sup>1,2</sup></b>						
Yes	1,288,629	1,169,585	1,407,674	2.1	1.9	2.3
No	60,720,208	60,140,418	61,299,998	97.9	97.7	98.1
<b>Total</b>	<b>59,742,972</b>	<b>59,105,870</b>	<b>60,380,075</b>	<b>100</b>		

Note. CI confidence interval; HIV human immunodeficiency virus; BRFSS Behavioral Risk Factor Surveillance System  
<sup>1</sup>Most recent year available was analyzed (BRFSS 2012).  
<sup>2</sup>HIV risks include intravenous drug use, sexually transmitted or venereal disease, given or received money or drugs in exchange for sex, or anal sex without a condom in the past year.

**967 Setting a Benchmark for HIV Testing at Visits to US Physician Offices**

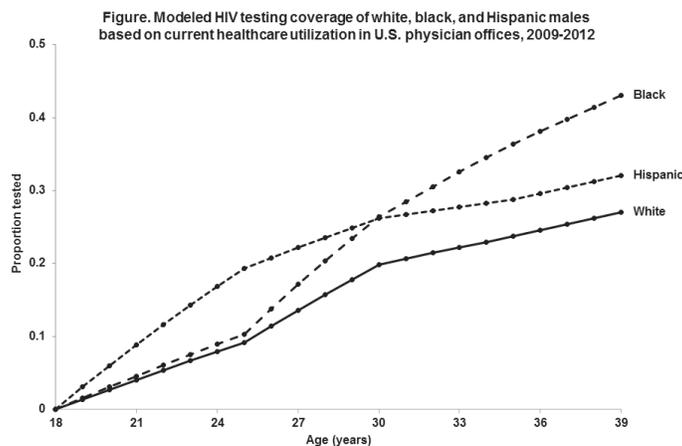
Karen Hoover; Charles Rose; Philip Peters  
 CDC, Atlanta, GA, USA

**Background:** Access to HIV testing in healthcare settings increased with implementation of the Affordable Care Act with its requirement for health plan coverage of preventive services, including HIV screening, without patient cost-sharing. We sought to estimate the frequency of HIV testing of males at visits to U.S. physician offices needed to achieve near-universal testing coverage by aged 39 years.

**Methods:** We analyzed data from the 2009-2012 National Ambulatory Medical Care Survey (NAMCS) and the U.S. Census. NAMCS is a nationally representative medical record abstraction of health services provided at physician office visits. We estimated the mean annual number visits by males aged 18-39 years, mean annual visits per person, and HIV testing at visits, stratified by age and race and ethnicity. We calculated the probability of an HIV test in a given year as  $1 - (1 - p)^r$  where  $p = \text{tests} / \text{population}$ , and  $r = \text{visits} / \text{population}$ , and modeled the proportion of males who would have been tested by aged 39 years based on current testing rates, and with a 2-, 4-, or 8-fold increase.

**Results:** Males aged 18-39 years had 58.4 million visits to physician offices, and HIV testing was performed at 754,280 (1.3%) of visits. White males aged 18-24 years had more mean annual visits per person (1.3) than black males (0.61) or Hispanic males (0.59). HIV testing rates were highest for black males aged 18-24 years (2.5% of visits) and 25-29 years (4.2%) and for Hispanic males aged 18-24 years (5.1%) and 25-29 years (2.7%) compared to white males aged 18-24 years (1.1%) and 25-29 years (2.0%). We found that with current testing rates of males aged 18-39 years, 27% of white, 43% of black, and 32% of Hispanic males would be tested at least once for HIV by aged 39 years (Figure); a 2-fold increase in testing would result in coverage of 47% of white, 68% of black, and 54% of Hispanic males; a 4-fold increase in coverage of 73% of white, 91% of black, and 80% of Hispanic males; and an 8-fold increase in coverage of 93% of white, 99% of black, and 97% of Hispanic males.

**Conclusions:** Increasing HIV testing at visits to U.S. physician offices by 4-fold could achieve high HIV testing coverage by aged 39 years, and by 8-fold could achieve near-universal coverage. Young black and Hispanic males visit physician offices less frequently so increased rates of HIV testing at these visits are needed to achieve optimal testing coverage for this group.



**968 Implementation of a Rapid HIV Testing Program in Psychiatric Inpatient Wards**

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**Background:** New York State law mandates the offer of HIV testing to every patient aged 13-64. However, acute mental health admittances into the psychiatric emergency room can be difficult settings in which to conduct HIV screening. Because individuals with mental illness have higher rates of HIV infection, routine testing of stabilized patients in psychiatric inpatient wards could be beneficial. This study sought to assess the implementation of a rapid HIV testing program in this nontraditional setting.

**Methods:** This prospective, descriptive study was conducted in a psychiatric inpatient ward over 23 days. Public Health Advocates (PHAs) recruited a convenience sample of patients, deemed to have capacity to consent by a team of physicians, for HIV testing. PHAs collected demographic characteristics and risk factors during counseling sessions. Chart reviews were conducted to assess psychiatric diagnoses and sexual/drug abuse history. Rates of test acceptances were tracked during the last nine days of the study.

**Results:** 405 patients were tested for HIV. Demographic characteristics of the participants were: 59.5% male, 43.0% Hispanic, and 40.2% Black. Mean age was 37.9 ± 13.0 years. 38.0% did not engage in regular medical care. As per CDC criterion, 26.4% of participants were high-risk for acquiring HIV. Risk factors were: multiple sex partners (22.2%), injection drug use (4.9%), sex for commodities (4.9%), sex with an injection drug user (3.2%), or sex with an HIV-positive partner (1.5%). Psychiatric diagnoses were: Schizophrenic/Psychotic/Affective (65.2%), Depression (15.6%), and Bipolar (13.8%). Drug abuse was noted in 33.1% of cases, and 7.4% reported sexual abuse history. One patient was confirmed HIV positive and linked to outpatient HIV care. In the last nine days of study, 294 of 394 patients able to consent were offered the HIV test (74.6%), of which 185 accepted (62.9%).

**Conclusions:** A rapid HIV testing program with multidisciplinary staff buy-in can test a large number of patients in a psychiatric inpatient ward. Expansion of HIV screening initiatives to nontraditional settings can increase access to testing for high-risk populations that may not otherwise engage in primary care, especially those that cannot consent while admitted in a psychiatric emergency room.

**969 Tailoring Mass Media MSM HIV Test Interventions to Reach High-Risk Men**

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**Background:** Mass media interventions have been shown to be effective in promoting HIV testing, yet their effectiveness may be attenuated among subgroups. We examined correlates of HIV testing among MSM immediately following a brief mass media intervention in order to identify gaps in intervention effectiveness and inform subsequent interventions.

**Methods:** MSM individuals in China were recruited online in 2014. MSM who were 16 years or older and had never received HIV testing viewed a one minute mass media video promoting HIV test uptake. Participants self-reported HIV testing behavior in the three weeks following the intervention. Odds ratios (OR) were used to identify factors associated with post-intervention HIV testing.

**Results:** In the study, 5339 online users clicked the banner on webpage and 721 eligible respondents completed the online questionnaire and watched HIV testing video. A total of 593 (82%) were followed up successfully and 215 (36%) reported HIV testing. The median age was 22 years old (interquartile range: 20-26 years old). Ethnic minorities and other non-Han individuals (proportion of HIV testing=22% vs 37%, OR=0.48, p=0.05) and students (32% vs 40%, OR=0.71, p=0.05) were less likely to be tested for HIV following the mass media intervention compared to their counterparts. No significant association was found between post-intervention HIV testing and age, education level, income level and residence status. Men who reported riskier behaviors had greater post-intervention HIV testing. Unprotected sex with female (61% vs 40%, OR=2.35, 95%CI=1.22-4.51) and drunk before having sex in the past 3 months (63% vs 43%, OR=2.19, 95%CI=1.13-4.24) were associated with post-intervention HIV testing. Men who had group sex (54% vs 35%, OR=2.16, 95%CI=1.13-4.16) and sex for money (63% vs 35%, OR=3.13, 95%CI=1.5-6.53) in the past 12 months were also more likely to have post-intervention HIV testing.

**Conclusions:** After an online mass media intervention, men with higher risk behaviors, who probably had higher perceived risk, were more likely to be tested for HIV. This suggests that subsets of high risk MSM can be effectively reached through online mass media interventions.

**970 Feasibility of a Social-Entrepreneurship Model to Promote HIV Self-Testing Among MSM**

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**Background:** HIV testing is critical to the surveillance and control of the HIV pandemic, and HIV self-testing (HIVST) offers an opportunity to increase HIV testing among people not reached by facility-based services. However, the promotion and implementation of HIVST is limited by insufficient community engagement. To fill this gap and to promote HIVST among MSM, we built a Social Entrepreneurship Model (SET) to promote HIVST and syphilis self-testing (SST) among Chinese MSM.

**Methods:** The Guangzhou Center for Disease Control (CDC) built an online SET model in cooperation with a local CBO (Guangzhou Tongzhi, GZTZ) using an online-mobile model. This model includes few key steps: First, participants that clicked on the link completed an online survey, including an informed consent. Participants then deposited \$23 USD (refundable) in exchange for a HIVST kit and a SST kit. After the participants performed HIVST and SST, the results were sent to the GZTZ which recorded testing results. The staff of the Guangzhou CDC then interpreted the results and gave feedback to the participants. Meanwhile, the deposit was automatically returned to the participants. Finally, the GZTZ contacted the individuals testing positive for HIV or syphilis to provide counseling services, confirmation testing and linkage to care.

**Results:** During April - June of 2015, a total of 380 participants finished the online survey, and 198 (52.1%) purchased self-testing kits online. Among those who purchased self-testing kits, the majority were aged under 30 (69.5%), received at least a college education (88.9%), and met their sexual partners online (93.1%). In addition, 72.2% of participants reported that they ever tested for HIV, and 20.2% had ever performed HIVST. Overall, feedback was received from 192 (97.0%) participants. Among these, 14 people did not use self-testing kits, and the HIV and syphilis prevalence among these users were of 4.5% (8/178) and 3.7% (6/178), respectively. All of the eight screened HIV positive sought further confirmation testing and were confirmed to be HIV positive, and all were linked to care.

**Conclusions:** Using an online SET model to promote HIV and syphilis among Chinese MSM is feasible, and an online SET model could be a good option to promote HIV and syphilis testing among MSM, particular in countries with good MSM online service targeting.

**971 HIV Self-Testing in the Seattle Transgender Community: A Mixed-Methods Evaluation**

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**Background:** Transgender communities are disproportionately affected by HIV. HIV prevention efforts that target cisgender men who have sex with men (cis MSM) may not reach or resonate with transgender communities. Several studies suggest HIV self-testing (HIVST) is acceptable in key populations, though information about uptake of HIVST outside research settings is limited, especially among transgender people. We evaluated reported uptake and perceptions of HIVST in the Seattle-area transgender community.

**Methods:** We analyzed cross-sectional Pride Survey data from 2014-15 to evaluate HIV testing behavior reported by cis MSM (collected at the Seattle Parade) and non-binary people, trans women, and trans men (collected at Seattle Trans\* Pride Festival; hereafter trans\*respondents). The analysis excluded known HIV-positive respondents; parade

survey data was weighted so that the age distribution was comparable to that in Trans\* Pride. In 2015, 7 focus groups of 3-6 participants were conducted to evaluate perceptions of local HIV/STD services among transgender people, including HIVST.

**Results:** Compared to cis MSM, a larger proportion of trans\* respondents reported an unknown HIV status and no prior HIV test (ever and in the past 2 years) (Table). The percent of cis MSM, trans women/non-binary people assigned male at birth, and trans men who had ever used HIVST was 20%, 7%, and 4%, respectively; use of HIVST at one's last test was reported by 8% of cis MSM and 0% of trans\* respondents. All the above differences were significant at  $p < .01$ .

In the transgender focus groups, perceptions of HIVST varied but tended to be favorable and often preferable to testing with local HIV test providers. Convenience and privacy were frequently cited by proponents of HIVST. Skeptics were concerned by perceptions of the test's cost, complexity, lower validity, longer window period, and that testing alone would be anxiety-provoking. The following misconceptions regarding HIVST were mentioned: it involves a finger-prick, specimens must be sent to a company, and a company stores users' personal information.

**Conclusions:** Pride survey data suggest that Seattle-area transgender people HIV test less frequently than cis MSM. Efforts to promote HIVST as a method for increasing HIV testing among transgender people should include educational campaigns to address concerns and misinformation and include transgender-friendly counseling resources.

**Self-Reported HIV-Testing Behaviors among Seattle Pride Survey Respondents in 2014-15, Stratified by Gender and Sex**

	Assigned <u>male at birth</u>		Assigned <u>female at birth</u>
	Cis MSM <sup>^</sup> (n=866)	Non-Binary/Trans Women (n=98)	Trans Men (n=66)
Reported unknown HIV status	3%	12%	10%
Never HIV-tested	15%	23%	28%
Number of tests in prior 2 years			
0	23%	41%	40%
1	22%	26%	37%
≥2	56%	33%	23%
Ever used self-test	20%	7%	4%
Last HIV test was a self-test	8%	0%	0%

Terminology: "cis" = current gender that corresponds to sex assigned at birth; "trans" = current gender that differs from sex assigned at birth; and "non-binary" = gender that does not fit within the binary male/female dichotomy.

<sup>^</sup>Cis MSM data were weighted so the age distribution was comparable to that of Trans\* respondents.

**972 MSM and TG Accessing HIV VCT in Bangkok: Spatial Characteristics, 2005-2015**

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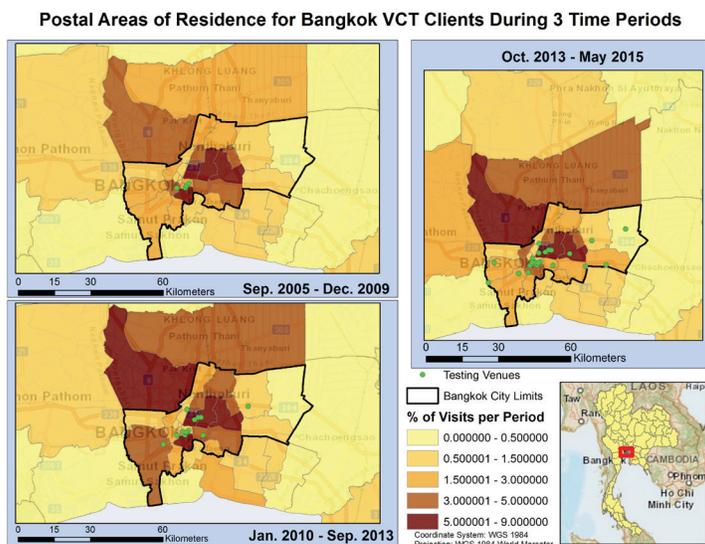
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**Background:** The Silom Community Clinic @TropMed (SCC @TropMed) is a voluntary counseling and testing (VCT) clinic for men who have sex with men (MSM), and transgender women (TG), in Bangkok. We assessed demographic and spatial features of our clients from 2005–2015.

**Methods:** We routinely collected demographic and spatial data from clients attending their first VCT at SCC @TropMed. Human immunodeficiency virus (HIV) VCT followed an algorithm of three rapid tests on blood. We divided visit dates into 3 periods based on the growth of public HIV testing venues for MSM in Bangkok; Period 1 (Sep. 2005–Dec. 2009), Period 2 (Jan. 2010–Sep. 2013), and Period 3 (Oct. 2013–May 2015). Clients provided postal zones of residence. We obtained geocoded HIV testing venues in Bangkok serving MSM and TG from the Bangkok Metropolitan Administration and U.S. Centers for Disease Control and Prevention. Using choropleth maps, we overlaid these spatial data and calculated distance between client residence and HIV testing venues using centroids of postal zones. We assessed differences in proportions across periods using chi-square tests of independence and trend, and spatial autocorrelation of prevalence and visit density using the Global Moran's I test.

**Results:** Among 8945 total clients (Period 1: 2802, Period 2: 3942, Period 3: 2201), at their first visit, 6681/8759 (76.3%) reported currently living in Bangkok, and 7429/8759 (84.8%) lived in postal zones located outside a 5-kilometer (km) radius of SCC @TropMed (Figure). Of 7728 tested, 2390 (30.9%) tested positive for HIV. Client age, HIV testing history, and current residence varied over time periods (all  $p < 0.01$ ). From Period 1 (12.6%) to 2 (37.5%) to 3 (41.0%), increasing proportions of clients lived in postal areas within 2 km of any testing venue ( $p < 0.01$ ), and increasing proportions of clients lived in postal areas >5 km from SCC @TropMed (Period 1: 83.4%, Period 2: 84.7%, Period 3: 87.0%,  $p < 0.01$ ). There was no evidence of clustered HIV prevalence (Moran's I 1.58,  $p = 0.11$ ) or clustered visit density (Moran's I 1.17,  $p = 0.24$ ).

**Conclusions:** Spatial data describe time-varying client demographics, including residence closer to a testing venue and residence farther from SCC @TropMed. This may represent greater access to MSM HIV testing venues in Bangkok and SCC @TropMed serving a broader geographic region. Spatial data can inform programmatic and research activities by providing meaningful data on populations and service accessibility.



## 973 Effect of Secondary Distribution of HIV Self-Tests on Womens Sexual Decision-Making

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**Background:** Providing multiple HIV self-tests to individuals for distribution to their sexual partners, i.e. 'secondary distribution', is a novel strategy that has the potential to increase knowledge of partner HIV status and enable safer sexual decision-making. By facilitating HIV testing at the time of high-risk sexual behaviors, self-testing might promote condom use and other HIV prevention behaviors. However, there is limited evidence on the feasibility of 'secondary distribution' of self-tests or the ability of individuals to make more informed sexual decisions with self-tests. We explored whether providing multiple self-tests to high-risk HIV-uninfected women influences their sexual decisions.

**Methods:** Antenatal and postpartum women and female sex workers (FSWs) who were HIV-uninfected and aged 18-39 years were recruited at 2 sites in Kisumu, Kenya. Following informed consent and instructions on using the OraQuick Rapid HIV 1/2 Test, women received 3-5 self-tests for own use or distribution to others. Structured interviews were conducted with the index participants (IPs) at enrollment and multiple times over 3 months to determine how self-tests were used. We examined IPs reported sexual behavior when their partners self-tested HIV-positive or HIV-negative.

**Results:** A total of 277 IPs (176 antenatal and postpartum, 101 FSWs) were enrolled. Most IPs distributed a self-test to at least one sexual partner (88% antenatal and postpartum, 96% FSWs), with FSWs giving self-tests to an average of 2.9 sexual partners. In total, 4% (4/144) of antenatal and postpartum women's partners and 14% (41/298) of FSWs' partners were reported to obtain an HIV-positive result from the self-test. Sexual intercourse was significantly less likely when a sexual partner tested HIV-positive versus HIV-negative (18% vs. 62%,  $p < 0.01$ ). Condom use among those who reported sexual intercourse was also significantly higher after a sexual partner tested HIV-positive versus HIV-negative (100% vs. 44%,  $p < 0.01$ ).

**Conclusions:** The results suggest that women who received multiple HIV self-tests were able to distribute tests to current or potential sexual partners and make positive sexual behavior decisions accordingly. Providing multiple self-tests for secondary distribution to high-risk women such as FSWs can lead to increased partner testing, results disclosure, and enable women to make safer HIV prevention decisions. The potential for secondary distribution as an HIV prevention strategy warrants further evaluation and consideration.

Table 1. Sexual decision-making among index participants who received multiple self-tests

	Study group		
	Full sample	ANC/PPC	FSW
<b>Number of index participants completing follow-up</b>	265	164	101
<b>Distributed self-tests to at least one sexual partner, No. (%)</b>	241 (91)	144 (88)	97 (96)
<b>Total number of self-tests used by sexual partners *</b>	447	144	298
<b>Total number of sexual partners who self-tested HIV-positive</b>	45 (10)	4 (3)	41(14)
<b>Sexual behavior when sexual partners self-tested HIV-negative</b>			
Had sexual intercourse with the partner since self-test, No. (%)	235 (62)	104 (75)	131 (54)
Used condom during last sexual encounter with the partner, No. (%)	104 (44)	13 (13)	91 (69)
<b>Sexual behavior when sexual partner self-tested HIV-positive</b>			
Had sexual intercourse with the partner since self-test, No. (%)	8 (18)	1 (25)	7 (17)
Used condom during last sexual encounter with the partner, No. (%)	8 (100)	1 (100)	7 (100)

Notes: ANC-antenatal care, PPC-postpartum care, FSW-female sex worker.

\*5 participants in ANC /PPC tested twice. 2 ANC/PPC participants and 15 FSW participants had indeterminate results and were not included in the analysis

## 974 Awareness of HIV Status: the Disclosure Gap in Rural Africa

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**Background:** Awareness of HIV infection is the first critical step in the continuum of HIV care. However, previous HIV status is not always disclosed to counselors and health personnel and little is known about the extent and causes of non-disclosure. The objective of this study was to measure the extent of non-disclosure of HIV status at a clinical and community setting in the Manhica District, Southern Mozambique.

**Methods:** This study was nested in a larger prospective observational study of linkage to care conducted between May 2014 and June 2015 in the Manhica demographic surveillance system (DSS). Randomly selected adults from the DSS were visited at home and offered HIV voluntary counseling and testing (HBT). Provider initiated (PICT) and voluntary testing (VCT) were offered at the Manhica district Hospital. All participants who did not know their HIV status and who tested positive at HBT, VCT or PICT were enrolled in the study. A history of previous HIV diagnosis was verified through the hospital electronic patient tracking system (ePTS).

**Results:** Among the 1401 adults screened in HBT, 323 (23.1%) disclosed their positive HIV status to the community counselor prior to testing. Of those tested, 108 were positive with an estimated prevalence of new HIV diagnoses of 10.0% (95%CI 8.4-12). However, 37 of the 108 positive (34.2%) were later identified through the ePTS as registered HIV-infected patients. The adjusted community prevalence of new HIV diagnoses was thus 6.8% (95%CI 5.5-8.5). The additional prevalence of new HIV diagnoses attributed to non-disclosure was higher in females (3.7%) than in males (2.4%) and highest among adults aged 35-44 years (5.3%). The overall HIV community prevalence of new and previous diagnoses was estimated at 30.8% (95% CI 28.4-33.2). The proportion of non-disclosures among participants testing positive at clinic-based testing sites and later identified through the ePTS was also high (12% at VCT and 29% at PICT  $p < 0.001$ ).

**Conclusions:** Non-disclosure of a previous HIV diagnosis among individuals testing positive is a frequent challenge encountered in all testing modalities. It can impact epidemiological population-based indicators relying on HIV self-disclosure. Inaccuracies in these indicators have implications on HIV program monitoring including estimating new diagnoses, real needs, coverage of ART and patient attrition.

Table 1: HIV testing and disclosure of HIV status at different steps in the diagnostic process across HIV

	VCT	PITC	HBT	p-value
Participants screened, N			1401	
Disclosure prior to testing, N (%)			323 (23.1%)	
HIV tested, N			1023	
HIV positive test result, N	910	1047	108	
Participants identified in EPTS post-test, N (%)	110 (12%)	303 (29%)	37 (34.2%)	< 0.001

### 975 Home-Based HIV Testing and New HIV Diagnoses in Chókwe District, Mozambique

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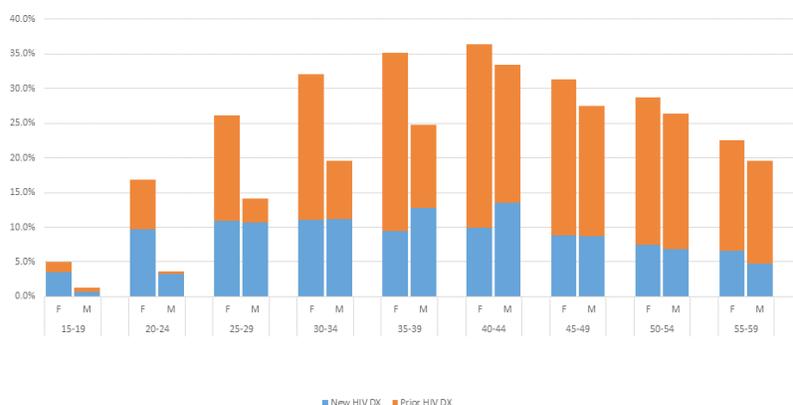
**Background:** Home-based HIV testing and counseling (HTC) has not been widely adopted because of high costs and low yield in new HIV diagnoses. As part of the Chókwe Mozambique Health Demographic Surveillance System (CHDSS), we report on the uptake of HTC offered at home, prevalence of HIV, and percentage of new diagnoses in a southern district of Mozambique.

**Methods:** CHDSS conducts an annual census of approximately 95,000 residents. As part of the first round of a multi-year combination-prevention project, in 2014-15, staff visited each CHDSS household and offered HTC to all residents. Consenting residents were screened for prior HIV diagnosis. Rapid HIV testing was conducted at home for residents without prior diagnosis, otherwise testing was conducted at the CHDSS laboratory using whole blood collected at home. Analyses are restricted to residents 15-59 years of age. Reported HIV prevalence is not adjusted to the CHDSS population.

**Results:** Of 53,227 residents 15-59 years of age, 25,344 (47.6%) tested for HIV. Proportionally more females than males (52% vs 41%), older (25-59) than younger adults (15-24) (50% vs 45%), and residents in rural than urban areas (50% vs 45%) tested for HIV. Of residents encountered, an estimated 82% of males and 88% of females accepted HTC. Of residents tested, 5119 (20.2%) were HIV-infected and 1945 (7.7%) were newly diagnosed. HIV prevalence increased markedly by age-group through 40-44 years, exceeding 30% for females and males in this age group, and steadily declined in older age groups (Figure). HIV prevalence was higher among females than males for each age group, particularly from 15 through 39 years. Percentage of new HIV diagnoses approached or exceeded 10% for females in age groups 20 through 44 years, and for males in age groups 25 through 44 years (Figure). For age groups 25 through 39 years, although HIV prevalence was substantially higher among females than males, percentage of new diagnoses (~10%) was similar in both groups.

**Conclusions:** In the first year of the combination-prevention project, nearly half of the estimated population 15-59 years of age were HIV tested, resulting in the new HIV diagnosis and referral to care of nearly 2,000 persons. Although testing rates were high among persons encountered, home-based HTC reached fewer men, younger persons, and persons who lived in urban areas. In high-prevalence populations, home-based HTC may be an important part of a comprehensive approach to achieve UNAIDS targets to diagnose 90% of HIV-infected persons.

**Figure.** Prevalence of HIV infection and percentage of new HIV diagnoses of 25,344 tested adults, by age-group and sex, Chókwe Mozambique Health Demographic Surveillance System, 2014-2015.



### 976 Increased Linkage to HIV Care After Clinic vs Community Testing in Rural Mozambique

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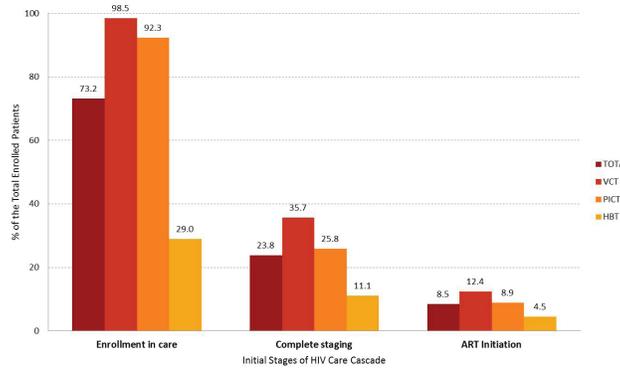
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**Background:** Improvements in testing services are needed if the global target of 90-90-90 is to be achieved. Client or provider-initiated and home-based HIV counseling and testing (VCT, PICT and HBT, respectively) are all complementary testing modalities to be considered when selecting local appropriate interventions. Linkage to HIV care throughout the cascade is a crucial indicator and yet there is little data on linkage across testing modalities. We aimed to compare the linkage rates between VCT, PICT and HBT in Southern Mozambique.

**Methods:** Between 2014 and 2015, we prospectively enrolled 341, 432 and 396 new adult HIV diagnoses through VCT, PICT and HBT respectively in a semi-rural area served by the Manhica District Hospital (MDH). Passive follow-up information was obtained through the MDH electronic HIV patient tracking and demographic surveillance system. Loss to Follow up (LTF) at each step of the care cascade was defined within 3 months of testing. Logistic regression was used to estimate the impact of testing modality on LTF at each step of the cascade.

**Results:** Among the 1169 enrolled patients, 56% were female with a median age of 34, 35 and 38.4 years in VCT, PICT and HBT respectively ( $p < 0.0001$ ). Linkage differed according to testing modality and cascade step. Of those tested at VCT, PICT and HBT, 99% ( $n=336$ ), 92% ( $n=397$ ) and 29% ( $n=113$ ) respectively enrolled in care ( $p=0, 0001$ ) while 51%, 42% and 53% of those enrolled attended the 1<sup>st</sup> clinic visit. PICT was significantly associated with a higher risk of LTF both at enrollment and 1<sup>st</sup> clinical visit ( $p=0.0001$  and  $0.0215$  respectively). Women, older participants and those reporting work absenteeism were less likely to be LTF for the 1<sup>st</sup> visit. Significant predictors of LTF at the staging step included being male ( $p=0.04$ ) and having individual testing ( $p=0.05$ ). Among those individuals eligible for ART, there was no significant difference in ART initiation between HIV testing cohorts (67, 63 and 68% for VCT, PICT and HBT respectively).

**Conclusions:** HBT participants were more likely not to enroll in care as compared to VCT and PICT, but there was no difference in LTF for initiating ART among those eligible. Areas relying on HBT should implement additional measures to ensure linkage to care after testing. Regardless of testing modality, there is a considerable block in the cascade of care before the 1<sup>st</sup> clinic visit leading to very low rates of ART initiation.



977 Effect of SMS, Phone-Call, and In-Person Reminders on Repeat HIV Test Uptake in Kenya

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**Background:** Following HIV-1 acquisition, most individuals develop an acute retroviral syndrome and a majority seek care. Available antibody testing cannot detect an acute HIV infection (AHI), but repeat testing after 2-4 weeks will detect seroconversion. This study assessed the effect of SMS, phone-call and in-person reminders on repeat HIV test uptake among patients evaluated for AHI.

**Methods:** The study was a non-blinded randomized control trial among patients 18-29 years of age seeking care at health facilities and community pharmacies in Coastal Kenya (NCT01876199, ClinicalTrials.gov). At the initial care-seeking visit, participants were tested for HIV antibodies and p24 antigen. Locator information included mobile phone number and home or workplace address. All participants who tested antibody and antigen negative were invited to report for repeat testing after two weeks. Participants were randomized 1:1 to either standard appointment (dated appointment card) or enhanced appointment which comprised a dated appointment card plus SMS and phone call reminders or in-person reminders (home or workplace visits by a fieldworker) for participants without a phone. Reminders were sent a few days before the appointment date and after a missed appointment by a single fieldworker. Phone reminders were sent using a basic feature phone. The primary outcome measure was visit attendance, i.e., the proportion of participants attending the repeat test visit. Factors associated with visit attendance were examined by bivariable and multivariable logistic regression.

**Results:** 410 participants were randomized. Visit attendance was 41% (85/207) for the standard group and 59% (117/199) for the enhanced group (odds ratio 2.0, p<0.001). There was no difference in visit attendance between participants in the phone reminders versus the in-person reminders sub-groups (58% vs. 60%, p=0.8). In the phone reminders sub-group, higher attendance was independently associated with older age, enrolling health facility and report of transactional sex in past 4 weeks (Table). Lower attendance was associated with reporting >1 sex partner in past 2 months.

**Conclusions:** Appointment reminders through SMS messages, phone calls and in-person contacts doubled the odds of repeat HIV test uptake. This low-cost intervention could help patients suspected of AHI report for repeat HIV testing 2-4 weeks after care seeking in settings that have no ability to test for p24 antigen or RNA.

Table: Factors associated with visit attendance for repeat HIV testing.

Characteristic	N	N	Bivariable analysis		Multivariable analysis (Full model)		
			Expected at follow-up visit	Attending follow-up visit (% of expected)	Odds ratio	Wald P value	Adjusted Odds ratio
<b>Treatment group:</b>							
Standard appointment	216	85 (42%)	Ref	Ref	Ref	Ref	
Standard appointment plus reminders	190	117 (58%)	1.9	0.001	2.0	0.001	
<b>Age:</b>							
18-24 years	258	118 (46%)	Ref	Ref	Ref	Ref	
25-29 years	148	84 (57%)	1.5	0.03	1.7	0.02	
<b>Level of education:</b>							
None	22	12 (55%)	1.5	0.4	1.3	0.6	
Primary	152	67 (44%)	Ref	Ref	Ref	Ref	
Secondary	172	93 (54%)	1.5	0.07	1.5	0.09	
Tertiary	60	30 (50%)	1.3	0.4	1.3	0.5	
<b>Treatment sought:</b>							
Fever							
No	210	114 (54%)	Ref	Ref	Ref	Ref	
Yes	196	96 (49%)	0.7	0.06	0.7	0.09	
<b>Enrolling health facility:</b>							
A	186	97 (52%)	6.9	0.001	6.8	0.001	
B	70	29 (41%)	4.3	0.01	4.1	0.02	
C	54	27 (50%)	6.2	0.002	7.1	0.002	
D	67	45 (67%)	12.8	<0.001	10.7	<0.001	
E	29	4 (14%)	Ref	Ref	Ref	Ref	
<b>&gt;1 sex partner in past 2 months</b>							
No	345	174 (50%)	Ref	Ref	Ref	Ref	
Yes	61	28 (46%)	0.8	0.5	0.5	0.04	
<b>Transactional sex in past 4 weeks</b>							
No	391	191 (49%)	Ref	Ref	Ref	Ref	
Yes	15	11 (73%)	2.9	0.07	4.9	0.03	
<b>Ever tested for HIV before</b>							
No	101	42 (42%)	Ref	Ref	Ref	Ref	
Yes	305	160 (52%)	1.6	0.06	1.5	0.1	

978 Effective, High-Yield HIV Testing for Partners of Newly Diagnosed Persons in Tanzania

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**Background:** With UNAIDS's 90-90-90 goal, high yield approaches to HIV testing and counseling (HTC) are critical. Partner tracing, where sexual partners of HIV-diagnosed clients are contacted by their partner or a health provider, is effective in identifying persons with undiagnosed HIV infection. A Malawi study found a 64% infection rate among sexual partners of index clients; and in a Cameroon study half (50.1%) were infected. Partner services for STI prevention have been utilized in the US and Europe, but this approach is underutilized in Sub-Saharan Africa (SSA). This study evaluated feasibility, acceptability and effectiveness of partner tracing in facility-based HTC in Tanzania.

**Methods:** The study was conducted in 3 hospitals in Njombe (Tanzania's highest HIV prevalence region) from June to September 2015. Newly HIV-diagnosed men and women, tested through PITC or VCT, were enrolled as index clients, and offered the choice of referring or bringing in their partner for HTC, or having a health provider anonymously contact their partner with the recommendation to come for HTC. Partners presenting to the facility were offered HTC and referred into HIV care and treatment if found HIV positive.

**Results:** 4,832 individuals were tested for HIV. 765 (15.8%) tested positive; 643 consented to participate. Of these, 251 were ineligible for partner referral including for being underage (31); not having a sexual partner in the last 12 months (148); being mentally unstable (18); being at risk of intimate partner violence (8) or other (46). 387 (99.7%) eligible index clients participated in the listing and referral process. 392 partners were listed, of whom 242 (61.7%) came to the facility; 222 (91.7%) through index client referral and 20 (8.3%) after a health provider contacted them. Of the 228 (94.2%) partners who were tested for HIV, 144 (63.1%) tested positive, all newly diagnosed. HIV positivity rate did not differ significantly ( $p=0.5$ ) among male (56.9%) and female partners (61.4%) (See Table 1)

**Conclusions:** This study demonstrated feasibility, acceptability and effectiveness of partner notification/referral for HTC in facility-based HTC in Tanzania. Given the need for high yield of identifying previously undiagnosed HIV infected individuals in the context of reaching 90-90-90 goals, and noting that notification was primarily by index clients, the findings present strong evidence for integrating partner notification and testing into facility-based HTC services in Tanzania and similar settings in SSA.

Table 1. Overview of HTC Partner Tracing Study findings (June – September 2015)

Facility	Makambako	Kibena	Ilembula	Total
People tested for HIV in the facility	2092	1634	1106	4832
HIV positive through HTC (PITC and VCT)	351	322	92	765
Percent testing positive through PITC and VCT	16.7	19.7	8.3	15.8
Index clients eligible and consenting to participate in the partner listing for referral to HTC	202	150	35	387
Index Client preference for sexual partner referral approach (n of listed partners):				
Client contacts / refers own sexual partner	194	147	32	373
Provider contacts / refers sexual partner	8	7	4	19
Partner comes to facility by type of referral:				
Client contacts / refers own sexual partner	91	110	21	222
Provider contacts / refers sexual partner	16	4	0	20
Partners agreeing to HTC	98	109	21	228
Partners testing HIV positive	49	81	14	144
HIV positive rate among testing partners	50	74.3	66.6	63.1

**979 94% Population HIV Testing Coverage With Repeat Hybrid Mobile Testing in East Africa**

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**Background:** In 2013-14, we reported 89% adult HIV testing coverage in 32 communities of 10,000 persons each in Uganda and Kenya using a hybrid mobile testing strategy (multi-disease community health campaigns [CHC], followed by home-based testing for CHC non-attendees [SEARCH:NCT01864603]). We repeated hybrid testing one year later and sought to determine: 1) adult testing coverage after two rounds of testing; 2) repeat testing rates; and 3) HIV prevalence, disease stage and testing coverage in year 2 among the 11% untested in year 1.

**Methods:** In 2014-15 (year 2), we repeated population-wide HIV testing using our hybrid approach in 16/32 communities. We offered new multi-disease services at CHCs, including urgent care and men's health education, and in Kenya, worked with the Ministry of Health to offer on-site medical male circumcision, family planning and cervical cancer screening. We determined the proportion of stable adults ( $\geq 15$  years; living in community for  $\geq 6$  months) that tested for HIV at least once during two rounds of hybrid testing, compared HIV prevalence and disease stage of adults who tested in year 2 but not year 1 vs. tested in year 1, and measured repeat testing in high-risk groups.

**Results:** Overall, 73,284/77,778 (94%) baseline census-enumerated, stable adult residents tested for HIV at least once during two rounds of hybrid testing. In Year 2, we tested 59,382/75,362 (79%) eligible stable adults (alive and HIV-/unknown): 46,633 (79%) tested at CHCs vs. 12,749 (21%) at HBT. If 10,085 adults (13%) reported to have moved out of community by an informant are excluded, year 2 testing coverage was 91%. Of 62,004 baseline HIV- adults eligible for repeat testing, 51,099 (82%) re-tested in year 2. Of 8,742 (11%) adults who did not test in year 1, 4,337 (50%) tested in year 2 and had similar HIV prevalence to those who tested in Year 1 (Table). In year 1 HIV- key populations who had not died or moved away, 95% of bar workers, 85% of transport workers, and 89% of fishing industry workers tested in both years 1 and 2. In qualitative data, new multi-disease services were a motivating factor for year 2 CHC participation.

**Conclusions:** With a repeat hybrid mobile approach, we achieved near universal HIV testing coverage, (94% of stable adults) over 12-15 months in 16 rural communities in Kenya and Uganda. Offering new multi-disease services in the repeat year contributed to ongoing high coverage (91%). This approach successfully reached transport, fishing and bar workers at high HIV risk for repeat test

**Table.** Comparison of HIV prevalence, and CD4 and viral load metrics among adults who tested HIV+ in Year 1 vs. adults who did not test in Year 1 but tested HIV+ in year 2.

	Adults who tested HIV+ in Year 1 (N=6,682)	Adults who did not test in year 1, but tested HIV+ in year 2 (N=421)	p value
<b>HIV Prevalence</b>	6,682/68,947 (9.7%)	421/4,337 (9.7%)	0.97
<b>Median CD4+ cell count at diagnosis (IQR)</b>	515 (356-698)	451 (292-633)	<0.001
<b>Mean log HIV RNA (SD)</b>	3.3 (1.3)	3.0 (1.4)	<0.001
<b>Median HIV RNA (IQR)</b>	2.7 (2.7-4.4)	2.7 (1.6-4.3)	<0.001

**980 Does a Male ChiP Increase Uptake of HIV Testing by Men? Lessons From HPTN 071 Study**

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**Background:** Male involvement in HIV testing programs can be pivotal in changing the dynamics of the HIV and AIDs epidemic. Factors attributing to low male involvement in Sub-Saharan Africa include culture, patriarchy and general reluctance to engage in health services. HPTN 071 is a 3 arm community-randomized trial in 21 communities in Zambia and South Africa that aims to study the impact of a combination prevention package on HIV incidence. The intervention is delivered in annual rounds of home-based door-to-door visits and includes voluntary HIV counselling and Testing (HCT) by a pair of Community HIV care Providers (ChiPs). Our objective was to determine whether having a male ChiP as part of a pair improved male uptake of the intervention and HIV testing in our communities.

**Methods:** CHiPs were paired up at the start of the intervention based on availability, gender balance, location of residence of the CHiP in relation to work area and personal characteristics. Every CHiP pair was assigned to a geographical zone consisting of 350–450 households. Process data of household visits was collected real time using hand-held electronic devices.

Outcome was defined as the proportion of males accepting the intervention and male uptake of HIV testing among those eligible. Primary exposure was the presence or absence of a male CHiP in the pair. Only pairs that worked together for the full duration of the study period were included. Crude proportions were calculated using cross-tabulation and crude ORs were calculated using logistic regression taking clustering on community level into account.

**Results:** Of the 206 pairs of CHiPs, 166 worked together since the start of the study: 53 female-female (FF) pairs, 107 male-female (MF) pairs and 6 male-male (MM) pairs. 25,206 out of 27,262 males (92.5%) accepted to take part in the intervention when counseled by MF or MM pairs compared to 49,581 out of 54,192 males (91.5%) with FF pairs (OR 0.88, 95% CI 0.55–1.40). Uptake of HIV-testing by eligible male participants (never tested or self-reported negative) was 29,348 out of 43,136 (68.0%) in pairs with minimally one male CHiP versus 17,857 out of 28,074 (63.6%) in FF pairs (OR 1.22, 95% CI 0.97–1.53).

**Conclusions:** We did not find convincing evidence that the presence of a male CHiP improves the uptake of the intervention or HIV-testing by male participants. Other factors such as the interaction between age of the CHiP and participants need to be considered.

## 981 Uptake of HIV Testing in the HPTN 071 (PopART) Trial in Zambia

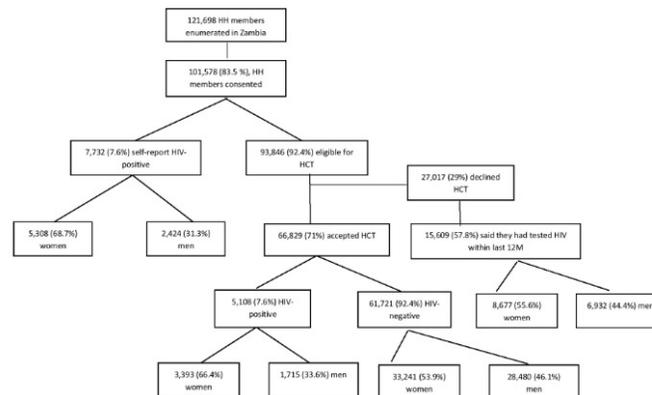
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**Background:** The HPTN071 (PopART) trial is a 3-arm community randomized trial in 12 communities in Zambia and 9 communities in South Africa evaluating the impact of a combination HIV prevention package, including a universal test and treat intervention, on HIV incidence. Arm A of the study provides the “full” combination HIV prevention package including home-based HIV voluntary counselling and testing, promotion of VMMC, and offer of immediate ART for those testing HIV-positive. The intervention in Zambia is offered in 8 of the 12 communities and is delivered in annual rounds by Community HIV Care Providers (CHiPs). We present data from the first annual round across the 4 Arm A communities in Zambia, which have an adult population of about 100,000.

**Methods:** Individuals who are contacted are offered participation in the study intervention, with verbal consent. Uptake of different components of the intervention, including HIV testing, was recorded electronically by the CHiPs during household visits. Data were analysed for the first annual round of the intervention, December 2013 to June 2015.

**Results:** 48,790 households (~100%) were visited by CHiPs during the first round, enumeration of individual household members was completed for 96% (46,899/48,790), and 83% (101,578/121,698) of adults (≥ 18 years) consented to participate. Refusal rate was 6.5% (7958/121,698) and 9% (10,962/121,698) were not contacted. Of those that consented to participate 45% (45,610/101,578) were men and the median age was 29. Prior to the CHiP visit, 47% (48,006/101,578) “knew their HIV status”, using a definition that they either reported they were HIV-positive (n=7,732), or that they had tested for HIV in the previous 12 months and were HIV-negative (n=40,274). Among those who did not self-report they were HIV-positive, 71% (66,829/93,846) accepted the offer of HIV counselling and testing from CHiPs, and 7.6% (5,108/66,829) tested HIV-positive. Among those consenting, 90% of women (50,619/55,968) and 87% (39,551/45,610) of men knew their HIV status by the end of Round 1; 16% (8,701/55,968) of women and 9% of men (4,139/45,610) self-reported or tested HIV-positive.

**Conclusions:** Through a home-based approach of offering a combination HIV prevention package the percentage of adults who knew their HIV status increased from ~50% to ~90%, among those who were contacted and consented to participate.



## 982 HIV Testing and ART Coverage Before a UTT Intervention: Findings in HPTN 071(PopART)

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**Background:** In 2014 UNAIDS set aspirational “90-90-90” global targets for knowledge of HIV status and ART coverage among people living with HIV. HPTN071 (PopART) is a 3-arm community randomised trial in 12 communities in Zambia and 9 communities in South Africa (SA), which tests the impact on HIV incidence of a combination HIV prevention approach compared with standard-of-care. Household-based interventions are provided in 2 trial arms, and include a universal offer of HIV testing and support for linkage to HIV care. ART is delivered through routine health care services; in one intervention trial arm, it is offered to all HIV-positive (HIV+) adults irrespective of CD4 count. We analysed baseline data on community-wide uptake of HIV testing and ART, to quantify the level of service expansion required to reach the 90-90-90 targets.

**Methods:** A randomly-selected cohort (PC) of adults aged 18–44 years was recruited from November 2013 to March 2015 to measure primary and secondary trial outcomes. Consenting participants provided a blood sample for laboratory HIV testing, and the research questionnaire included questions on previous HIV testing, knowledge of HIV status, and uptake of ART.

**Results:** 38,383 adults were enrolled in the PC, ~2000 in each trial community. A total of 23,362 were enrolled before they were offered the household-based interventions, of whom 21,854 had a laboratory HIV test result available by mid-2015 and 21,776 answered questions on prior HIV testing. HIV prevalence was 12% in men (806/6674) and 27% (4048/15102) in women; 69% of men and 88% of women reported previously testing for HIV, with wide variation among communities. In Zambia, among HIV+ men 46% (172/373) reported they were HIV+ and 31% (117/373) that they were on ART; among HIV+ women 55% (970/1760) reported being HIV+ and 36% (636/1760) were on ART. In SA, among HIV+ men 27% (116/433) reported they were HIV+ and 18% (78/433) that they were on ART; among HIV+ women 48% (1103/2288) reported being HIV+ and 32% (724/2288) were on ART. Knowledge of HIV status and ART uptake varied considerably across communities, and increased with age.

**Conclusions:** Among HIV+ men around one-third knew their HIV status and one-quarter were on ART, and among HIV+ women around half knew their HIV status and one-third were on ART, across the trial communities. These levels are far below 90-90-90 targets; the HPTN071 trial will determine whether, with household interventions, the targets can be reached.

### 983 HIV Testing and Linkage to Care in the Botswana Combination Prevention Project

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**Background:** To identify 90% of PLHIV, effective community models to test and link men and women are needed. The Botswana Combination Prevention Project (BCPP) is a randomized controlled trial designed to evaluate the impact of high coverage of a combination prevention package on population level HIV incidence in 30 communities. The trial is ongoing; we describe preliminary data on uptake of HIV testing and linkage to care (LTC) in the first 7 intervention communities.

**Methods:** HIV testing campaigns conducted October 2013–May 2015 included home-based and mobile testing. Enumeration was attempted at all inhabited households, and interviews and HIV testing were offered to residents  $\geq 16$  years who did not have documentation of an HIV test in the previous 3 months. Newly identified and known HIV-positive persons not on ART were given point of care CD4 tests, referrals, and LTC interventions if they did not register at the HIV clinic.

**Results:** Of the 19,043 enumerated community residents ages 16–64, 17,282 completed HTC interviews; 70% (12,183/17,282) through home-based testing and 30% (5099/17,282) at mobile venues. Overall refusal rate for testing was 4%. Home-based testing reached more women (62%; 7496/12183); mobile testing reached more men (56%; 2879/5099). The rate of newly identified HIV-positives was 7% for both home and mobile testing. The highest rate of newly identified HIV-positives for both males and females was in the 31–40 age group (range 10–13%) for both home and mobile testing. Sixteen percent (2,743/17,284) of those who completed HTC interviews had documentation of prior HIV-positive status; 84% (2,317/2,743) of them were on ART. More women (72%; 1973/2743) already knew their HIV-positive status than men (28%; 770/2743). Among the newly diagnosed, known HIV-positive persons not on ART and ART defaulters referred to the HIV clinic, 77% (1138/1479) registered at the local clinic.

**Conclusions: Discussion:** Preliminary data from BCPP indicates that both home-based and mobile testing campaigns are effective modalities for finding newly identified HIV-positive persons and known HIV-positive persons not on ART. Both modalities reach men and women; however, efforts to target age groups where new infections are highest and to increase testing among men should be strengthened and prioritized to reach the goal of identifying 90% of PLHIV. Tracking referrals to the clinic and providing follow up to those who do not link initially are important for high LTC rates.

### 984 Cross-Sectional HIV Incidence at Scale-up of ART in 24 Rural Communities in Botswana

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**Background:** Direct real-time estimates of HIV incidence using cross-sectional sampling can provide critical information for design and evaluation of HIV prevention interventions. The ongoing scale-up of ART in southern African countries presents a substantial challenge to the accuracy and reliability of cross-sectional methods used for identification of HIV recency.

**Methods:** HIV recency was estimated at the baseline of the Botswana Combination Prevention Project (BCPP). Cross-sectional data were collected during household surveys in 24 rural communities from Nov 2013 to Aug 2015. An HIV incidence testing algorithm combining the Limiting-Antigen Avidity Assay (LAG-Avidity EIA) with ART status and level of HIV-1 RNA load (multi-assay algorithm described in Rehle et al., PLoS One 2015;10:e0133255) was used. The LAG cut-off normalized optical density was 1.5. ART status was documented. The HIV-1 RNA cut-off was 400 cps/mL. The Mean Duration of Recent Infection was 130 days and the False Recent Rate was set to zero.

**Results:** A total of 2,727 individuals tested HIV-positive among 9,745 individuals with definitive HIV status (28.3% HIV prevalence after adjustment for study design; 95% CI: 25.6%–31.2%) during the baseline household surveys. About 70% of HIV-positive individuals were already on ART. LAG-Avidity EIA data was generated for 2,710 of 2,719 (99.7%) HIV-positive individuals with research blood draw available, and 234 cases were identified as LAG-Avidity EIA recent. Among those, 198 individuals were considered chronically infected based on their documented ART status. Eleven of 36 LAG-Avidity EIA recent, ARV-naïve individuals had an HIV-1 RNA load  $\leq 400$  cps/mL, and were classified as having long-term HIV infections. Thus, 25 LAG-Avidity EIA recent, ARV-naïve individuals with HIV-1 RNA above 400 cps/mL were classified as individuals with recent HIV infections. HIV incidence across 24 communities was estimated at 1.00% (95% CI 0.60%–1.41%).

**Conclusions:** The increasing scale-up of ART in southern African communities requires adjustment of cross-sectional methods for identification of HIV recency. An algorithm that combines LAG-Avidity EIA testing with ART status and HIV-1 RNA data was used to estimate baseline HIV recency in 24 rural communities at baseline of the BCPP. This algorithm should be validated by longitudinal HIV incidence data in the future. HIV incidence in rural communities in Botswana was estimated at 1.0% in 2013–2015.

### 985 Local Social Network Features Predict HIV Testing Uptake in a Rural Ugandan Community

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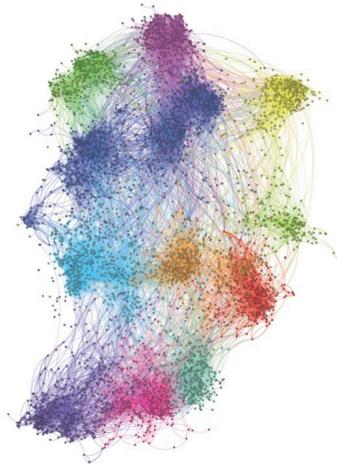
**Background:** Understanding social dynamics behind HIV testing may provide novel ways to achieve high testing coverage more efficiently. We tested whether social network data predicted testing uptake in a community wide mobile HIV testing campaign.

**Methods:** We built a social network for a rural Ugandan community with 4810 adults where, as part of the SEARCH Trial (NCT01864603), a hybrid approach of community health campaigns (CHC) followed by home based testing (HBT) for non-attendees tested 94% of adult stable residents (82% by CHC, 12% by HBT). In a baseline census, adult residents ( $\geq 15$  years old) provided contact information for up to 6 friends in each of five social domains (health, money, emotional, food, and free time). Named contacts were matched to a census enumeration to build the network. Associations between testing uptake and network characteristics, including local network density (proportion of realized edges among all possible), clustering (proportion of trios that are fully connected), and number of named contacts from each social domain, were evaluated using logistic regression with cluster-robust standard errors, adjusting for age, gender, occupation, education, marital status, and wealth. Structural features of a subnetwork of HIV+ adults were compared to 1000 simulated populations of the same prevalence and network topology.

**Results:** The community-wide network contained 25148 links across all domains, with 96% of individuals connected in a single cluster (Figure 1). After adjusting for demographics, subjects with fewer named contacts in any domain and subjects with a denser local network were both significantly less likely to attend CHC and more likely to fail to test at either CHC or HBT. The greatest risk for not testing was conferred by higher network density (aOR 2.9, 95%CI 1.47, 5.74) and fewer named free time contacts (aOR 1.24, 95%CI 1.11, 1.38). Increased risk for CHC nonattendance was also associated with high local network clustering (aOR 1.78, 95%CI 1.06, 2.97). A subnetwork of 100 HIV+ adults was denser and had more clusters of size  $\geq 4$  than expected by chance, and showed multiple social links to non-testers.

**Conclusions:** Network-based testing strategies that target dense and clustered local networks and increase social outreach may achieve high coverage more efficiently. Social network data identified clusters of HIV+ individuals with social links to multiple non-testers, potentially informing identification of hidden high-risk individuals.

**Fig. 1:** Complete social network of 4810 adults (25148 relations across all 5 social domains) in a rural community in Uganda. Nodes (persons) are color-coded by village, lines represent social links between persons. 96% of nodes lie within one connected cluster, with 99.9% of all links; clustering coefficient 0.17, average degree of separation 4.7.



#### 986 Combination HIV Prevention and HIV Incidence in a Ugandan Hotspot Fishing Community

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**Background:** Targeted combination HIV prevention interventions have been advocated to control the HIV epidemic in high HIV incidence and prevalence hotspots in sub-Saharan Africa. However, there is limited empirical evidence on the population-level effects of these interventions. We present data on the rapid scale-up of treatment as prevention (TasP) and medical male circumcision (MMC) and trends in HIV incidence in a fishing community with high HIV prevalence (41%) in Rakai District, Uganda.

**Methods:** Between November 2011 and May 2015, four survey rounds of the Rakai Community Cohort Study (RCCS), a population-based longitudinal study of HIV incidence, risk behaviors and HIV service utilization among residents aged 15-49, were conducted in a large Lake Victoria fishing village. HIV incidence was estimated as the number of new infections per 100 person years (py) between visits. We used multivariable Poisson regression models with generalized estimating equations, to estimate adjusted incidence rate ratios (aIRR) and 95% confidence intervals (95%CI) of HIV incidence comparing the period before the start of TasP and rapid scale-up of MMC (2011-2012) to periods after 2012.

**Results:** 4,071 persons participated in at least one RCCS survey round. Average ART coverage among HIV-positive persons increased from 18.6% in November 2011 to 67.3% by May 2015, while MMC coverage among non-Muslim men increased from 24.3% to 49.2% over the same time period. HIV incidence was 4.0/100py (95%CI: 2.6-5.7) between November 2011 and October 2012, falling to 3.3/100 py (95%CI: 2.4-4.5) between October 2012 and October 2014, and to 2.9/100 py (95%CI: 2.2-3.8) between October 2014 and May 2015. The aIRR comparing the period 2011-2012 to 2014-2015 was 0.75 (95% CI=0.47-1.20); the aIRR for men was 0.75 (95% CI=0.39-1.43) and 0.77 for women (95% CI=0.38-1.56).

**Conclusions:** Rapid scale-up of combination HIV prevention in very high risk fishing communities on Lake Victoria is feasible, and there is preliminary empirical evidence of the effects of these interventions in decreasing HIV incidence.

#### 987 Genital Inflammation and HIV Shedding Post-Male Circumcision in Rakai, Uganda

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**Background:** Among HIV infected men undergoing medical male circumcision (MC), resumption of sexual intercourse prior to wound healing is associated with increased risk of HIV transmission to female partners. We previously reported that penile HIV shedding increases post-MC. In this study, we examined the association between pro-inflammatory cytokines and penile HIV shedding.

**Methods:** A prospective cohort study of 223 HIV-infected men undergoing dorsal slit MC was conducted between June 2009 and April 2012 in Rakai, Uganda. Preoperative and weekly penile lavages from 1-4 weeks post-MC (n=1034 visits) were tested for pro-inflammatory cytokines (IFN- $\gamma$ , IL-10, IL-12p70, IL-13, IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-8, and TNF- $\alpha$ ) by an electrochemiluminescence immunoassay (Meso Scale Discovery Inc.). Cytokines with <1% detection (IFN- $\gamma$ , IL-12p70, & IL-4) were excluded from analyses. Modified Poisson regression with generalized estimating equations and robust variance estimators were used to estimate prevalence risk ratios (PRRs) for penile HIV shedding at weeks 1-4 post-MC. Models were run for each cytokine individually. Among detectable samples, log<sub>10</sub> cytokine levels were compared by penile HIV shedding status using the Wilcoxon rank-sum test and correlations with penile log<sub>10</sub> HIV RNA were determined by Spearman's rank-order test corrected for multiple comparisons (Bonferroni method).

**Results:** Penile HIV and HSV-2 shedding was detected among 13.7% (141/1026) and 10.8% (87/805) of sample visits, respectively. Relative to baseline, detection of each cytokine was higher during wound healing weeks 1-3 post-MC (P<0.01), except for IL-2 (P=0.072; n=1031). Detection of all cytokines was lower among healed wounds (P<0.01; n=791). Baseline plasma HIV VL, CD4 count, genital ulcer disease, and antiretroviral therapy were not associated with cytokine detection. HIV shedding 1-4 weeks post-MC was associated with the detection of IL-1 $\beta$ , IL-2, IL-6, IL-8, IL-13, and TNF- $\alpha$  after adjusting for HSV-2 shedding and study visit (P<0.05; n=630). Log<sub>10</sub> IL-1 $\beta$  and IL-8 levels were higher among visits with HIV shedding than non-shedding visits 1-4 weeks post-MC (P<0.01). Log<sub>10</sub> IL-1 $\beta$ , IL-6, IL-8, IL-13, and TNF- $\alpha$  levels were positively correlated with penile log<sub>10</sub> HIV RNA levels (P<0.01).

**Conclusions:** An increase in genital pro-inflammatory cytokines is associated with HIV shedding from MC wounds. Strategies to reduce genital inflammation post-MC and HIV shedding during wound healing may help to prevent male to female transmission.

**988 Home-Based HIV Counseling and Testing Using Index Patients in Lesotho**

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**Background:** HIV testing coverage in Lesotho has expanded in recent years with the percent of persons aged 15-49 who have ever tested increasing from 2009 (39.3% of men; 68.6% of women) to 2014 (65.2% of men; 85.5% of women). To achieve universal HIV testing coverage, novel approaches are needed. The use of clinic-based index patients (IPs) for home-based HIV counseling & testing (HBCT) has the potential to expand testing coverage by facilitating access to homes and persons at risk for HIV.

**Methods:** Between December 2012 and September 2013, 192 IPs were recruited from 2 health centers in Mafeteng and Mohale's Hoek districts as part of the NIH-funded Enhanced Prevention in Couples (EPIC) study. IPs were asked to approach their household (HH) members regarding testing. Home visits were scheduled with IPs and HBCT was offered to both IPs and HH members who did not self-report HIV+. Demographic and acceptability measures were collected by in-person surveys.

**Results:** At recruitment, 119 (62%) of 192 IPs self-reported as HIV+ and 73 IPs self-reported as HIV- or status unknown and were offered HBCT; 58 (81%) accepted and of these, 2 IPs were identified as HIV+. A total of 661 HH members were identified through IPs; 98 (15%) HH members self-reported as HIV+. HBCT was offered to the remaining 563 HH members and 495 (88%) accepted (90% of women and 84% of men); 54 (10.9%) HH members were newly diagnosed HIV+ via HBCT. In total, 56 (10%) of 553 tested IPs and HH members were newly diagnosed as HIV+; 70% of newly diagnosed were women. HIV+ IPs (both self-reported and newly diagnosed) yielded a higher percent of new HIV diagnoses among HH members than HIV-/status unknown IPs (13% vs. 7% p=0.04). Of 123 couples with at least one partner tested by HBCT, 57 serodiscordant couples were identified: 51 couples included a partner who was diagnosed as HIV+ prior to this study and for whom one HIV- partner was identified via HBCT; 6 couples included a partner who was newly identified as HIV+ via HBCT. Nearly all tested persons (99%) were "very satisfied" with HBCT; 97% said they would recommend HBCT to family and friends.

**Conclusions:** The study demonstrated the feasibility of utilizing IPs to access HH members and provide HBCT. Using HIV+ IPs to reach HH members and conduct HBCT may be an efficient approach to advance HIV prevention through identification of other HIV+ persons in HHs and identification of new discordant couples. HBCT was highly acceptable through this approach.

Table. Demographics, testing outcomes, and acceptability among IPs and HH members who accepted HBCT testing

	HBCT Participants (IP and HH)		
	Total (N=553)	Female (n=367)	Male (n=186)
Index participant (%)	58 (10)	50 (14)	8 (4)
Age (range)	33 (18-88)	33 (18-88)	34 (18-86)
Ever tested for HIV	451 (82)	313 (86)	138 (74)
Self-reported risk of HIV			
No risk	179 (32)	112 (30)	67 (36)
Small risk	83 (15)	54 (15)	29 (16)
Moderate risk	27 (5)	15 (4)	12 (6)
Great risk	213 (39)	149 (41)	64 (34)
Don't know	51 (9)	37 (10)	14 (8)
Tested HIV+	56 (10.1)	39 (10.6)	17 (9.1)
Will disclose (among newly HIV+)	22 (39)	17 (44)	5 (29)
To Primary Partner (of those plan to disclose)	14 (64)	10 (59)	4 (80)
Will seek HIV care & treatment (among newly HIV+)	25 (45)	17 (44)	8 (47)*
Recommend HBCT to family/friends/	538 (97)	360 (98)	178 (96)
Very satisfied with HBCT	545 (99)	361 (98)	184 (99)

All findings are either: N (column %) or median (range).

**989 Prevention for HIV-Infected Persons in HPTN 065: Room for Improvement**

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**Background:** HPTN 065 examined the feasibility of a test, link-to-care, plus treat strategy for HIV prevention in the Bronx, NY and Washington, DC. Few data are available on patient perspectives about the effect and use of antiretroviral therapy (ART) for prevention. As part of HPTN 065, we surveyed a sample of HIV-infected patients to assess knowledge and attitudes on ART use for treatment and prevention.

**Methods:** We recruited patients at 10 HIV care sites (Bronx 4, DC 6). Patients completed a computer tablet-based 21 question survey during December 2013 to December 2014.

**Results:** Characteristics of 725 patients who participated in the survey were as follows: median age 52 years (range 18-77); 69% male; 62% African American; 42% men who had sex with men, and 95% on ART. Regarding reasons for taking ART, 90% indicated they would take ART "to improve their own health" and 62% "to lower the chance of passing HIV to a sex partner." Respondents also indicated ART should be started "when someone is sick from HIV" (76%) and "when a doctor tells a person they need HIV medicines" (73%); fewer (54%) indicated ART should be started "to lower the chance of passing HIV to sex partners." With regards to the chance of transmitting HIV during sex while taking ART versus not taking ART, 24% indicated that risk was higher, 35% that it was lower and 32% indicated the same risk. With regards to chances of transmission with a low versus high viral load, 11% indicated higher, 41% lower, and 38% the same risk. Most (87%) indicated it was possible to transmit HIV with an undetectable viral load. Among the 614 (85%) patients who saw an HIV provider within the last 3 months, 48% had talked with the provider about sex partners or condom use, 30% about sex partner HIV status, and 29% about disclosing their HIV-positive status to sex partners. Only 49% had been asked about alcohol use and 24% about injection drug use. With regards to sexual behavior among 691 patients on ART, 2% reported more sex partners and 4% indicated condom use less often since starting ART (Table).

**Conclusions:** Overall, patients favored using ART for their own health but had more limited knowledge about using ART to reduce risk of HIV transmission to partners. HIV providers need to discuss with patients HIV risk behaviors and the importance of viral suppression for prevention of HIV transmission. We also found little evidence in our study for risk compensation among patients on ART in HIV care.

Table. Sexual risk behaviors reported by survey participants on ART (n=691)

Survey Item	Percent
"Since you have started taking HIV medicines, have you changed the number of sex partners you have?"	
No, I have the same number of partners	43
I am not having sex now	37
Yes, I have fewer partners	18
Yes, I have more partners	2
Do not know/ Refuse to answer	1
"Do you think that taking HIV medicines has changed how often you use condoms when having sex?"	
No, I use condoms the same amount	45
Yes, I use condoms more often	39
I do not use condoms whether or not I am on HIV medicines	6
Yes, I use condoms less often	4
Do not know/ Refuse to answer	5

**990 Increased Levels of HIV Baseline Drug Resistance Testing & Early Treatment Initiation**

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**Background:** National HIV treatment guidelines have evolved greatly in the past decade. Current guidelines recommend early initiation of antiretroviral treatment (eART) and baseline drug resistance testing (bDRT) upon diagnosis and linkage to care. bDRT and eART patterns were assessed with respect to national treatment guidelines.

**Methods:** San Francisco residents diagnosed with HIV/non-AIDS between 2001 and 2012 who were linked to care at publicly-funded facilities were included in the analysis (N=2,565). Cases were stratified by era of treatment guidelines based on diagnosis year: Era 1=2001-03 (n=657), Era 2=2004-06 (n=716), Era 3=2007-09 (n=640) and Era 4=2010-12 (n=552). bDRT and eART were defined as occurring within 3 months of diagnosis.

**Results:** The proportion of cases with bDRT increased from 2001 to 2012 (p<0.001). The nadir was 4% in 2001, increased to 9% in 2002, dipped to 8% in 2003, rose to 12% in 2004, and then continued to increase to a peak of 56% in 2012. bDRT increased across the 4 eras, from 7% to 15% to 33% to 49% (p<0.001). The proportion of cases with eART increased from 2001 to in 2012 (p<0.001). The proportion was 16% in 2001, decreased over the next 3 years to a nadir of 4% in 2004, and then began to increase steadily to a peak of 45% in 2012. eART increased across the 4 eras, from 11% to 6% to 16% to 36% (p<0.001). eART was associated with an increased likelihood of bDRT (p<0.001). Gender, age, race/ethnicity and HIV transmission risk were not associated with bDRT overall; persons <20 years and Latinos were more likely to have been tested in Era 4 (p<0.05).

**Conclusions:** Baseline drug resistance testing increased steadily from 2004 to 2012, though not all genotyped cases initiated treatment within 3 months of diagnosis. Nevertheless, baseline genotyping can be useful for informing regimen selection upon the subsequent decision to initiate ART, since a transmitted resistant mutant present early in the course of infection may still be in viral archives but no longer detectable as the most abundant variant. eART increased substantially in Era 4 which may reflect the adoption of treatment as prevention, whereas the observed decrease in late Era 1 and in Era 2 may stem from concerns during that time about the toxicities associated with ART. Lack of disparities in baseline drug resistance testing by demographic or risk group may be due to federal and municipal support that facilitated access to primary care services.

**991 HIV Prevalence and Care in the New York State Department of Corrections**

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**Background:** In 1988, an unlinked seroprevalence study showed that nearly 1 in 5 incoming inmates to New York State (NYS) Department of Corrections and Community Supervision (DOCCS) facilities had HIV infection. Enormous challenges have been addressed in DOCCS facilities with extensive programs for HIV testing, medical care, prison medical staff education, transition planning and stigma reduction. Continuing studies have shown decreasing HIV seroprevalence in incoming inmates with 2.4% of males and 3.7% of females seropositive in 2012. With changing NYS laws on use of surveillance data and NYS's "Ending the Epidemic" initiative, this project was undertaken to understand the extent and experience of diagnosed HIV infected persons under custody and to further intervention opportunities.

**Methods:** An electronic file of DOCCS inmates under custody inclusive of HIV medical diagnosis codes was matched to the NYS Department of Health (DOH) HIV Registry using probabilistic matching software. CD4 lymphocyte (CD4) and viral load (VL) data routinely reported to the HIV registry were used as a proxy for HIV-related medical care. Evidence of HIV care was defined as ≥1 CD4 or VL. Prevalence was based on inmates under custody on 12/31/2014, with HIV care assessed for July – December, 2014.

**Results:** 49,224 persons were under custody on 12/31/2014. 1,109 matched to cases in the HIV Registry, for an HIV prevalence of 2.3% (men 2.2%, women 3.7%). HIV care was assessed for 887 who were diagnosed before July 2014 and under custody continuously from July to December, 2014. Medical diagnoses codes documented that 796 (89.7%) of those were known to DOCCS as having HIV. 776 (87.4%) had evidence of HIV care, and 774 (87.2%) had a VL. VL closest to yearend was ≤200 copies/ml in 721 (81.2%).

**Conclusions:** This work represents the first direct measure of the prevalence of diagnosed HIV in those under custody in NYS DOCCS. Among those with diagnosed HIV, nearly 9 of 10 had disclosed their diagnosis to DOCCS and were receiving care. Viral suppression of those receiving any HIV care (90.6%) compares favorably to a similar statewide rate of 82% in 2013. The match provides important opportunities for further public health interventions, including interventions with out-of-care persons who have previously disclosed status to DOCCS, intervention with diagnosed HIV-infected incarcerated persons not in care and not known to DOCCS, and NYSDOH follow-up to assess and intervene for linkage to care post-incarceration.

**992 Frequency and Duration of Churn Among Persons Living With HIV in Washington, DC**

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**Background:** Measuring retention in care is an essential component of the HIV care continuum. However, traditional retention measures often require extensive resources but also tend to focus on one point in time, consequentially failing to adequately capture the care dynamics of those who cycle in and out of care. It is unclear whether this cycling, or "churn," results in poorer clinical outcomes. The objective of this analysis was to characterize the frequency of churn and duration of gaps in care among persons living with HIV, as well as identify individual characteristics associated with varying churn patterns.

**Methods:** Analyses were restricted to routinely collected HIV and laboratory surveillance data for persons diagnosed with HIV in the District of Columbia prior to 2008 and in care at the end of 2013 with ≥1 visit in 2008 and ≥1 visit in 2013. Churn was defined as being out of care for ≥6 months. Frequencies, univariate, bivariate, and nonparametric analyses, as well as multivariate logistic regression were conducted to assess associations between individual characteristics and churn.

**Results:** Among 3,364 people, 91% (n=3,064) had evidence of churn. The mean duration of churn was inversely related to the number of gaps: persons with one gap in care had a mean gap of 63 weeks compared to 36 weeks among persons with seven gaps in care. Among the 3,064 individuals who churned, 26% had one gap, 28% two gaps, 22% three gaps, and 25% had 4-7 gaps in care between 2008 and 2013. Frequency of churn varied significantly by sex (p=0.04), race/ethnicity (p<0.01), age (p<0.01), and duration of HIV infection (p=0.01). Multivariate logistic regression found that, compared to persons with no churn, those 45-64 years old were significantly less likely to experience two or more gaps in care than those under 35 years of age. Blacks were also significantly more likely to churn than whites. Persons diagnosed with HIV ≤1 year prior to their 2008 visit were significantly more likely to churn compared to those diagnosed more than 15 years prior. There was a significant change between reported CD4 and viral loads between 2008 to 2013 across all churn categories.

**Conclusions:** These results add to our understanding of the impact of taking periodic breaks from care and help quantify the large amount of churn observed among people living with HIV. Our data on churn duration may also assist in more efficient allocation of relinkage resources by focusing on those persons who are younger and have been more recently infected.

Adjusted odds ratios (aOR) and corresponding 95% confidence intervals (95% CI) for significant variables of interest with churn levels (reference = no churn), n=3,364				
	1 Gap aOR (95% CI)	2 Gaps aOR (95% CI)	3 Gaps aOR (95% CI)	4-7 Gaps aOR (95% CI)
<b>Race/Ethnicity</b>				
White, non-Hispanic	Ref.	Ref.	Ref.	Ref.
Black, non-Hispanic	1.61 (1.09, 2.36)	1.63 (1.11, 2.41)	1.43 (0.96, 2.12)	1.32 (0.89, 1.94)
Other or Unknown	1.35 (0.78, 2.34)	1.07 (0.61, 1.89)	0.82 (0.46, 1.48)	0.69 (0.39, 1.24)
<b>Age as of December 31st, 2008</b>				
Under 35	Ref.	Ref.	Ref.	Ref.
35-44	0.81 (0.50, 1.30)	0.65 (0.41, 1.05)	0.53 (0.33, 0.86)	0.65 (0.40, 1.04)
45-54	0.67 (0.42, 1.08)	0.53 (0.33, 0.84)	0.46 (0.28, 0.73)	0.50 (0.31, 0.80)
55-64	0.69 (0.40, 1.21)	0.57 (0.33, 0.98)	0.46 (0.26, 0.80)	0.42 (0.24, 0.74)
65 and over	1.32 (0.52, 3.37)	0.73 (0.28, 1.90)	0.43 (0.15, 1.20)	0.37 (0.13, 1.06)
<b>Duration of HIV Infection Prior to 2008 Lab (years)</b>				
More than 15	Ref.	Ref.	Ref.	Ref.
11 - 15	1.46 (0.93, 2.30)	1.02 (0.65, 1.60)	1.14 (0.72, 1.82)	1.23 (0.77, 1.95)
6 - 10	1.41 (0.92, 2.16)	1.27 (0.84, 1.94)	1.26 (0.81, 1.94)	1.53 (0.99, 2.36)
1 - 5	1.36 (0.89, 2.09)	1.23 (0.81, 1.88)	1.23 (0.80, 1.91)	1.25 (0.81, 1.93)
1 or less	15.50 (2.05, 117.31)	16.89 (2.26, 126.52)	15.97 (2.11, 120.80)	15.92 (2.11, 120.33)

### 993 UCARE4LIFE: Mobile Texting to Improve HIV Care Continuum Outcomes for Minority Youth

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**Background:** Minority youth living in the Southern U.S. face higher HIV rates and faster disease progression than non-minority youth. According to the Pew Institute, over 75% of youth use cell phones and the mean number of texts sent and received per day is 60. This study examined the utility of a text message program as an HIV self-management tool.

**Methods:** A prospective, randomized two-group pilot study was conducted from Fall 2014 thru Summer 2015 to assess the promise of text messaging among minority youth to increase their retention in care and HIV medication adherence. Eligible participants included English-speaking HIV-positive youth aged 15 to 24, who had personal cell phones with text-messaging capability whom received their HIV medical care at one of three clinics located in Louisiana, Alabama, or North Carolina. A total of 341 unique, culturally appropriate text messages were delivered from domains such as treatment and appointment adherence, HIV basics, clinical visits, social support and risk reduction. Data were collected via medical record abstraction and pre/post-intervention assessment focus groups and behavioral assessment surveys.

**Results:** A total of 164 youth were eligible and 146 voluntarily enrolled. The demographics of those enrolled were 86% male, 77% African American, 66% homosexual, and 85% were aged 21 thru 24. The intervention group received a mean number of 12 texts a week; the control received standard of care that did not include text messaging. T-tests, chi-squares, and McNemar statistical analysis were employed for data analysis. Post-intervention data showed that the viral load was significantly statistically lower for the intervention group compared to the control group at three (p=.039) and six months (0.003) post-baseline among youth who were non-adherent or new to ART at baseline. There was no statistically significant difference between retention in medical care for the intervention group versus control group. Six months post-baseline, the intervention youth demonstrated a statistically significant better understanding of the effects of substance use on their ability to remember to take their ART (p=.007) than among the controls.

**Conclusions:** Delivering culturally appropriate, timely, tailored text messages to HIV positive youth is a promising new intervention. Youth who were non-adherent or new to taking ART benefited most with lower viral loads and understanding of the effects of substance use on their health.

### 994 Missed Opportunities: Adapting the HIV Care Continuum to Reduce HIV-Related Deaths

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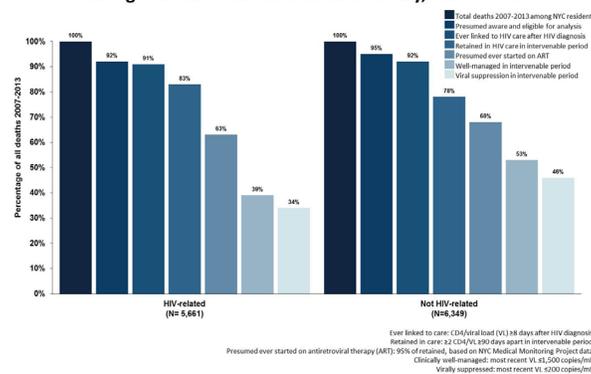
**Background:** With optimal care and treatment, persons with HIV (PWH) can lead healthy lives. However, HIV-related deaths among PWH remain common in New York City (NYC). We developed a novel continuum of HIV care that estimates care outcomes before death to identify opportunities to prevent HIV-related death.

**Methods:** We selected PWH from the NYC HIV Surveillance Registry who died during 2007-2013, resided in NYC at death, and who died  $\geq 15$  months after HIV diagnosis. The primary analysis used CD4 and viral load (VL) tests from surveillance to measure outcomes during the "intervenable period," the period from 15 months to 3 months before death. Outcomes included the proportion of patients who were: ever linked to care; retained in care; presumed to have ever started antiretroviral therapy (ART); clinically well-managed (VL  $\leq 1500$  cc/mL); and virally suppressed (VL  $\leq 200$  cc/mL). We stratified the continuum by underlying cause of death (COD) (HIV-related vs. other), and then the HIV-related continuum by sex, race/ethnicity, age at death, and transmission risk.

**Results:** 11,187 PWH died during 2007-2013 and were eligible for analysis. Overall, 98% (N=11,007) linked after HIV diagnosis; 80% (N=8,992) were retained in care during the intervenable period; 66% (N=7,376) had ever started ART; 47% (N=5,217) were clinically well-managed; and 40% (N=4,518) were virally suppressed (VS). Half (47%) of all deaths were HIV-related (Figure). Retention in care was higher among PWH with HIV-related COD (83% vs. 78%), but VS was substantially lower (34% vs. 46%). The stratified HIV-related continuum revealed disparities in VS. Despite comparable retention rates, whites had higher VS (42%) than blacks (32%) and Hispanics (33%). Retention and VS increased with increasing age at death: 75% of persons  $< 30$  years were retained and 30% had VS, vs. 88% and 56%, respectively, among persons  $\geq 60$ . By risk, men who have sex with men had the lowest retention rate (81%), and persons with a history of injecting drugs had the highest VS (37%).

**Conclusions:** Although retention was high among NYC PWH who died during 2007-2013, VS was low, at nearly half that among persons living with HIV (64% in 2013). High retention coupled with low VS suggests the need to develop strategies to improve VS and address psychosocial and structural barriers to optimal clinical management. The HIV mortality reduction continuum is a novel framework for evaluating pre-death care outcomes among PWH and identifying opportunities for intervention.

HIV Mortality Reduction Continuum by Underlying Cause of Death among Persons with HIV in New York City, 2007-2013



### 995 A Novel Practical Spatial Analysis of HIV Care Outcomes, Metro Atlanta, 2012-2014

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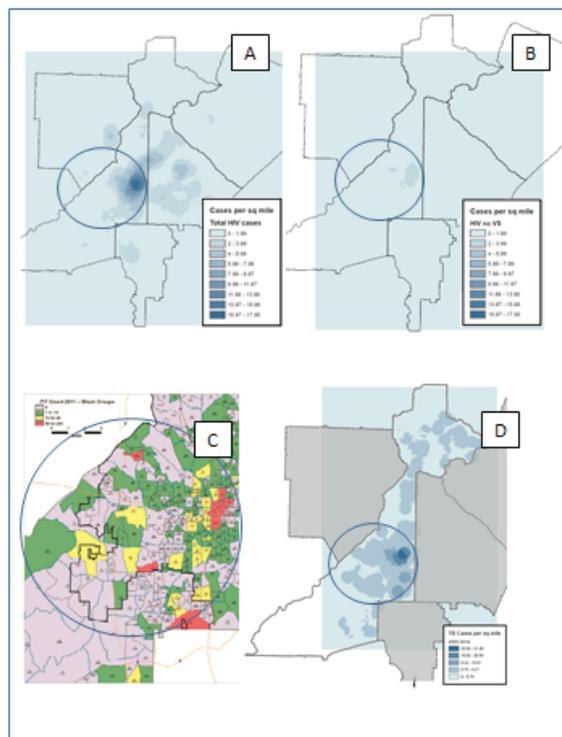
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**Background:** Spatial tools may facilitate strategic resource deployment to address local HIV continuum outcomes (linkage to care, viral suppression) in a targeted way, particularly when cases are visualized as absolute numbers per square mile. We aimed to characterize spatial (1) clustering of newly diagnosed HIV patients in metropolitan Atlanta, (2) clustering of their continuum outcomes with surveillance data collected over an 18-month period, and (3) overlap with tuberculosis (TB) and homelessness.

**Methods:** We analyzed HIV cases diagnosed in 2012 and TB cases diagnosed 2009-2014 reported to Georgia Department of Public Health (GDPH) HIV/AIDS Epidemiology and TB programs, and homeless persons from Atlanta's 2011 homeless count. With GDPH-reported CD4 and HIV viral loads (VL), "poorly linked to care" was defined by absence of CD4 or VL by three months, and "virally unsuppressed" by absence of a VL  $< 200$  copies/mL by 18 months from diagnosis. We evaluated for spatial clusters with K-function. Bernoulli spatial scan was applied to locations of co-infected persons to assess for clustering of excess co-infection ( ArcGIS 10.3.1, SatScan 9.4.2).

**Results:** Among 1,617 new HIV diagnoses, we identified statistically significant spatial clustering of new diagnoses in multiple metro Atlanta counties, smaller clusters of poorly linked individuals in two counties, and a single significant poor viral suppression cluster in a single county. In the single virally unsuppressed cluster, there were 5 cases of virally unsuppressed persons per square mile (vs 0 in other areas). Within this cluster, there were 60-200 homeless persons per census block group (vs <60 outside cluster). From 2009-2014, there were 20-30 cases of active TB per square mile (vs 1-4 cases/square mile outside cluster). We also found significant spatial clustering of excess HIV/TB co-infection, and overlap with homeless service organizations.

**Conclusions:** The local spatial cluster we identified of absolute number of virally unsuppressed persons within a single county serves as a prime target for focused service delivery, potentially with collaboration of geographically proximal service organizations. The spatial overlap with TB disease and with a high density of homeless underscores that control of chronic HIV infection remains inextricably linked with TB and homelessness, and also suggests opportunities may exist to partner with TB and homeless service providers to improve local HIV outcomes.



**Figure. Spatial overlap of HIV, homelessness and TB in Metropolitan Atlanta, GA.** (a) HIV cases diagnosed in 2012 reported to the Georgia Department of Public Health (GDPH) HIV/AIDS Epidemiology Section; (b) Virally unsuppressed HIV cases (diagnosed in 2012 and did not achieve a VL <200 copies/mL by 18 months from diagnosis); (c) Locations of homeless persons identified through Atlanta's 2011 Point-in-Time Homeless Count; (d) Tuberculosis (TB) cases diagnosed 2009-2014 reported to the GDPH TB program.

## 996 Factors Associated With Retention and Engagement in HIV Care (the REACH Survey)

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**Background:** The life expectancy for successfully treated people living with HIV in the UK is now similar to that in the general population but patients who engage poorly with care are at risk of poorer health outcomes and death. Engaging patients in HIV care remains a major challenge with little evidence available on the factors that need to be addressed.

**Methods:** We conducted a cross-sectional survey on experience of care and living with HIV for REACH (Retention and Engagement Across Care services for HIV in the UK). Patients attending seven London HIV clinics (May 2014–August 2015) completed the survey (N=990). We systematically recruited 557 regular attenders (RA: all appointments attended in past year), 269 irregular attenders (IA: one or more missed appointments in past year) and 164 non-attenders (NA: returned to care in past year after absence of a year or more). The sample was stratified to over-represent IA and NA.

**Results:** The median age of patients was 44 years (IQR 37–51). 27.4% were female; 36.8% identified as heterosexual and 57.0% as homosexual; 53.4% were white, 28.1% were black African and 18.5% were from other ethnic groups; 41.0% were born in the UK; and 16.0% had no post-16yrs education.

Women were more likely to be NA (34.0%) or IA (29.3%) than RA (24.6%,  $p=0.05$ ). Older people (>45yrs) were more likely to be RA (49.9%) than IA (37.6%) or NA (36.6%,  $p<0.001$ ). Those who identified as homosexual were more likely to be RA (59.9%) or IA (56.9%) than NA (47.1%) whereas those who identified as heterosexual were more likely to be NA (40.8%) or IA (38.5%) than RA (34.9%,  $p=0.003$ ). NA were more likely to have no post-16yrs education (20.5% vs IA=13.8%, RA=15.8%,  $p=0.02$ ). There were no significant differences ( $p>0.05$ ) in attendance pattern by ethnic group, country of birth, language or relationship status.

Table 1 shows the proportion of IA and NA reporting the listed reasons for ever missing appointments (sometimes or often) at the HIV clinic. IA and NA also reported missing appointments because of drinking alcohol (IA=6.4%, NA=6.5%) and taking drugs (IA=13.2%, NA=12.6%). IA and NA with caring responsibilities sometimes or often missed appointments because of this (IA=41.2%, NA=52.3%).

**Conclusions:** Engagement in HIV care is associated with gender, age, sexual orientation and education. Different patterns of attendance are also associated with multiple underlying causes. Our findings suggest no one-size-fits-all method of improving engagement and support the use of a range of approaches.

**Table 1: Reasons for ever missing an appointment at HIV clinic**

	IA (%age)	NA (%age)	p value
Simply forgot	32.7	31.1	0.73
Felt depressed / overwhelmed	29.4	25.0	0.33
Felt too tired	24.5	23.8	0.86
Felt too sick or ill	24.2	16.5	0.06
Didn't want to think about being HIV positive	19.3	28.0	0.04
Didn't have enough money	17.8	19.5	0.66
Couldn't get time off work	15.6	23.2	0.05
Didn't have transport	14.1	17.1	0.41
Had enough medication	13.4	24.4	0.003
Felt well	13.0	20.7	0.03
Were afraid to be seen at the clinic	10.0	18.9	0.009
Had not followed doctor's advice	10.0	14.6	0.15
Didn't think a doctor could help	6.3	10.4	0.13

**997 Computer-Based Prevention Counseling for HIV-Infected Persons (HPTN 065)**

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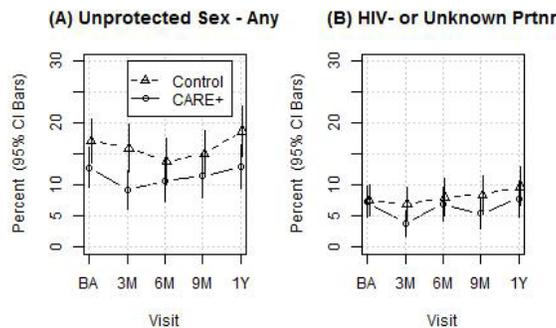
**Background:** HPTN 065 examined the feasibility of an enhanced Test, Link to Care, plus treat (TLC-plus) approach for HIV prevention in the Bronx, NY and Washington, DC. One component of the study evaluated a computer-based prevention counseling intervention (CARE+) for HIV-infected persons in care, to determine its effect on reducing unprotected sex.

**Methods:** HIV patients at 10 study clinics (6 in DC, 4 in NY) were randomized 1:1 to either the intervention of CARE+ plus standard of care (SOC) prevention counseling or to the control arm of ACASI-risk assessment only plus SOC prevention counseling. Participants completed the assigned computer-based session at baseline, 3, 6, 9, and 12 months. Generalized estimating equation models were used to analyze the proportion of participants reporting unprotected vaginal or anal sex the last time they had sex.

**Results:** Of 948 participants who completed at least one follow-up, 643 (68%) were men; 581 (61%) African-American; 190 (20%) Latino; 391 (41%) men who have sex with men; median age was 51 years (range 18-77 years). Eighteen percent (n=173) had less than high school-education and 28% (n=266) had an annual household income under \$10,000. The majority of respondents, 88% (n=834), were on ART. Retention at month 12 was 75% (n=354) in the CARE+ arm and 78% (n=370) in the control arm.

At baseline, 499 (53%) participants reported any sex in the last 3 months (236 [50%] CARE+ participants and 263 [55%] controls). The frequency of reported unprotected sex with any partner did not change over time in CARE+ participants (13% at baseline, 12% at 12 months, odds ratio (OR): 0.995 (95% CI: 0.91, 1.1), p=0.91) (Image 1A). No difference occurred between CARE+ and control participants in unprotected sex over time (OR comparing time trends, control versus CARE+: 1.03 (95% CI: 0.91, 1.2), p=0.67). At baseline, 33/471 (7%) of CARE+ participants reported unprotected sex with an HIV-negative or unknown status partner compared to 35/477 (7%) among control participants. At 12 months, the numbers were 26/354 (7%) among CARE+ and 34/370 (9%) among controls (difference not significant) (Image 1B).

**Conclusions:** The computer-based HIV prevention intervention (CARE+) did not reduce the reported frequency of unprotected sex among these HIV-infected patients in care at participating HPTN 065 study sites. Overall rate of reported unprotected sex was low.



**998 The Swedish HIV Treatment Cascade**

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**Background:**

Access to antiretroviral treatment (ART) has dramatically reduced HIV-related mortality and morbidity, but for viral suppression and good clinical outcome, HIV-infected individuals must fulfill several steps along the HIV care continuum.

It has been estimated that only a minority of HIV-infected individuals in the United States have suppressed HIV-1 RNA levels. The process of achieving virologic suppression proceeds through 5 stages: HIV diagnosis, linkage to care, retention in care, receipt of ART, and virologic suppression. This progression is often called the cascade of care or the HIV care continuum.

**Methods:** All patients in Sweden diagnosed with HIV are included in the InfCare HIV database. We used InfCare HIV data reported through May 2015 to estimate the HIV care continuum for the complete Swedish HIV-infected adult cohort. All adult patients ever diagnosed with HIV and still alive were included.

**Results:** Using HIV surveillance data reported to the Public Health Agency of Sweden it was estimated that 10% of all HIV-infected subjects in Sweden remain undiagnosed. Among 6794 diagnosed patients, >99.9% were linked to care and >99% of those stayed in care. 94.3% were on ART and of those 95.5% had a viral load <50 copies/mL (snapshot analysis). The vast majority of patients with a viral load >50 copies/mL had a so called viral blip and a subsequent viral load <50 copies/mL.

**Conclusions:** Of estimated 8000 HIV-infected subjects in Sweden, 90% were diagnosed with HIV, and 80% were on suppressive ART with a viral load <50 copies/mL.

**999 Antiretroviral Treatment Among Commercially Insured Persons With HIV in the United States**

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**Background:** Antiretroviral treatment (ART) is now recommended for all HIV-infected persons in the United States. This analysis describes antiretroviral use among commercially insured HIV-infected adults from a large administrative claims database.

**Methods:** We analyzed 2013 Truven Health MarketScan Commercial Claims and Encounters<sup>®</sup>, a large database derived from administrative claims for healthcare services provided to commercial health plan enrollees. Among enrolled persons aged 18–64 years, we identified HIV-infected persons who had at least one inpatient or outpatient medical claim with an ICD–9–CM diagnosis code for HIV infection. We linked and examined their outpatient pharmacy claims for antiretroviral use using National Drug Codes. We compared persons prescribed antiretrovirals with those who did not have prescribed antiretrovirals to identify differences by age, sex, geographic region, and type of insurance. Chi-square and t-test were done for statistical significance.

**Results:** In 2013, there were a total of 43,737,217 persons in this commercial insurance database, including 62,185 (0.14%) HIV-infected adults. Overall, 38,939 (63%) HIV-infected adults were prescribed antiretrovirals and 23,246 (37%) were not prescribed any antiretrovirals. There were significant overall differences (p-value <0.05) in age, gender, residence, geography and health insurance plan among HIV-infected adults prescribed and not prescribed antiretrovirals (Table). Notably, higher proportion of persons who were not prescribed antiretrovirals were female (28.5% vs 15.8%) and at ages 18–34 (27.1% vs 16.5%) compared with those who were prescribed antiretrovirals.

**Conclusions:** Despite having commercial insurance, a significant proportion of HIV-infected adults were not prescribed ART. Insurance-based strategies such as electronic reports to physicians regarding patients not receiving ART could be important novel methods to increase the percentage of HIV-infected adults who receive optimal care in the United States.

Table 1. Antiretroviral treatment of commercially insured persons aged 18–64 years, MarketScan 2013

Characteristic	No antiretrovirals prescribed	Antiretrovirals prescribed	p-value
<b>Age group (years)</b>			
18–34	6,307 (27.1%)	6,444 (16.6%)	<0.001
35–44	5,447 (23.4%)	9,624 (24.7%)	
45–54	7,341 (31.6%)	15,180 (39.0%)	
55–64	4,151 (17.9%)	7,091 (19.8%)	
<b>Sex</b>			
Male	16,623 (71.5%)	32,771 (84.2%)	<0.001
Female	6,623 (28.5%)	6,168 (15.8%)	
<b>Residence</b>			
Urban	21,577 (92.8%)	36,620 (94.0%)	<0.001
Rural	1,114 (4.8%)	1,571 (4.0%)	
Missing	556 (2.4%)	748 (1.9%)	
<b>Geographic region</b>			
Northeast	7,922 (34.1%)	8,368 (21.5%)	<0.001
North Central	2,612 (11.2%)	4,841 (11.9%)	
South	7,864 (33.8%)	16,533 (42.6%)	
West	4,276 (18.4%)	8,640 (22.2%)	
Unknown	572 (2.5%)	757 (1.9%)	
<b>Health plan type</b>			
FFS	1,575 (6.8%)	1,056 (2.8%)	<0.001
HMO	3,279 (14.1%)	7,768 (19.9%)	
POS	2,131 (9.2%)	3,605 (9.3%)	
PPO	14,326 (61.6%)	22,022 (56.6%)	
Other	1,605 (6.9%)	3,756 (9.6%)	
Missing	330 (1.4%)	693 (1.8%)	

**1000 Increases in Health Insurance Coverage Among MSM: 20 US Cities, 2008–2014**

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**Background:** The Affordable Care Act (ACA), passed in 2010, is intended to improve access to health insurance coverage for all Americans, including those at risk for or living with HIV; the ACA includes provisions for prevention and treatment of HIV, which can result in improved health outcomes. CDC’s National HIV Behavioral Surveillance (NHBS) monitors health behaviors in populations at risk for HIV, including gay, bisexual, and other men who have sex with men (MSM). We assessed changes in reported health insurance coverage among MSM participating in NHBS in 2008, 2011, and 2014.

**Methods:** We analyzed NHBS data from sexually active MSM aged 18 years or older who were recruited and interviewed at venues in 20 U.S. cities. We compared the percentages reporting health insurance coverage in 2008, 2011, and 2014, using chi-square tests. To determine if interview year was associated with health insurance status, we used a Poisson model with robust standard errors to calculate adjusted prevalence ratios (aPRs) and 95% confidence intervals (CIs). The model was adjusted for demographic characteristics and self-reported HIV status; we included individual interaction terms for each covariate by interview year.

**Results:** Among included MSM (2008: 8,903; 2011: 9,256; 2014: 9,640), the percentage with health insurance coverage increased from 68% in 2008 to 79% in 2014 (aPR 1.14 [2014 vs 2008], CI: 1.12, 1.16, P value for trend including all three years < 0.001). By age, the increase in health insurance coverage was significant for all groups but was higher among MSM aged 18–29 (aPR 1.17, CI: 1.14–1.21) and 30–39 years (aPR 1.16, CI: 1.12–1.20). By education, the increase was greatest for MSM with no more than a high school education (aPR 1.28, CI: 1.22, 1.35). By income, the increase was greatest for MSM reporting an annual income less than \$20,000 (aPR 1.33, CI: 1.26, 1.39). Health insurance coverage increased regardless of self-reported HIV status (Table).

**Conclusions:** Corresponding with the passage of the ACA, health insurance coverage increased among MSM participating in NHBS from 2008 to 2014. Increases were greatest in key demographic segments with historically lower health insurance coverage. While causality cannot be established using NHBS data, these findings are encouraging. As health insurance coverage increases among MSM, it will be important to also monitor progress in HIV prevention and treatment outcomes while assessing for any continued barriers to care.

Table: Health Insurance Status among Men Who Have Sex With Men—National HIV Behavioral Surveillance System, 20 U.S. Cities, 2008, 2011, 2014

	2008		2011		2014		aPR <sup>b</sup>	95% CI	P-value
	No. insured <sup>a</sup>	(%)	No. insured <sup>a</sup>	(%)	No. insured <sup>a</sup>	(%)			
<b>Race or ethnicity</b>									
Black or African American	1354	(63)	1682	(68)	1975	(75)	1.15	(1.10,1.20)	<0.001
Hispanic or Latino <sup>c</sup>	1370	(61)	1468	(61)	1860	(74)	1.15	(1.10,1.20)	<0.001
White	2857	(74)	2817	(77)	3082	(84)	1.11	(1.09,1.14)	<0.001
Other or multiple races	457	(69)	491	(73)	607	(82)	1.19	(1.12,1.26)	<0.001
<b>Age group (years)</b>									
18–29	2271	(62)	2621	(64)	2997	(74)	1.17	(1.14,1.21)	<0.001
30–39	1635	(66)	1532	(70)	1947	(78)	1.16	(1.12,1.20)	<0.001
40–49	1421	(74)	1424	(76)	1386	(82)	1.09	(1.05,1.13)	<0.001
≥ 50	716	(83)	895	(82)	1234	(89)	1.07	(1.03,1.10)	<0.001
<b>Education</b>									
≤ High school	1345	(51)	1527	(56)	1638	(66)	1.28	(1.22,1.35)	<0.001
> High school	4698	(75)	4945	(76)	5926	(83)	1.10	(1.08,1.12)	<0.001
<b>Annual household income</b>									
≤ \$19,999	1297	(49)	1596	(55)	1961	(67)	1.33	(1.26,1.39)	<0.001
\$20,000–\$39,999	1387	(62)	1496	(66)	1708	(75)	1.17	(1.12,1.21)	<0.001
\$40,000–\$74,999	1739	(80)	1760	(80)	2007	(86)	1.06	(1.03,1.09)	<0.001
≥ \$75,000	1528	(88)	1530	(88)	1795	(91)	1.04	(1.02,1.06)	0.001
<b>Self-reported HIV status</b>									
HIV-negative	4612	(68)	4954	(70)	5776	(78)	1.13	(1.11,1.16)	<0.001
HIV-positive	802	(76)	987	(79)	1351	(85)	1.14	(1.10,1.19)	<0.001
Unknown	599	(58)	515	(60)	420	(70)	1.17	(1.09,1.26)	<0.001
Total	6043	(68)	6472	(70)	7564	(79)	1.14	(1.12,1.16)	<0.001

aPR = Adjusted Prevalence Ratio, CI = Confidence Interval

<sup>a</sup>Includes public, private, other, and multiple insurances

<sup>b</sup>Overall model is adjusted for race or ethnicity, age group, education, income, HIV status, and city. Model for each covariate is adjusted for race or ethnicity, age group, education, income, HIV status, city, and an interaction term for that individual covariate by year (2014 vs 2008).

<sup>c</sup>Hispanics or Latinos can be of any race.

**1001 Reliance on Ryan White Coverage for Provider Visits Following the Affordable Care Act**

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**Background:** Prior to 2014, over 100,000 low-income, uninsured adults living with HIV relied on a safety net of Ryan White Program support, local charities, or uncompensated care (collectively, "RWP/Uncomp") to cover visits to HIV providers. The Affordable Care Act (ACA) may have reduced reliance on RWP/Uncomp through shifts into Medicaid or private health insurance coverage. We compared coverage for provider visits before and after the ACA (2011-2013 vs. 2014) in 10 geographically diverse clinics stratified by state Medicaid expansion status.

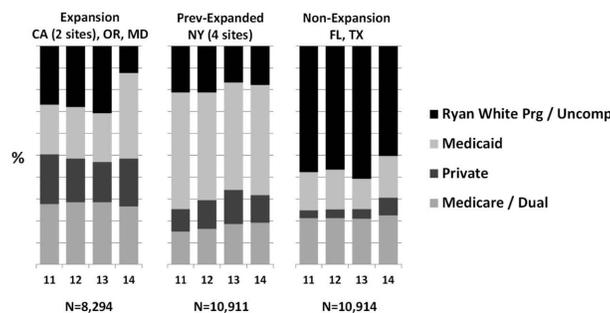
**Methods:** Analyses included all patients engaged in care at 4 sites in Medicaid expansion states (CA [2 sites], OR, MD), 4 sites in a state (NY) that expanded Medicaid in 2001, and 2 sites in non-expansion states (TX, FL). Visit coverage was classified as RWP/Uncomp, Medicaid, private insurance, or Medicare. Multinomial logistic models were used to examine changes in coverage patterns using Medicare as a referent and adjusting for age, race, gender, HIV risk factor, and site of care.

**Results:** In total, 30,121 patients contributed 80,770 person years (PY). The cohort was 76% male, 44% Black, 26% Hispanic, 27% White, 47% MSM, 12% IDU, and had a median age of 45 (IQR 35-52) years in 2011. RWP coverage constituted 86% of RWP/Uncomp across the 4 year interval.

Clinics in states with Medicaid expansion experienced a decrease in RWP/Uncomp from 29% of PY during 2011-2013 to 12% in 2014, adjusted relative risk ratio (ARRR) 0.41 (0.38, 0.45); Medicaid coverage increased from 23% to 39%, ARRR 2.07 (1.94, 2.21), and private coverage increased modestly from 20% to 22%, ARRR 1.20 (1.12, 1.29) (Figure). In NY sites, RWP/Uncomp decreased slightly (20% to 18%, ARRR 0.88 [0.83, 0.94]). In non-expansion sites, RWP/Uncomp was the dominant form of coverage and decreased from 58% to 50%, ARR 0.82 [0.78, 0.86]), with increases in both Medicaid (16% to 19%, ARRR 1.14 [1.07, 1.21]) and private (4% to 8%, ARRR 1.92 [1.73, 2.12]).

**Conclusions:** In expansion state sites in 2014, shifts from RWP/Uncomp to Medicaid were substantial. In NY sites and in non-expansion state sites, decreases in RWP/Uncomp were relatively small. Overall, many PLWH in all sites continued to rely entirely on RWP/Uncomp for provider visits, with the greatest reliance occurring in non-expansion state sites. The ACA has not eliminated the need for the RWP's safety net provider visit coverage.

Figure. Coverage for HIV Provider Visits 2011-2014 by Medicaid Expansion Category



**1002 Receipt of Ryan White Care Services Is Associated With Improved Long-Term Outcomes**

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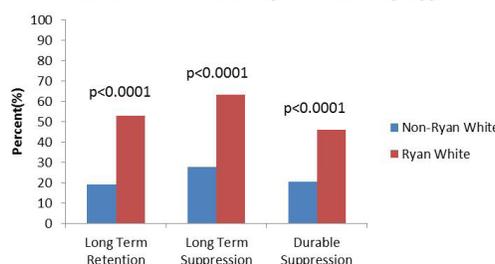
**Background:** The HIV care continuum is an effective framework for improving the care of persons living with HIV (PLWH). We sought to examine how utilization of Ryan White (RW) care, medical case management (MCM) and other supportive services predicted long-term retention in care, viral suppression (VS), and durable VS.

**Methods:** Data were used from the electronic HIV/AIDS Reporting System (eHARS) from 2010-2014 for Philadelphia residents 18+ years old who had at least 1 viral load (VL) or CD4 completed in 2009. Retention was defined as 1 lab in each 6 months of the year with at least 60 days between the two. VS was defined as evidence of 1 VL/year and the last VL of each year  $\leq 200$  copies/mL. Durable VS was defined as evidence of 1 VL/year and all VLs  $\leq 200$  copies/mL. eHARS data was linked with RW service utilization data. Univariate and stepwise logistic regression determined predictors for long term retention and VS, and durable VS. Models were adjusted for RW care status, race, sex, gender, age, risk, AIDS status, RW ambulatory care, MCM, and average number of RW contacts and services/year. Insurance status and FPL were also included in a sub-analysis.

**Results:** 8,375 PLWH were included, 3,410 of which received care at a RW funded facility and 4,965 who did not. Those who received RW care were more likely to be retained for all years (OR: 3.40; CI: 2.87-4.04), suppressed at the end of each year (OR: 3.77; CI: 3.36-4.23) and durably suppressed (OR: 2.92; CI: 2.45-3.47) compared to those who did not receive RW care. Those who received MCM during each year of follow-up were more likely to be retained each year (OR: 1.70; CI: 1.44-2.01) but significantly less likely to be suppressed each year (OR: 0.72; CI: 0.61-0.85) or durably suppressed (OR: 0.58; CI: 0.50-0.67). Multivariate logistic regression among those who received RW care showed that individuals with an average of 20+ contacts a year were more likely to be retained in care each year (OR: 3.26; CI: 2.35-4.52), but less likely to be durably suppressed (OR: 0.68; CI: 0.48-0.88). Poverty level was a significant predictor of VS and durable VS, but not retention in care.

**Conclusions:** Utilization of the RW system is a significant predictor for long term retention in care, VS, and durable VS. Use of multiple RW services or increased contact with the RW system may be a marker for greater social and medical complexity leading to difficulty maintaining VS. Data will inform improvements to the system of care and drive further evaluation of supportive services on outcomes.

Treatment Measures by Care Facility Type



### 1003 The Causal Impact of ART Initiation on Household Food Security

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**Background:** There are several plausible mechanisms from ART to household welfare, and the direction of net effects is unclear: on the one hand, patients incur costs when utilizing ART (e.g., for travel, even where ART is free of charge); on the other hand, patients recover health and employment on ART. This study examines the impact of ART on one aspect of household welfare – household food security.

**Methods:** We use routinely collected longitudinal data collected by the Africa Centre for Health and Population Studies and employ a regression discontinuity design over 2300 observations (collected between 2004 and 2012), to assess the causal impact of ART on three food security outcomes: probability of an adult in the household missing any food for financial reasons, probability of an adult in the household missing a meal for financial reasons, and probability of a child in the household missing a meal for financial reasons.

**Results:** Our results show that for each outcome ART causes a significant increase in the probability of food insecurity in the year following ART initiation, which diminishes to 0 between 2 and 4 years after ART initiation, depending on the outcome examined. In the first year after initiation, ART initiation yielded a significant increase in the probability of an adult in the household missing food by 5.5% (coefficient = 0.055, 95% CI = [0.0190, 0.0904]), a significant increase in the probability of an adult in the household missing a meal by 6.5% (coefficient = 0.065, 95% CI = [0.0156, 0.1147]), and a significant increase in the probability of a child in the household missing a meal by 4.6% (coefficient = 0.046, 95% CI = [0.0036, 0.0892]). The upper bound on these causal estimates is an approximately 10% increase in household food insecurity as a result of ART initiation, with the effect size in all scenarios diminishing to zero within 3 years after initiation.

**Conclusions:** ART initially places a significant burden on household food security; however, this effect disappears over time. It is likely that the financial burden of utilizing ART, which are high relative to income in this community, initially outweigh the longer-term beneficial ART effects on employment and income. Food and financial support programs should be considered to alleviate the temporary loss in food security following ART initiation, especially in the context of the expanding ART rollout and treatment-as-prevention strategies.

### 1004 Increased STD Testing Among HIV-Infected Adults in Care United States, 2009-2013

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**Background:** STD prevention services are important for HIV prevention efforts because STDs can be a marker for condomless sex and increase HIV viral load and genital shedding, which can increase HIV transmission. Current guidelines recommend that all sexually active HIV-infected persons be tested at least annually for *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (GC), and syphilis. However, little is known about temporal trends in STD testing among HIV-infected adults in the United States. We examined CT, GC, and syphilis testing trends among HIV-infected adults receiving medical care in the United States from 2009 to 2013.

**Methods:** The Medical Monitoring Project (MMP) is a surveillance system designed to produce nationally representative behavioral and clinical estimates for HIV-infected adults receiving medical care in the United States. Using weighted MMP data collected from 22,125 persons from the project's 2009 to 2013 data collection cycles, we analyzed medical record data to examine testing for CT, GC, and syphilis by year and stratified by sexual behavior and race/ethnicity.

**Results:** From 2009 to 2013, the proportion of sexually active HIV-infected adults tested for all three STDs in the year preceding interview increased from 20% to 36% ( $\beta=0.05$ ,  $P$  for trend  $< 0.01$ ); however, the majority of sexually active adults (64%) were not tested for all three STDs in the most recent year. Testing for all three STDs was highest among men who have sex with men (MSM) (29% to 39%,  $\beta=0.04$ ,  $P$  for trend  $< 0.01$ ), men who have sex with women (25% to 33%,  $\beta=0.04$ ,  $P$  for trend  $< 0.01$ ), and women who have sex with men (26% to 35%,  $\beta=0.03$ ,  $P$  for trend  $< 0.01$ ) (figure 1a). Statistically significant increases were also found for all race/ethnicity groups: non-Hispanic whites (23% to 30%,  $\beta=0.04$ ,  $P$  for trend  $< 0.01$ ), non-Hispanic blacks (28% to 35%,  $\beta=0.04$ ,  $P$  for trend  $< 0.01$ ) and Hispanics (34% to 47%,  $\beta=0.05$ ,  $P$  for trend  $< 0.01$ ) (figure 1b).

**Conclusions:** Testing for STDs among sexually active HIV-infected adults receiving medical care in the United States significantly increased from 2009 to 2013. While this increase indicates progress, testing for all STDs remains far below recommended guidelines. Improvements in testing were seen across sexual behavior and race/ethnicity groups; testing was highest among MSM and among Hispanics. Our findings suggest that enhanced efforts may be warranted to screen all HIV-infected sexually active adults for CT, GC, and syphilis.

Figure 1a

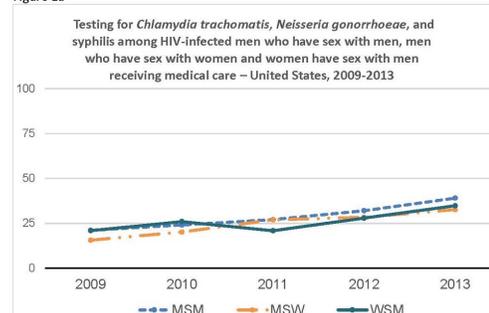
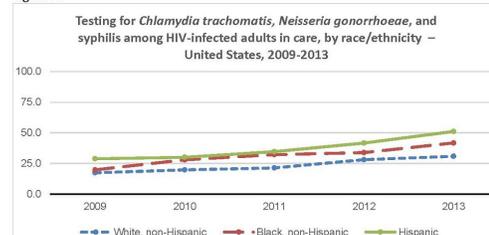


Figure 1b



•**Chlamydia trachomatis testing:** Defined as documentation in the medical record, during the 12 months before the interview, of a result from culture, direct fluorescent antibody (DFA), enzyme immunoassay (EIA) or enzyme-linked immuno-assay (ELISA), NAAT, or nucleic acid probe. •**Neisseria gonorrhoeae testing:** Defined as documentation in the medical record, during the 12 months before the interview, of a result from culture, gram stain, nucleic acid amplification test (NAAT), or nucleic acid probe. •**Syphilis testing:** Defined as documentation in the medical record, during the 12 months before the interview, of a result from non-treponemal syphilis tests (rapid plasma reagin [RPR], Venereal Disease Research Laboratory [VDRL]), treponemal syphilis tests (Treponema pallidum hemagglutination assay [TPHA], T. pallidum particle agglutination [TP-PA], microhemagglutination for antibody to T. pallidum [MHA-TP], fluorescent treponemal antibody absorption [FTA-ABS] tests), or dark-field microscopy.

**1005 Increased Gonorrhea and Chlamydia Case Detection in a Multisite US HIV Cohort**

**Julia Goldberg Raifman**<sup>1</sup>; Anne K. Monroe<sup>2</sup>; Kelly A. Gebo<sup>3</sup>; Khalil Ghanem<sup>4</sup>; Allison L. Agwu<sup>3</sup>; Todd Korthuis<sup>5</sup>; Wm. Christopher Mathews<sup>6</sup>; Aditya Gaur<sup>7</sup>; Stephen A. Berry<sup>3</sup>; for the HIV Research Network

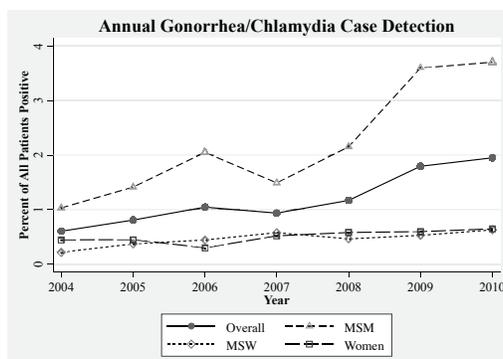
<sup>1</sup>Johns Hopkins Bloomberg Sch of PH, Baltimore, MD, USA; <sup>2</sup>Johns Hopkins Univ Sch of Med, Baltimore, MD, USA; <sup>3</sup>Johns Hopkins Univ, Baltimore, MD, USA; <sup>4</sup>Johns Hopkins Bayview Med Cntr, Baltimore, MD, USA; <sup>5</sup>Oregon Hlth & Sci Univ, Portland, OR, USA; <sup>6</sup>Univ of California San Diego, San Diego, CA, USA; <sup>7</sup>St Jude Children's Rsr Hosp, Memphis, TN, USA

**Background:** U.S. guidelines recommend annual Gonorrhea (NG) and Chlamydia (CT) screening for persons living with HIV. Detecting these diseases may decrease HIV transmission by leading to sexual risk counseling and treatment. In some HIV clinics in the U.S. and Canada, increased NG/CT screening has not increased case detection, prompting consideration of more targeted screening approaches. During 2004-2010, annual NG/CT testing increased steadily from 22% to 39% of patients in the HIV Research Network multisite U.S. clinical cohort. We evaluated, 1) trends in the proportion of patients who tested positive among those tested each year (test positivity), and 2) trends in the proportion of patients positive among all patients in care, whether tested or not (case detection).

**Methods:** Seven adult clinical sites had NG/CT test result data. We included all patients with at least one calendar year of active clinical follow-up and restricted the analysis to person-years (PY) of active follow-up. We tested for linear trends in test positivity and case detection using generalized estimating equations and adjusting for clustering within person. We adjusted for age, race, injection drug use, CD4 count, number of HIV provider visits, viral load, and HIVRN clinical site.

**Results:** Of 19,368 patients, 40% were men who have sex with men (MSM), 31% were men who have sex with women (MSW), and 29% were female. The median age in the first year of inclusion was 42 years. During 68,458 PY of follow-up, NG/CT tests were done in 21,561 PY. As testing increased, test positivity increased from 2.7% in 2004 to 4.9% in 2010, adjusted odds ratio (AOR) per year 1.13 (1.09-1.18). Case detection increased from 0.6% in 2004 to 2.0% in 2010 (Figure), AOR per year 1.28 (1.23-1.33). Case detection increased among all sexual risk groups, with the greatest increase among MSM (AOR per year 1.31, 1.25-1.38), followed by MSW (1.18, 1.06-1.31), and by women (1.17, 1.05-1.29). For two clinical sites with available data to distinguish body site of testing, test positivity was greater for the rectal (7.9%) than the genital site (4.0%).

**Conclusions:** As NG/CT testing increased, the test positivity also increased, yielding an overall improvement in case detection in this large cohort. Test positivity may have increased because providers were more successfully targeting higher risk patients and/or body sites. Increasing the testing rate above 39% may further improve case detection. There is a need to further promote NG/CT screening in HIV clinics.



**1006 Gonorrhea and Chlamydia Testing in US HIV-Infected Men Who Have Sex With Men**

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**Background:** Testing and treating HIV-infected patients for *Neisseria gonorrhoeae* (GC) and *Chlamydia trachomatis* (CT) infection benefits their health and has been shown to reduce HIV transmission risk in some cases. Men who have sex with men (MSM) are disproportionately affected by GC and CT infection and (per US guidelines), if HIV-infected, should be screened for them at least annually. GC and CT infections in MSM occur most often in the anorectum or pharynx. Extragenital testing substantially improves diagnostic yield, yet rates of extragenital GC and CT testing have been low historically.

**Methods:** We used 2009-2012 data from the Medical Monitoring Project (MMP) to estimate proportions of sexually active MSM who were tested in the previous 12 months for GC and CT infection in urine and anorectal and pharyngeal sites. We estimated the positivity for each test type; patients could be tested at multiple sites, precluding statistical comparisons. MMP is a US surveillance system producing nationally representative estimates about HIV-infected adults in medical care. GC and CT testing data were abstracted from medical records. Sexual behavior data were self-reported in interviews.

**Results:** Of 6,079 HIV-infected MSM, 29.9% (95% CI: 26.8 – 33.0) were tested for GC and 30.1% (95% CI: 27.0 – 33.2), for CT; positivity was 5.3% (95% CI: 4.2 – 6.4) for GC and 5.7% (95% CI: 4.6 – 6.8) for CT. Approximately 8.5% were tested for GC or CT with no site specified. For GC, lower proportions of MSM were tested at anorectal (3.5%) and pharyngeal (2.8%) sites than in urine (17.6%); however, positivity was higher among anorectal (9.9%) and pharyngeal (8.4%) samples than among urine samples tested (3.8%). For CT, lower proportions of MSM were also tested at anorectal (3.6%) and pharyngeal (2.2%) sites than in urine (18.0%), and positivity was higher for anorectal samples (11.6%) than for urine samples (4.5%), but similar for pharyngeal samples (5.9%) and urine samples.

**Conclusions:** Less than one-third of sexually active MSM in HIV medical care in the US were tested for GC and CT in the previous 12 months, per current recommendations. Fewer than 5% of patients were tested at extra-genital sites, and GC and CT test positivity was more than twice as high for anorectal compared with urine tests. Providers should perform routine extragenital screening for GC and CT among sexually active HIV-infected MSM to diagnose treatable infections and decrease HIV transmission risk.

Table 1. Gonorrhea and chlamydia testing by anatomical site among sexually active HIV-infected men who have sex with men, 2009-2012 Medical Monitoring Project (N=6079)

	N	Gonorrhea (GC)		Chlamydia (CT)	
		% <sup>a</sup>	95% CI <sup>a</sup>	% <sup>a</sup>	95% CI <sup>a</sup>
Total tested, any test	1929	29.9	26.8 – 33.0	30.1	27.0 – 33.2
Diagnosed, any GC or CT	97	5.3	4.2 – 6.4	5.7	4.6 – 6.8
Tested with no site specified	549	8.7	7.1 – 10.3	8.5	7.0 – 10.1
Urine tested	1116	17.6	15.2 – 20.1	18.0	15.5 – 20.5
Diagnosed	39	3.8	2.5 – 5.1	4.5	3.0 – 5.9
Anorectal tested	262	3.5	2.5 – 4.5	3.6	2.6 – 4.7
Diagnosed	23	9.9	5.8 – 14.0	11.6	6.9 – 16.3
Pharyngeal tested	211	2.8	1.8 – 3.9	2.2	1.6 – 2.9
Diagnosed	18	8.4	4.9 – 11.8	5.9	2.6 – 9.3

<sup>a</sup>Percentages and 95% CIs are weighted to account for unequal selection probabilities and non-response

**1007 Incident Syphilis, Gonorrhea, and Chlamydia Infection Among a Cohort of MSM**

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**Background:** Implementing HIV treatment as prevention (TasP) may potentially influence risk behavior among key groups such as men who have sex with men (MSM). We measured the incidence and determinants of chlamydia (CT), gonorrhea (GC) and syphilis infection within a prospective cohort of MSM in Vancouver, Canada where TasP has been policy since 2010.

**Methods:** Eligible participants were recruited from 2012-2015, aged  $\geq 16$  years of age and reported recent sex with another man. Participants completed study visits every 6 months, which included a computer-assisted self-interview on demographics, sexual behavior and substance-use, and a nurse-administered clinical questionnaire. A rapid HIV test was administered and venous blood sample taken for serology for syphilis. Urine NAAT tests and/or pharyngeal, rectal or urethral swabs for chlamydia and gonorrhea were offered as an optional service. We used generalized estimating equations to identify factors associated with any incident STI infection, either diagnosed through a study visit or reported between study visits by the participant. A multivariable model was built using backward selection, Type III p-values ( $p < 0.05$ ), and QIC minimization.

**Results:** Of 575 MSM (29.4% HIV-positive and 70.6% HIV-negative at enrollment) with follow-up, 134 (23.3%) had an incident STI. Prior STI diagnosis was strongly associated with an incident STI (relative risk [RR]: 25.07, 95% CI: 19.03-33.03,  $p < 0.001$ ). During a median of 1.98 person-years of follow-up 77 chlamydia, 69 gonorrhea, and 37 syphilis cases were reported/diagnosed, for an incidence rate of 7.14 per 100 person-years (PYRs) for CT, 6.40 per 100 PYRs for CT and 3.43 per 100 PYRs for syphilis. Any STI incidence did not differ by HIV status ( $p = 0.85$ ). Factors independently associated with incident STI were younger age (adjusted RR [aRR]=0.98 per year older; 95% CI: 0.96-0.99), group sex event attendance (aRR=1.49, 95% CI: 1.08-2.07), anal sex with casual partners (aRR=2.78, 95% CI: 1.87-4.14), poppers use (aRR=1.61, 95% CI: 1.16-2.22), and injection drug use behavior (aRR=1.89, 95% CI: 1.20-2.97). Further, MSM who reported only having condomless anal sex with HIV-positive men on treatment or with low viral loads were more likely to have an incident STI infection (aRR=1.40, 95% CI: 0.98-1.98).

**Conclusions:** STI incidence and re-infection was common. This may be a partial concomitant effect of TasP scale-up, and may reduce some of its benefits without an additional focus on primary prevention of other STIs.

**1008 The Importance of Linkage and Engagement of Care in Post HIV STI Acquisition**

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**Background:** It is well documented that HIV/sexually transmitted infections (STI) coinfections increase the risk of transmitting HIV. STI infections post HIV diagnosis can also serve as a proxy of ongoing high risk sexual behaviors. This analysis sought to quantify the prevalence of and timing of HIV/STI infections and assess the association of linkage and engagement in care on STI acquisition post HIV diagnosis.

**Methods:** HIV diagnoses reported to the District of Columbia Department of Health (DOH) surveillance system between 2007 and 2013 were matched to new chlamydia, gonorrhea and primary/secondary syphilis infections reported to the DOH during the same time period. Coinfection was defined as an STI that occurred 3 months after HIV diagnosis; monoinfected HIV cases had not had an STI reported to the DOH. Demographics, linkage to care within 3 months, engagement in care ( $> 2$  visits per year, 30 days apart) and the interval between HIV infection and first STI were calculated. Descriptive analysis of the covariates and Cox regression were used to describe the risk of STI acquisition after HIV infection.

**Results:** Among the 6,719 HIV cases identified between 2007 and 2013; 411 (6%) individuals had new STI coinfections after their HIV diagnosis and 5,236 were monoinfected with HIV. Median time to first STI infection was 2 years after HIV diagnosis. Of the 411 coinfecting persons, 88.3% were male, 66.9% were Black, mean age at HIV diagnosis was 31 yrs., and 63.5% were HIV infected through MSM. Individuals had a mean of 1.5 STIs (range, 1-11) after HIV diagnosis and the most common coinfection was Chlamydia (42%). The adjusted regression model found that individuals ages 15-19 at HIV diagnosis had more than 7 times the risk of STI acquisition (aOR 7.2: 95%CI 4.8-10.9). Persons never linked to care had  $> 6$  times the risk of an STI coinfection (aOR 6.6: 95%CI 4.9-8.9), with a median time to STI acquisition of 2 years. Those not engaged in care had  $> 2$  times the risk of contracting an STI (aOR 2.6: 95%CI 1.7-4.0), with a median time to STI acquisition of 1.35 years.

**Conclusions:** Persons never linked to care, those poorly engaged in HIV care, and younger persons had a higher risk for STI acquisition after HIV diagnosis, indicative of ongoing high-risk sexual behaviors. These data further support the importance of linkage and engagement in care among HIV-infected persons and emphasize the need for continued education regarding safe sex and secondary prevention.

**1009 Evidence of HIV Care Following STD Clinic Visits by Out-of-Care HIV-Positive Persons**

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**Background:** In 2012, over 1,200 HIV-positive patients accessed services at New York City's (NYC) nine sexually transmitted disease (STD) clinics. Counseling, linkage, and social services are offered by medical providers and public health advisors at these facilities to help patients in need of HIV care services engage (or re-engage) in HIV primary care.

**Methods:** We matched data from the STD clinic electronic medical record and the NYC HIV/AIDS Surveillance Registry for HIV-positive persons who sought services at NYC STD clinics in 2012. We identified patients who were out of HIV care ( $< 2$  viral load [VL] or CD4 results) in the 365 days preceding their STD clinic visit and looked for subsequent evidence of HIV care ( $\geq 1$  VL or CD4 result) within 3 months after the STD clinic visit. We compared patient characteristics (demographics, receipt of an HIV test on day of clinic visit, sexual risk behaviors, and STD diagnoses) among those with and without evidence of HIV care within 3 months after the STD clinic visit.

**Results:** Among 378 out-of-care patients, 164 (43%) had evidence of HIV care during the 3 months after the STD clinic visit. Within 12 months, 99% (162/164) of these patients had a VL recorded (with 69% virally suppressed); of 214 patients who did not have evidence of HIV care during the 3 months after the STD clinic visit, 42% (90/214) had a VL within 12 months and 50% were virally suppressed ( $p < 0.01$  for VL report). Evidence of HIV care was more common among those who received an HIV test during their STD clinic visit (largely persons originally reported to the HIV Registry by a non-STD clinic provider in the year prior) than among those who did not receive an HIV test (66% vs. 40%,  $p < 0.01$ ). Evidence of HIV care following the STD clinic visit was also more common among Hispanic than non-Hispanic patients (51% vs. 41%,  $p = 0.08$ ) and women vs. men (67% vs 42%,  $p = 0.02$ ). Lack of evidence of HIV care did not differ significantly among persons reporting  $\geq 5$  sexual partners in 3 months prior to visit compared to those with  $< 5$  partners (69% vs. 57%,  $p = 0.10$ ), but was more common among patients diagnosed with gonorrhea on the day of the clinic visit than those not diagnosed (69% vs. 54%,  $p = 0.03$ ).

**Conclusions:** STD clinic visits provide an opportunity to link or re-link patients who may be unengaged in HIV care. Targeting patients less likely to access HIV care in the months after their visit may help reduce disparities in subsequent VL suppression and onward transmission.

**1010 Successful Implementation of Extended ART Initiation Criteria in Rural South Africa**

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<sup>1</sup>Médecins Sans Frontières, South Africa & Lesotho, Cape Town, South Africa; <sup>2</sup>Médecins Sans Frontières, Cape Town, South Africa; <sup>3</sup>Médecins Sans Frontières, South Africa & Lesotho, Eshowe, South Africa; <sup>4</sup>Médecins Sans Frontières, Southern Africa Med Unit, Cape Town, South Africa; <sup>5</sup>Médecins Sans Frontières, Roma, Lesotho

**Background:** There is concern that earlier initiation of antiretroviral treatment (ART) in lower resource settings may compromise access to care for patients with lower CD4 counts, and that patients with higher CD4 counts may have lower retention in care (RIC). In July 2014, we extended ART initiation criteria from CD4 cell counts of  $\leq 350$  to  $\leq 500$  copies/ $\mu$ l in

9 primary health clinics in KwaZulu-Natal, South Africa. Here we assess whether any compensatory reduction in initiation of sicker patients was seen and whether retention among those newly eligible was satisfactory in this public sector setting.

**Methods:** In this retrospective cohort analysis we compare proportions initiated on ART and RIC at 6 months among pre-ART patients with baseline CD4 taken between July 1 and December 31, 2014 (CD4≤500 cohort) and between July 1 and December 31, 2013 (CD4≤350 cohort). Pregnancy, TB, age <15 years and WHO stage 3 or 4 were exclusion criteria. Outcomes were determined from time of baseline CD4 and analysed using survival analysis.

**Results:** There were 1,932 patients in the CD4≤350 and 1,657 in the CD4≤500 cohorts. In both cohorts median age was 29 years and 71% were female. Mean baseline CD4 counts were 398 (95%CI: 389-410) and 410 (95%CI: 397-422) in the CD4≤350- and CD4≤500 cohorts. Among participants with CD4 351-500, percentage initiated on ART within 3 months increased from 7% to 70% (Table 1); among those with CD4≤200 this increased from 68% to 86%. From baseline CD4 RIC at 6 months was 82% (95%CI: 79%-85%) in the CD4≤500 cohort and 80% (95%CI: 76%-84%) in the CD4≤350 cohort.

**Conclusions:** Expanding eligibility for ART to CD4≤500 resulted in rapid change in time to ART initiation among those with baseline CD4 351-500 without any reduction in initiation among those with a CD4 ≤ 350. This early analysis suggests that staff and patients can effectively implement extended initiation criteria if clinic resources are sufficient; partly achieved in our setting through decongestion using community models of care. ART uptake remained higher in patients with CD4 201-350 than among those with CD4 351-500, indicating room to improve uptake among those initiating with higher CD4 counts as guidelines shift towards test-and-treat. High RIC across time and groups suggests that 'healthier' patients do not have increased loss to follow-up. It was possible to implement earlier ART initiation in our high HIV prevalence, low resource-setting without compromising access to care for more vulnerable patients.

**Table 1:** Time from baseline CD4 to ART initiation among pre-ART patients by CD4 count, before and after CD4 eligibility criteria were extended from ≤350 to ≤500 copies/µl

CD4 at ART initiation (copies/µl)	Time from baseline CD4 to ART initiation (months)	CD4≤350 cohort			CD4≤500 cohort		
		Baseline CD4 July 1 – December 31, 2013			Baseline CD4 July 1 – December 31, 2014		
		Percent initiated	95% CI		Percent initiated	95% CI	
		Lower limit	Upper limit	Lower limit	Upper limit		
≤200	One	56.6	43.9	70.1	77.5	67.9	85.9
	Three	67.9	55.3	79.9	86.3	77.8	92.7
	Six	69.8	57.3	81.5	90.0	82.3	95.3
201-350	One	59.7	48.7	71.0	63.7	54.9	72.5
	Three	73.4	63.1	83.1	77.0	68.9	84.3
	Six	76.4	66.1	85.4	84.1	76.8	90.1
351-500	One	3.1	1.2	8.1	48.6	39.5	58.5
	Three	7.0	3.4	13.0	69.5	60.6	78.0
	Six	10.0	6.0	16.7	74.3	65.7	82.2
≥500	One	2.4	0.99	5.6	4.7	2.5	8.4
	Three	2.8	1.3	6.2	7.0	4.3	11.3
	Six	4.3	2.2	8.0	9.3	6.1	14.1

**1011 The Real-World Impact of CD4-Eligibility Criteria on Retention in HIV Care**

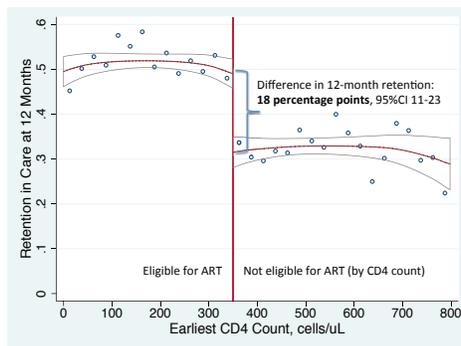
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**Background:** Countries are considering whether to offer antiretroviral therapy (ART) to all HIV patients regardless of CD4 count. Clinical trials have found modest health benefits to early ART. However, these trials may underestimate the benefits. By seeking to minimize attrition, they fail to investigate an important behavioral pathway through which deferred ART eligibility may affect health in real world settings: non-retention among patients not yet eligible for therapy. We address this critical gap by assessing the effect of immediate (vs. deferred) ART eligibility on retention in HIV care in rural South Africa.

**Methods:** All patients (n=11,307) presenting to the public sector ART program in Hlabisa sub-district with a first CD4 count between 12 August 2011 and 31 December 2012 were included in the analysis. Patients were eligible for immediate ART if CD4<350 cells/µL; patients not yet eligible for ART were referred to pre-ART care and instructed to return every 6 months for CD4 monitoring. Because of measurement error in the CD4 laboratory assay, assignment to immediate versus deferred ART was effectively random near the threshold. We used a regression discontinuity design to recover causal effects. We assessed the effect of immediate eligibility on retention in HIV care at 12 months, as measured by the presence of a clinic visit, lab test, or ART start date in the interval 6 to 18 months (intent-to-treat effect). In addition, we assessed the causal effect of eligibility on retention in the subgroup of patients whose treatment uptake was determined by their CD4 count (complier causal effects).

**Results:** Immediate eligibility increased 12-month retention from 32% to 50% (intent-to-treat effect: 18% points; 95%CI 11-23; p<0.001) among patients with first CD4 counts close to the 350-cell threshold. Having an eligible CD4 count increased the probability of initiating ART within six months from 18% to 43% (25% points; 95%CI 20-31; p<0.001). In patients whose uptake of ART was determined by the value of their CD4 count, having an eligible CD4 count increased 12-month retention from 21% to 91% (complier effect: 70% points; 95%CI 42-98; p < 0.001).

**Conclusions:** Deferred ART eligibility resulted in dramatically lower retention in HIV care among otherwise similar patients who just missed the cutoff for immediate eligibility. The results suggest that clinical trials may underestimate the benefits of early ART and, consequently, the clinical and population health benefits of eliminating CD4 initiation criteria.



**Figure.** Intent-to-treat effect: ART eligibility at first CD4 count and 12-month retention in care. Due to measurement error in the CD4 laboratory assay, eligibility for ART was as-good-as-randomly assigned for patients close to the 350-cell threshold. Thus, the difference in retention at the 350-cell threshold can be interpreted as the causal effect of having an eligible CD4 count.

**1012 Do ART Eligibility Expansions Crowd Out the Sickest? Evidence From South Africa**

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**Background:** In August 2011, South Africa expanded adult antiretroviral therapy (ART) eligibility from CD4  $\leq$ 200 to CD4  $\leq$ 350 cells/ $\mu$ L. While this policy was designed to increase access, it is possible that an influx of newly eligible patients could have crowded out sicker patients due to clinic capacity constraints. We assessed whether the 2011 eligibility expansion led to treatment delays among those previously eligible in 17 rural clinics and one sub-district hospital in KwaZulu-Natal.

**Methods:** We included all patients seeking care for the first time in the Hlabisa HIV Treatment and Care Programme between February 2011 and February 2012. Our primary outcome was days from registration to ART initiation. We used proportional hazards regression with a regression discontinuity design, controlling for continuous linear trends before and after the policy change, with an indicator to identify a proportional shift in hazards at the time of the policy change. Person-time began at clinic registration and continued until ART initiation, transfer to another clinic, or the end of the study period. Analyses were stratified by first CD4 count to assess direct effects of the expansion on newly eligible patients and spillover effects on patients with CD4 counts < 200 or >350.

**Results:** 1,363 patients registered at the clinics in the six months before the guideline expansion, and 2,467 patients registered in the six months after. Newly eligible patients with CD4 200-350 saw a 109% increase in initiation rates (HR: 2.092; 95%CI 1.52-2.88). Meanwhile, rates did not change for always-eligible patients with first CD4 <200 (HR: 1.14; 95%CI 0.91-1.44), and decreased among never-eligible patients with CD4 >350 (HR: 0.45; 95%CI 0.24-0.85).

**Conclusions:** We found that, in the short term, this ART eligibility expansion successfully increased ART initiation rates among newly eligible patients, and did not bring about negative spillover effects in the always-eligible group. However, the never-eligible group with CD4 >350 did see a decrease in initiation rates, possibly resulting from capacity constraints. It will be important to monitor the long-term impact of eligibility expansions for extended crowd-out effects.

**1013 Imputing Clinical Records From Routine Laboratory Data: Date of ART Initiation**

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**Background:** Lack of nationally representative data hinders assessment of national HIV treatment programs in many low-resource settings. Where laboratory data are collected on a national scale, such data could be used to create a national monitoring cohort but only if information on treatment initiation can be determined. We developed a novel method to impute dates of antiretroviral treatment (ART) initiation from routine laboratory data in South Africa's public sector HIV programme that could be applied to a national labs database such as South Africa's National Health Laboratory Service (NHLS) database and assessed validity of this approach.

**Methods:** We analyzed data from two large clinical HIV cohorts: Hlabisa (rural primary care clinics and one sub-district hospital in KwaZulu-Natal) and Right to Care (network of urban clinics in Gauteng). Both cohorts contain known ART initiation dates and lab test results are imported directly from NHLS. While the ART initiation date for patients was known (gold standard), we imputed ART start dates using only lab data that would be available in a laboratory database. To do this, we identified the date of "ART workup"; the lab tests used to determine treatment readiness in national HIV treatment guidelines (first documented hemoglobin or alanine transaminase test among patients with a CD4 count in the 12 months prior to these tests). We then calculated the median time from the ART workup to ART initiation and imputed ART start date as the date of ART workup plus that median time. We calculated sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) of our imputed start date to be within 6 months of the actual ART start date.

**Results:** We analyzed data from >80,000 HIV-positive adults of whom >90% had an ART workup. Among patients who had a workup and initiated ART, the median time to initiation was 16 days (IQR 7, 31) in Hlabisa and 21 (IQR 8, 43) in RTC cohort. Among patients with known ART start dates, SE of the imputed start date was 83% in Hlabisa and 88% in RTC, indicating this method will correctly estimate the ART start date for about 85% of those with a known ART workup. In Hlabisa, PPV was 95%. SP (100%) and NPV (92%) were also very high.

**Conclusions:** Routine lab data can be used to infer ART initiation dates in South Africa's public sector with high rates of classification. Lab data can be used to monitor and evaluate health systems performance and improve the accuracy and completeness of clinical records.

**Table 1:** Sensitivity, specificity, positive and negative predictive value of ART start date imputation method in RTC and Hlabisa cohorts

		HGB/ALT + CD4	HGB/ALT only
<b>Sensitivity (RTC cohort)</b>		Truly initiated ART = yes	
ART workup in 6mo prior to known ART start date?	Yes	61,105	61,632
	No	8,178	7,651
	<b>Sensitivity</b>	<b>88.2%</b>	<b>89.0%</b>
<b>Sensitivity (Hlabisa cohort)</b>		Truly initiated ART = yes	
ART workup in 6mo prior to known ART start date?	Yes	17,970	18,393
	No	3,796	3,373
	<b>Sensitivity</b>	<b>82.6%</b>	<b>84.5%</b>
<b>Specificity</b>		Truly initiated ART = no	
Ever had an ART workup?	Yes	79	84
	No	35,556	35,551
	<b>Specificity</b>	<b>99.8%</b>	<b>99.8%</b>
<b>Positive Predictive Value (PPV)</b>		Had ART workup = yes	
Known ART start date in 6mo after ART workup date?	Yes	17,970	18,393
	No	866	963
	<b>PPV</b>	<b>95.4%</b>	<b>95.2%</b>
<b>Negative Predictive Value (NPV)</b>		Had ART workup = no	
Ever initiated ART?	Yes	3009	2,521
	No	35,556	35,551
	<b>NPV</b>	<b>92.2%</b>	<b>93.4%</b>

**1014 Prospective Multisite Cohort Study of Pre-ART Losses and ART Refusal in South Africa**

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**Background:** Failure to initiate antiretroviral therapy (ART) is a significant barrier to population-level viral suppression. We quantified losses in pre-ART care from presentation for voluntary counseling and testing (VCT) through 3 months post-VCT among adults at two urban testing centers in South Africa (RSA). In addition, we measured and identified factors associated with treatment refusal at baseline.

**Methods:** We prospectively surveyed HIV-infected ART-eligible (CD4 < 500 cells/mm<sup>3</sup>) adults who presented for VCT between July 2014-July 2015 in Soweto and Gugulethu over a 3 month period post-VCT. Trained interviewers administered a structured questionnaire to participants to understand psychosocial, clinical and structural factors associated with ART refusal at baseline. Bivariate analyses were performed to identify factors associated with ART refusal. All covariates with p < 0.2 were included in a multivariable model. The model was further stratified by social support based on our prior qualitative findings.

**Results:** Across sites, 1071 individuals presented for testing and were found to be HIV-infected and ART-eligible over the study period. Of those, 360 (33.6%) did not return to collect their CD4 results within 6 weeks of testing (Median CD4: 194 cells/mm<sup>3</sup>). Among those who returned, 500 (70.3%) were enrolled in the prospective cohort. Median age at testing was 35 years-old, of whom 62.6% were women, 57.2% were unemployed, and 75.6% had not completed high school. Median CD4 was 244 cells/mm<sup>3</sup>. We found a 6.6% (n=33) refusal rate at baseline. Multivariable analyses showed fatalism was associated with ART refusal at baseline (AOR: 1.16, p=0.02). Additional exploratory stratified analyses found that high social support attenuated the association of fatalism with ART refusal (**Table 1**). After three months, 77.8% (n=389) of participants were retained. Of those, 1.3% (n=5) died in 3 months prior to ART initiation, and 14.9% (n=58) reported no ART initiation by 3 months post-VCT.

**Conclusions:** Among over 1000 HIV-infected, ART-eligible individuals presenting for VCT at 2 sites in RSA, one-third did not return for their CD4 count. Of the 500 ART-eligible individuals enrolled in our cohort, nearly 7% initially refused ART at baseline. Among those tracked for 3 months, 16% (n=63) died or had yet to initiate ART. Future interventions should be designed to focus on decreasing gaps in pre-ART care, and addressing fatalistic beliefs by optimizing social support.

**Table 1. Multivariable Model of Factors Associated ART Refusal at Baseline (N=483)**

Variable	Low Social Support N=133		High Social Support N=350	
	Adjusted Odds Ratio	95% CI	Adjusted Odds Ratio	95% CI
Age ≥ 35	1.49	0.35 – 6.30	1.20	0.47 – 3.07
Baseline CD4 < 350	1.02	0.22 – 4.81	1.31	0.39 – 4.34
Less HS Education	1.66	0.25 – 11.17	1.08	0.29 – 3.97
Female Gender	1.11	0.22 – 5.47	0.96	0.36 – 2.53
Denial	0.75	0.48 – 1.16	1.16	0.90 – 1.49
Stigma	1.32	0.84 – 2.08	1.14	0.88 – 1.47
<b>Fatalism</b>	<b>1.40</b>	<b>1.05 – 1.86</b>	1.12	0.96 – 1.30

**1015 6-Year Retention and Immunological Response to ART by Gender: leDEA West Africa**

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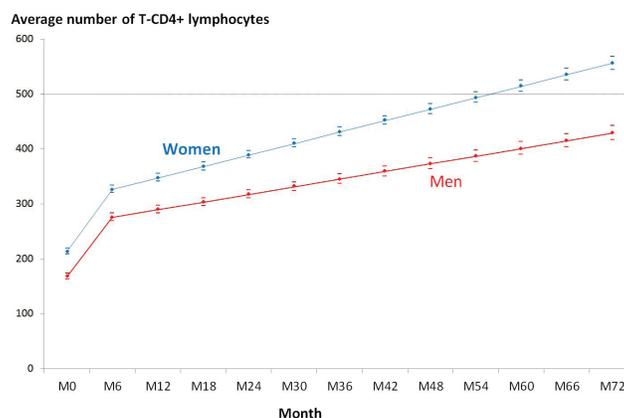
<sup>1</sup>INSERM U897, Bordeaux, France; <sup>2</sup>CePreF, ACONDA, Abidjan, Côte d'Ivoire; <sup>3</sup>CHU de Treichville, Abidjan, Côte d'Ivoire; <sup>4</sup>Univ of Abuja Teaching Hosp, Abuja, Nigeria; <sup>5</sup>Cntr de Prise en Charge des Personnes Vivant Avec le VIH, CHNU, Cotonou, Benin; <sup>6</sup>Cntr Médical de Suivi de Donneurs de Sang /CNTS/PRIMO-CI, Abidjan, Côte d'Ivoire; <sup>7</sup>INSERM U897, ISPED, Univ de Bordeaux, Bordeaux, France

**Background:** Gender differences were previously reported in sub-Saharan Africa for retention and immunological response to antiretroviral therapy (ART), but follow up was usually limited to the first 2-3 years after ART initiation. West Africa is among the regions of the world with the least well-performing ART programs. We evaluated gender differences on retention and immunological response in the 6 years following ART initiation in West African adults.

**Methods:** We included all the patients of the leDEA West Africa collaboration who initiated ART between 2002 and 2014 and had a baseline T-CD4+ lymphocyte measurement. A logistic regression analysis assessed the risk of no follow-up after ART initiation; survival analyses with Cox models assessed the risk of 6-year retention failure (defined as either dead or lost-to-follow-up). Year 2-3 and 5-6 were pooled to comply with proportional hazards assumption. The evolution of the immunological response by gender was investigated by linear mixed models.

**Results:** A total of 49,677 patients (66.2% women) contributed 197,953 person-years of follow-up after ART initiation in 16 clinics in 9 countries. At ART initiation men were older than women (median age 40.8 years vs. 34.0 years) and had a lower median CD4 count (140/μl vs. 184/μl). Being male was associated with an increased risk of no follow-up after starting treatment (4.2% vs. 3.3%, adjusted OR (aOR) = 1.22, 95% CI = [1.12; 1.33]) and with an increased retention failure probability over the first 4 years of follow-up: 17.2% vs. 13.1%, adjusted HR (aHR) = 1.22, CI = [1.17; 1.28] in Year 1; 9.6% vs. 7.3%, aHR = 1.11, CI = [1.05; 1.18] in Y2-3; 8.4% vs. 6.4%, aHR = 1.15, CI = [1.04; 1.28] in Y4. Retention failure did not differ by gender in Y5-6 (6.7% vs. 5.8%, aHR = 0.95 [0.87; 1.04]). The evolution of the immunological response was similar in men and women during the 4 first months of follow-up. From month 5 up to month 72, women had a better immunological response than men, allowing them to reach the 500 CD4 threshold by 54 months on average, while this was never reached for men by 72 months (Figure 1).

**Conclusions:** In the West African context, a better retention and immunological response are achieved by women in comparison to men for long periods of follow up after ART initiation. This difference on retention may disappear from the fifth year following ART initiation. Interventions targeting gender-related factors need to be tailored to improve HIV care programs' effectiveness as soon as possible after ART initiation.



**Figure 1. Evolution of the average number of T-CD4+ lymphocytes (95%CI) in women and men in the 6 years following ART initiation in West Africa**

**1016 Barriers to Care and 1-Year Mortality in Newly Diagnosed HIV+ Persons in South Africa**

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**Background:** Despite increasing availability of antiretroviral therapy (ART), only a fraction of those newly diagnosed with HIV enter care promptly in South Africa, leading to premature mortality among those not linked to care. Our objective was to evaluate the impact of self-perceived barriers to receipt of health care at the time of HIV diagnosis on 1-year mortality among newly diagnosed HIV-infected individuals in South Africa.

**Methods:** We surveyed adults (≥18y) prior to HIV testing at four sites (two hospital outpatient departments and two primary health clinics) in Durban from August 2010 to January 2013. HIV-infected participants were offered CD4 testing and underwent TB screening. We used Cox proportional hazards models to determine the association between the number of perceived barriers to care and time to death within one year of HIV diagnosis. Perceived barriers included: 1) service delivery (wait too long to see a provider, not treated with respect by clinic staff); 2) financial (could not afford medication or transport); 3) personal health perception (not sick enough or too sick); 4) logistical (could not get off of work, care responsibilities for others); and 5) structural (could not get to clinic due to hours or transport, did not know where to find care). We assessed deaths via phone calls to family members and confirmed these through the South African death registry.

**Results:** Among 4,903 participants enrolled, 1,899 (39%) were HIV-infected and 521 (28%) were co-infected with TB. Mean age was 35 years (SD 10), 49% were female, and median CD4 count was 192/μl (IQR: 72-346/μl). 1,057 participants (56%) reported no barriers, 370 (20%) reported 1-3 barriers, and 460 (24%) reported >3 perceived barriers to care. By one year after enrollment, 250 (13%) of participants had died. Adjusting for age, sex, distance to clinic, TB status and baseline CD4 count, participants who identified 1-3 barriers (adjusted hazard ratio [aHR] 1.49, 95% CI 1.06, 2.08) and >3 barriers (aHR 1.81, 95% CI 1.35, 2.43) had higher risk of 1-year mortality compared to those without self-identified barriers.

**Conclusions:** HIV-infected individuals in South Africa who reported multiple perceived barriers to medical care at the time of diagnosis were nearly twice as likely to die within one year. Targeted structural interventions such as extended clinic hours, travel vouchers, and more streamlined clinic operations may improve linkage to care and ART initiation for these patients.

**1017 Retention of Clinically Stable ART Patients in a Rapid Model of Care in Haiti**

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**Background:** Long clinic waiting times are a major contributor to attrition among antiretroviral (ART) patients. We evaluated outcomes for clinically stable ART patients enrolled in a new rapid model of care, the "Rapid Pathway" (RP), in one ART clinic at the GHEKIO Center in Port-au-Prince, Haiti.

**Methods:** Clinically stable patients who had received at least 6 months of ART were eligible for RP care. Once enrolled in RP, patients were scheduled for clinic appointments every two months. At each visit, patients were contacted one day in advance by a community health worker. Patients who were asymptomatic were eligible for RP care, and those with symptoms or poor adherence were referred to a physician for evaluation. Patients in RP received care by a nurse, who evaluated health status, dispensed ART and other medications, and completed a visit form in the electronic medical record. Patients spent a median of 29 minutes from arrival to discharge from clinic, including the dispensing of ART.

**Results:** From June 1, 2014 to August 31, 2014, 1,799 eligible patients initiated RP care. Of these, 950 (53%) were women and 632 (35%) lived on <\$US 125 per year. In the 6 months prior to RP initiation, patients were a mean of 12 (SD: 23) and a median of 2 (IQR: 0, 12) days late to scheduled clinic visits and ART re-fills. We evaluated 12-month retention in care, defined as having at least one visit during the period from 9 to 12 months after the date of RP eligibility; timeliness of visits; and ART adherence, defined as medication possession ratio (number of pills dispensed/number of pills that would have been dispensed with perfect adherence over the follow-up period). 1,663 patients (92%) were retained in care for 12 months; retention was 96% among patients with timely adherence in the 6 months prior to enrollment in RP. The mean adherence over the study period was 89% (SD: 17) and 42% of patients missed no more than 5 days of ART during the study period. The median CD4 T-cell count increased from 447 at baseline to 514 at 1 year. With multivariable analysis, significant predictors of 12-month retention were greater time on ART prior to RP enrollment (OR 1.13; 95% CI: 1.06,1.21) timeliness of visits in the 6 months prior to RP enrollment (OR 1.01;

**Conclusions:** Clinically stable ART patients had outstanding retention with expedited, nurse-led care in Haiti. This program may also serve as a model for other resource-poor settings.

**1018 Evaluating Appointment Patterns to Improve Sustainability of HIV Treatment in Zambia**

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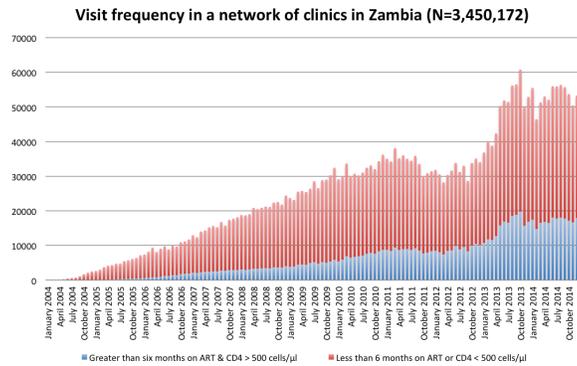
**Background:** Prevailing facility-based models for HIV treatment in high-prevalence settings require substantial contact with clinically stable individuals, thus incurring high costs for patients (e.g., transport, lost wages) as well as systems (e.g., staffing, provider burn out). De-intensification of services through emerging community-based models for clinically stable patients is needed to sustain and expand treatment capacity. We examined patient appointment histories in a network of clinics in Zambia to characterize visit patterns and quantify the potential reductions in visit burden obtainable through community-based models.

**Methods:** We evaluated a cohort of HIV-infected adults on ART who made at least one clinic visit between March 1, 2013 and February 28, 2015 at 61 clinics in Zambia.

Appointment dates and clinical data were obtained from the electronic medical record system used in routine clinical care. We evaluated time between scheduled appointments and stratified this interval by time on ART and WHO stage and CD4 count at enrollment. We quantified the proportion of visits among patients on ART for greater than six months and with a most recent CD4 count > 500/μl or > 350/μl.

**Results:** Overall, 150,213 patients, observed over a maximum of nine years, made 3,450,172 visits after ART initiation. The median appointment interval was 31 days in the first 6 months on ART and 61 days subsequently, even after five or more years on treatment. Median appointment interval did not differ by enrollment WHO stage nor CD4 count. In this cohort, 868,841 of all visits (25%) occurred among patients on ART for greater than six months and who had a most recent CD4 count >500 cells/μl; while 1,507,835 visits (44%) were made by patients on ART for more than six months and who had a most recent CD4 count > 350 cells/μl. Visits made by patients with CD4 count > 500 cells/μl increased over time from 5% (2971/58,059) in 2005 to 32% (205,389/639,799) in 2014.

**Conclusions:** Current facility-based care applies a one-size-fits-all appointment interval not tailored to clinical changes or time on treatment. Between one-quarter to nearly one-half of all visits occurred in patients who are likely clinically stable. Evolution from facility-based care towards a differentiated system that includes community-based models is urgently needed to enhance efficiency and sustainability.



**1019 Identifying Hotspots of Poor Adherence Among Patients on ART in Zambia**

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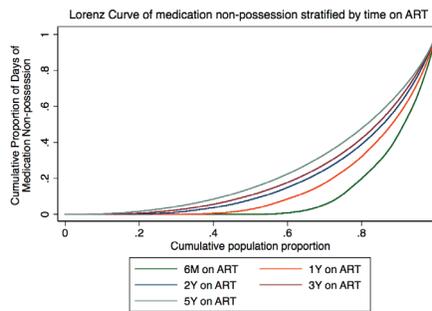
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**Background:** The distribution of poor adherence to antiretroviral therapy (ART) in a patient population indicates whether barriers to medication use are concentrated within sub-populations or systematic. We quantify the medication possession ratio (MPR) and characterize the distribution of medication non-possession in a network of clinics in Zambia to identify “hotspots” of poor adherence.

**Methods:** We analyzed a population of adults on ART for more than 3 months who made at least one clinic visit between March 1, 2013 and February 28, 2015. Pharmacy refill and clinical information were obtained through the electronic medical record system used in routine care. MPR was calculated as the number of days of ART dispensed over the total number of days the patient should have been on ART using pharmacy records. We constructed a Lorenz curve, plotting the cumulative proportion of days of medication non-possession against the cumulative proportion of patients to visualize the distribution of poor adherence. We used a multi-level linear regression model to examine clinic and individual-level factors associated with MPR.

**Results:** Among 133,827 patients in 56 clinics (64% female, median age 34 years [IQR 29–41], median baseline CD4 count 208 cells/μl [IQR 111–333]), the median MPR was 80.7 [IQR 64.4–91.4] indicating patients were not in possession of ART 19.3% of the time. After 1 year on ART, 31% of patients had 100% medication possession, the next 57% account for 50% of medication non-possession and the final 12% contribute the remaining 50%. Over time, a greater proportion of patients contribute to days of non-possession (Figure). In multi-level regression, disclosure of HIV status (3.3%, p<0.001) and ART initiation 2012–2015 (16.2%, p<0.001) were associated with higher MPR, while enrollment WHO Stage 4 (-2.58%, p<0.001) and male (-1.16%, p<0.001) were linked to lower MPR. Across clinics, median MPR ranged from 43.2 to 94.7 and clinic accounted for 16% of the total variability in MPR after adjusting for individual and clinic-level characteristics.

**Conclusions:** A small fraction of patients account for the majority of days of medication non-possession, especially early on. Further characterization of these patient sub-populations where non-adherence is concentrated is needed to target individual-level interventions. Clinic, however, explained the greatest amount of variability in MPR. Health systems interventions targeting clinic “hot spots” may represent an efficient use of resources to improve ART adherence.



**1020 Late ART Initiation and 12-Month Mortality After ART Initiation in Sub-Saharan Africa**

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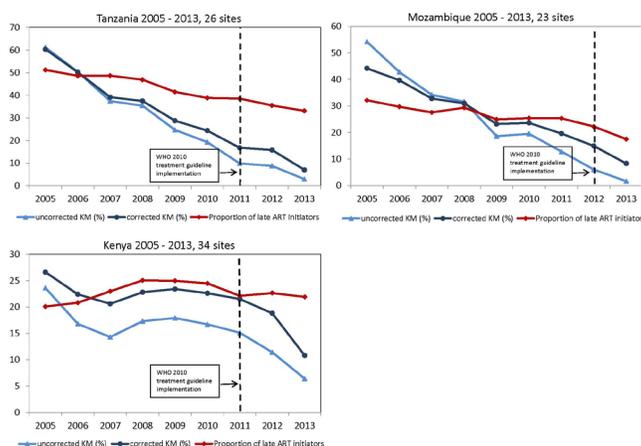
**Background:** The effect of late antiretroviral therapy (ART) initiation and changing ART guidelines on patient outcomes has not been fully characterized due to under-ascertainment of mortality. We examined changes in mortality rates before and after the adoption of WHO 2010 treatment guidelines after accounting for unascertained deaths.

**Methods:** Kaplan-Meier 12-month mortality estimates were calculated for 113,118 patients who had initiated ART at 83 clinics between 2005–2013 in Tanzania (n=26), Mozambique (n=23), and Kenya (n=34). We examined corrected 12-month mortality estimates that reflect unascertained deaths using a correction factor from patient tracing studies in the region. Changes in mortality rates (percent per year) by country both before and after adoption of WHO 2010 treatment guidelines (from CD4≤200 to ≤350) were estimated using a linear regression model. We calculated relative risks (RR) of late ART initiation (CD4≤100 or WHO stage 4) and mortality at 12 months following ART initiation by comparing these outcomes during the year before and the year after guideline changes.

**Results:** Late ART initiation significantly decreased after guideline changes in Tanzania (RR<sub>2012 vs. 2010</sub>: 0.91, 95% CI: 0.86–0.97, p<.05), and Mozambique (RR<sub>2013 vs. 2011</sub>: 0.69, 95% CI: 0.65–0.73, p<.0001) but not in Kenya (RR<sub>2012 vs. 2010</sub>: 0.92, 95% CI: 0.82–1.04, p=0.21). Over the period prior to the guideline change (secular trend), the corrected mortality rates declined by 1.2% per year in Tanzania (95% CI 1.2–2.2, p<0.05), 4.2% per year in Mozambique (95% CI 3.6–4.7, p<0.0001) and 6.2% per year in Kenya (95% CI 5.3–7.1, p<0.0001). The corrected mortality estimates showed a significantly reduced risk of deaths after versus before guideline change in Tanzania (RR<sub>2012 vs. 2010</sub>: 0.65, 95% CI: 0.58–0.71, p<.0001), Mozambique (RR<sub>2013 vs. 2011</sub>: 0.42, 95% CI: 0.39–0.46, p<.0001) and Kenya (RR<sub>2012 vs. 2010</sub>: 0.83, 95% CI: 0.73–0.96, p<.05). These changes exceeded secular trends in all three countries, but to a lesser extent in Kenya (Figure 1).

**Conclusions:** Among large number of patients who initiated ART, one-year mortality rates after ART initiation declined after country-level implementation of WHO 2010 treatment guidelines at rates greater than secular trends in mortality.

Figure 1. Changes in corrected and uncorrected 12-month mortality after ART initiation in Sub-Saharan Africa.



**1021 Declining Mortality in Patients on ART Lost to Follow-up in Sub-Saharan Africa**

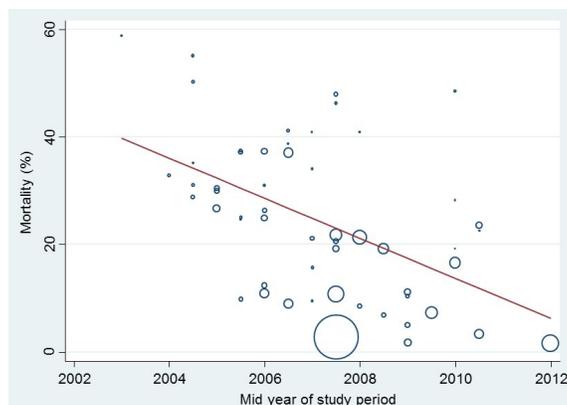
Matthias Egger<sup>1</sup>; Anne Mooser<sup>1</sup>; Kathrin Zürcher<sup>1</sup>; Denis Nash<sup>2</sup>; Olga Tymejczyk<sup>2</sup>; Margaret Couvillon<sup>1</sup>; for the leDEA and MESH Consortia  
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**Background:** Mortality is high in patients lost to HIV treatment and care programs in sub-Saharan Africa, but may have declined with the continued scale up of facilities providing antiretroviral therapy (ART). We did a systematic review and meta-regression analysis of studies of vital status in patients on antiretroviral therapy (ART) who were lost to follow-up.

**Methods:** We searched the Medline, Embase, and African Index Medicus (AIM) databases and the abstracts of CROI and IAS conferences for studies that traced patients lost to follow up during the scale-up of ART in sub-Saharan Africa. Mortality in patients successfully traced was the main outcome. Transfers to other clinics and the CD4 cell count at the start of ART were also analyzed. We used random-effects meta-(regression) models to examine trends over time.

**Results:** We identified 59 studies (34 articles, 24 conference presentations) published 2005-2015, including 6 studies in children. Loss to follow-up was typically defined as not having returned to the clinic for >3 months after a missed appointment. A total of 47,530 patients were traced using telephone calls, home visits, social networks or, in the Republic of South Africa, by linkage to the mortality registry. The vital status of 26,730 (56.2%) patients lost to follow-up could be ascertained. Overall 9,105 (34.1%) patients had died. Among those alive, 4,049 (21.2%) had transferred to another facility. Mortality decreased over time, from around 40% in 2003 to 8% in 2012 (Figure). In meta-regression mortality declined by 3.9% per year (95% CI 2.1% to 5.7%, p<0.001). Trends were similar in adults and children. The median CD4 count at the start of ART increased by around 10 cells per year both in patients lost to follow up (11.6 cells; 95% CI 1.6-21.6; p=0.028) and in all patients (10.2 cells; 95% CI 4.0-16.4, p=0.005). No change over time in transfers to other clinics over time was noted (p=0.79).

**Conclusions:** Mortality in patients lost to follow-up has declined substantially with the scale up of ART in sub-Saharan Africa. The decline in mortality appears to be explained by earlier start of ART, with higher CD4 cell counts, rather than by an increase in silent transfers to other ART providers.



**1022 Lost to Found: The Silent Transfer of Antiretroviral Therapy Patients in South Africa**

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**Background:** Incorrect reporting of Lost to Follow-up (LTF) patients who silently transfer (STF) to another health facility negatively affects the ART program's retention in care (RIC) figures. Identifying the proportion of LTF patients who STF and the reasons patients STF is a priority in order to ensure patient continuity of care, improve policy and treatment guidelines, and provide accurate ART programme outcomes.

**Methods:** A mixed methods approach was conducted using quantitative and qualitative data sourced from an ART longitudinal electronic patient monitoring system and patient and healthcare provider (HCP) interviews. Adults on ART with a LTF outcome between 2008 and 2012 were included. Thirty patient and five HCP interviews were completed.

**Results:** Of the 4182 patients identified as LTF 36% were identified as STF. Sixty-eight percent were female and 51% were between 25-34 years of age. Eighty-four percent transferred once with a mean time to transfer of 870 days from ART start.

Patients were interviewed to ascertain understanding of the transfer process and identify STF reasons. Although the majority of patients (n=27/30) believed it necessary to inform staff of the intent to transfer, more than half suggested challenges in requesting a transfer, including fear of negative staff attitudes (n=11), emergencies and life events (n=5) and long transfer documentation waiting times (n=3).

HCPs acknowledged patients STF due to challenges with the transferring process and fear of repercussions when defaulting treatment. HCPs stated they would not prevent patients from requesting a transfer nor turn STF patients away who had interrupted treatment or arrived without adequate documentation. The HCPs' open acceptance of transferring patients is inconsistent with patients' perceptions that staff negatively react to patients requesting transfer or those transferring to another facility without request.

**Conclusions:** Our study showed that incorrect reporting of STF patients negatively affects RIC data and RIC in the ART program could be underestimated by as much as one third. Operational service challenges and staff attitudes contribute to STF. Cumbersome transfer documentation processes hinder HCPs' ability to effectively manage a STF. The ART program needs to review current transfer guidelines and develop processes that ease the burden on staff and are conducive to the needs of patients to prevent STF. Understanding the STF process will improve the quality of RIC data and the continuity of patient care.

**1023 Countries With Lower HIV Prevalence Have Lower ARV Coverage: UNAIDS 2015 Database**

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**Background:** Countries with a higher prevalence of HIV (at least 5% infected) have been prioritized in PEPFAR and Global Fund sponsored antiretroviral treatment programmes. However 50% of HIV-infected people live in countries with lower HIV prevalence (<5% infected). The aim was to compare uptake of HIV testing and treatment in adults and children between countries with higher or lower HIV prevalence.

**Methods:** The UNAIDS 2015 database includes country-level information on epidemic size, prevalence of HIV infection, antiretroviral treatment coverage, Antenatal Clinic (ANC) visits and Early Infant Diagnosis (EID). The analysis included results from 52 low and middle income countries with at least 50,000 people infected with HIV included. Least squares linear regression was used to correlate national adult HIV prevalence HIV with estimated rates of treatment coverage (adults, pregnant women and children), ANC, and EID. The analysis was weighted by epidemic size and controlled for GDP/capita and region (African vs non-African countries).

**Results:** Of the 52 low or middle income countries in this analysis, 40 had a lower prevalence of HIV <5% (total 16 million HIV infections), while 12 had a prevalence of at least 5% (total 16.1 million HIV infections). As shown in the summary Table, the lower prevalence countries had significantly lower rates of treatment coverage in adults, pregnant women and children (p<0.01 for each comparison). In addition, lower prevalence countries had a smaller percentage of women attending antenatal clinic visits and Early Infant Diagnosis (EID) for infants (p<0.01). The annual death rate for people with HIV was 4.5% in the lower prevalence countries versus 2.5% in the higher prevalence countries. The HIV transmission rate (total new infections divided by HIV epidemic size) was 6.2% in lower prevalence countries versus 5.4% in higher prevalence countries.

**Conclusions:** In this analysis of the UNAIDS 2015 database, including 32.1 million HIV infected people in 52 low or middle income countries, lower prevalence countries had significantly lower treatment coverage in adults, pregnant women and children, lower rates of Antenatal Clinic Visits and Early Infant Detection, and higher annual death rates. Countries with lower HIV prevalence need to upscale HIV and testing and treatment further, to meet the UNAIDS 90-90-90 targets by 2020.

Countries	Lower prevalence (<5% HIV+) N=40 Total HIV+: 16.0 million	Higher prevalence (≥5% HIV+) N=12 Total HIV+: 16.1 million
Mean HIV prevalence	1.6%	14.6%
HIV+ adults on ART	31.7%	48.3%
HIV+ children on ART	22.4%	48.3%
HIV+ pregnant women on ART	46.7%	89.1%
Early Infant Diagnosis	20.1%	72.3%
At least 4 ANC visits	55.3%	68.1%
Death rate /year	4.5%	2.5%

**1024 Feasibility of the Third 90-90-90 Target: Viral Load Coverage and Outcomes in Rwanda**

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**Background:** UNAIDS set the ambitious 90-90-90 targets to end the AIDS epidemic by 2020. To reach the third "90", viral load (VL) monitoring needs to be reinforced as many of resource-limited setting continue to use CD4 to monitor success or failure of ART. As HIV services have expanded in Rwanda, annual VL monitoring was added to the guidelines in 2007 and the number of VL machines increased from X to X. This study aimed to assess the number of patients with a VL test over one-year in Rwanda, proportion of patients with treatment failure, and the healthcare provider's (HCP) response.

**Methods:** A cross-sectional, mentorship activity was completed countrywide in 2015 in all health facilities (HF) providing ART. A doctor and nurse pair of mentors visited HF and supported them through onsite coaching. During mentorship, mentors collected data from the VL register between 1 Jan and 31 Dec 2014 and recorded: number of VL samples taken, VL results received and coded as <20 copies/ml (undetectable VL), 21-999 copies/ml, ≥1000 copies/ml (treatment failure) and missing results. For samples with VL ≥1000, mentors reviewed their medical files to assess whether the HCP responded based on the national HIV guidelines (if ≤10 files, all files were assessed; if >10 files, a random sample was selected).

**Results:** Data were collected from 485 HF representing 96.4% of all HFs in the country. 117,226 VL samples taken from Jan-Dec 2014. Results received for 89.1% (n=104,546), however 5.0% (n=5,274) were inconclusive due to insufficient/inappropriate samples. Majority of patients with results had an undetectable VL 86.6% (n=85,969) and 9.7% (n=9,606) had treatment failure. The remaining 3.7%(n=3,697) had a VL between 21 and 999 copies/ml. Among patients with treatment failure, 3,164 (32.9%) patient files were reviewed. Concerning the decision of HCP, 31.7% (n=1,003) were counseled for medication adherence, 19.6% (n=620) reinforced with adherence and control VL in three months and 12.2% (n=386) shifted to the next regimen. However, 36.5% (n=1155) of files of patients assessed with treatment failure, HCP did not intervene at all.

**Conclusions:** VL test is feasible to monitor patients on ART in resource-limited countries. More than 85% of patients who performed VL suppressed their VL. However, more efforts still needed to reach all patients in need of VL, reinforce adherence to reach the suppression rate as set by UNAIDS and to strengthen capacity of HCP in treatment failure management.

**1025 ART Coverage and Viral Load in Tanzania: Bukoba Combination Prevention Baseline Study**

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**Background:** The Bukoba Combination Prevention Study is an evaluation of a two-year HIV prevention program in Bukoba Municipality, Kagera Region, Tanzania. A population-based baseline survey took place November 2013-January 2014 prior to program implementation. The program aim is to reduce new HIV infections through improving uptake of HIV testing and counselling (HTC), linkage to HIV care, and anti-retroviral therapy (ART) initiation.

**Methods:** All persons aged 18-49 years residing in 53 randomly selected enumeration areas (EAs) were eligible for the study. Enrolled participants were interviewed about past HIV tests and current HIV status. For those who reported HIV-positive status, interviewers ascertained current ART use by observing a pill bottle or clinical care card. All participants were offered HTC after the interview, and CD4 and viral load (VL) tests were conducted for those who tested HIV-positive. Viral suppression (VS) was defined as VL <1000 c/ml. Wald chi-square tests accounting for EA-level clustering were used to evaluate differences in proportions. All analysis is unweighted.

**Results:** Of the 5,696 household residents who were contacted and eligible, 5,397 (95%) enrolled in the study. HTC was accepted by 4,797 (89%) participants, and 436 (9%) of those tested HIV-positive. Men in the sample had lower HIV prevalence than women (6% vs 11%, p<.001). Among those who tested HIV-positive, 166 (38%) were aware of their diagnosis and 55 (13%) were using ART. A lower proportion of men were aware of their diagnosis (31% vs 41%, p=.052). Among HIV-positive participants, median CD4 was 486 (IQR 326-679) cells/mm<sup>3</sup> and median VL was 22,989 (IQR 120-100,515) c/ml. Among ART non-users, 107 (28%) had CD4 ≤350 cells/mm<sup>3</sup> and were ART-eligible under national guidelines, and 196 (52%) had CD4 ≤500 cells/mm<sup>3</sup>. Among ART users, 52 (95%) had VS.

**Conclusions:** In this population-based survey of a large urban area of Tanzania, HIV prevalence for both sexes was higher than the regional and national prevalence. While a high proportion of ART users had VS, the majority of participants who tested HIV-positive were unaware of their diagnosis, and ART usage was uncommon overall. The insufficient ART coverage in this community underscores the need for effective testing and linkage-to-care strategies, including targeted methods to test and link men. The combination prevention program that is underway has the potential to increase the prevalence of VS by improving HIV testing coverage and expanding ART initiation criteria.

**1026 Heterogeneity in Local Population Viremia Despite >50% Suppression in East Africa**

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**Background:** Prior reports estimate 25% of HIV+ populations in Sub-Saharan Africa are virally suppressed. To better understand VL metrics, assess major drivers of transmission in East Africa, and inform global epidemic modeling, we studied individuals and discordant couples and determined 1) % of adults with viral suppression, 2) heterogeneity of communities, 3) local prevalence of viremia in communities (which expresses transmission risk from random contacts), and 4) VL suppression in male-female discordant couples.

**Methods:** In 303,461 persons in 30 Ugandan and Kenyan communities (N~10,000 each; HIV prevalence 10.1%; SEARCH Study:NCT01864683), we determined HIV serostatus in 117,711/132,030 (89%) of stable adult residents. We measured plasma VL (Abbott) in 74% of HIV+ adults and calculated the % with VL that was: 1) undetectable <500 c/mL; 2) low, 500-10,000 c/mL; 3) moderate, 10,001-100,000 c/mL; and 4) high, ≥100,000 c/mL. In communities, we estimated the local prevalence of viremia (% of adults [regardless of HIV status] with detectable VL). Predictors of undetectable VL were evaluated via logistic regression. Male-female couples were self-identified pairs in one household.

**Results:** VL was undetectable in 4,490/8,828 (50.9%) of all adults and in 3,427/4,202 (82%) of adults on ART by self report. VL was low, moderate and high in 14.9%, 21.7%, and 12.5% of adults, respectively. Predictors of undetectable VL included region (W. Uganda: odds ratio (OR) 1.34 [95% CI, 1.14-1.58] and Kenya: OR 1.49 [1.29-1.72] vs. E. Uganda), older age (OR 3.26 [2.55-4.17] for age 30-40 vs. 15-20), female (OR 1.48 [1.34-1.63]), and higher wealth (OR 1.52 [1.32-1.77] for highest vs. lowest quintile). Local population prevalence of viremia was higher in Kenya (range 2.1%-11.2%, median 5.6%) than in Uganda (range 0.5%-5.3%, median 2.3%). Discordancy existed in 492/16,023 (3.1%) of Ugandan and 859/8,616 (10.0%) of Kenyan couples. In 58% of Ugandan and 53% of Kenyan discordant couples, the HIV+ partner was viremic. In 14% and 15%, respectively, the HIV+ partner had VL>100K, indicating marked transmission risk.

**Conclusions:** In this large population VL assessment in Uganda and Kenya, >50% of HIV+ adults had undetectable VL. However, nearly 5-10% of the entire adult population of some Kenyan communities had HIV viremia, and >50% of all Ugandan and Kenyan discordant couples had one partner with viremia. Thus, although half of all HIV+ adults have undetectable VL, there are localized groups with high potential transmission risk.

**1027 High Rate of Viral Suppression in Late Mortality on First-Line ART in Uganda/Zimbabwe**

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**Background:** Early mortality (<48 wks) after ART initiation in resource-limited settings is well recognised, but less well understood are the causes of later mortality, which are widely assumed to be due to virological failure (VF) or non-adherence. We investigated HIV viral load (VL) in patients who died after 48 weeks of continuous first-line ART in the DART study.

**Methods:** The DART trial randomised 3,316 Ugandan and Zimbabwean patients to laboratory monitoring (LCM; CD4 cell count every 12 weeks) or clinically driven monitoring (CDM). Prospective VL testing was not undertaken. Previous analyses found that low pre-ART CD4 cell count was strongly associated with higher mortality during the first year of ART, which was predominantly from infectious causes. All late mortalities had stored plasma samples from the closest visit to the date of death retrospectively tested for VL. Logistic regression models were used to determine predictors of mortality with virological suppression (VS) status (VL<200 copies/mL) in patients who died. Fractional polynomials were used for continuous variables, but non-linear risk was not found.

**Results:** 210/382 (55%) deaths during the DART study occurred after week 48; 112 were on continuous first-line and 78 on second-line ART. The late mortality rate was low (10.7/1,000 PY). VL data were available for 105/112 (94%) mortalities at a median (IQR) of 10 (6-14) weeks before death. 43/105 (41%) patients were virologically suppressed (VS) at the time of death. VF deaths were more often due to opportunistic infections (26% vs 12%; p=0.09). CD4 cell count was significantly lower at the time of death in patients with VF than VS (Median: 62 vs 238 cells/mm<sup>3</sup>; p<0.001) and a greater proportion had CD4<100 cells/mm<sup>3</sup> (66% vs 19%). In multivariate logistic regression analyses (Table), patients in the CDM arm had reduced odds of 0.28 (95% CI: 0.11 - 0.68) death with VS, with no evidence of a change over time. The odds of death with VS were almost 4 times higher for each additional 100 cells/mm<sup>3</sup> increase in baseline CD4 count. Gender, age, initial ART regimen, CD4 cell count at week 48, baseline VL and opportunistic infections were not associated with VS at death in multivariate analyses.

**Conclusions:** 40% of late deaths on ART occurred without VL criteria for treatment switch being fulfilled. There were significantly more deaths with VS among patients who received CD4 cell count monitoring. Further research is required to elucidate the cause of deaths in those without VF.

Variable	N (%)	N (%) with virological suppression	Univariate OR (95% CI)	Multivariate OR (95% CI)	p-value
<b>Trial Arm</b>					
LCM	45 (43%)	26 (58%)	1.00	1.00	<0.01
CDM	60 (57%)	17 (28%)	0.29 (0.13 – 0.65)	0.28 (0.11 – 0.68)	-
<b>Centre</b>					
Entebbe, Uganda	33 (31%)	17 (52%)	1.00	1.00	0.06
Kampala, Uganda	40 (38%)	18 (45%)	0.77 (0.31 – 1.94)	1.07 (0.37 – 3.04)	-
Harare, Zimbabwe	32 (30%)	8 (25%)	0.31 (0.11 – 0.90)	0.30 (0.09 – 0.98)	-
<b>Death with opportunistic infection</b>	21 (20%)	5 (24%)	0.38 (0.13 – 1.13)	-	-
	<b>Median (IQR)</b>	<b>Median with VS (IQR)</b>			
<b>Baseline CD4 Count</b> (per 100 Cells/mm <sup>3</sup> increase)	0.72 (0.25 – 1.22)	1.07 (0.56 – 1.42)	3.42 (1.65 – 7.10)	3.83 (1.69 – 8.69)	<0.01
<b>Week 48 CD4 Count</b> (per 100 Cells/mm <sup>3</sup> increase)	1.61 (0.89 – 2.14)	1.99 (1.23 – 2.67)	1.57 (1.07 – 2.30)	-	-
<b>Baseline Viral Load</b> (per 1 Log <sub>10</sub> copies/mL increase)	5.44 (5.00 – 5.77)	5.47 (5.01 – 5.66)	3.42 (1.65 – 7.10)	-	-

**1028 Measuring Viral Load Suppression in South Africa Using a Novel, National Database**

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**Background:** South Africa has embraced UNAIDS' ambitious goal of 90% of people on antiretroviral treatment (ART) having a suppressed viral load (VL). One strategy has been to decentralize ART services by down-referring patients to smaller facilities, supported by the Nurse Initiated and Managed ART program. There is limited information on VL suppression levels among ART patients and on outcomes by site of care. Using a novel patient matching algorithm, we merged 2 existing databases to create a national marker of VL suppression for all clinics in South Africa to monitor ART effectiveness and understand influences of clinic success.

**Methods:** The National Health Laboratory Services' database, which contains all public sector VL tests in South Africa by facility, was merged with the District Health Information System database, which reports on the Total number of patients Remaining on ART (TROA) by facility. We analyzed the last VL test of patients in a 12-month period for each facility. We used the TROA to categorize facility size into quartiles. We report the proportion of patients receiving a VL test in a 12-month period, the results of those tests (<400, 400-1000, >1000, and >10,000 copies(cp)/ml) and how these differ by province and facility size.

**Results:** From April 2014-March 2015, 3,775 public facilities reported 2,993,125 patients on ART. During the same period, 2,199,890 unique patients received 2,995,133 VL tests. Nationally, 75% of ART patients had a VL test in the last 12 months and 78% were suppressed (VL <400 cp/ml). 19% and 12% of patients had a VL >1000 and >10000 cp/ml respectively. The proportion of patients with suppressed VL ranged from 69 to 82% across provinces (Table 1). In 3 provinces, >25% of patients had a VL result >1000 cp/ml. VL suppression was associated with facility size (TROA), controlling for % of patients tested and province. Two-thirds of all ART patients are seen in the 25% largest facilities and a greater proportion of them were VL suppressed compared to those seen in the 25% smallest facilities (difference of 14.5% (95% CI: 13.1-15.9)). Overall, 3.7% of facilities met the 90% target for VL suppression and these were distributed across facilities of all sizes.

**Conclusions:** There is great geographic diversity in VL testing and suppression levels in South Africa. While most facilities need to increase the proportion of patients tested and suppressed, utilizing VL suppression data to target interventions will help South Africa reach the 90% viral suppression goal.

Table 1. Viral load (VL) suppression for people in care and on ART results by province, April 2014 to March 2015

Province	Total Remaining on ART (Oct/Nov 2014)	Number of Patients with VL in past 12 months	Proportion of Patients Tested in past 12 months	VL<400 cp/ml % (N)	VL 400-1000 cp/ml % (N)	VL>1000 cp/ml % (N)	VL>10000 cp/ml % (N)
Entire Country	2,951,159	2,199,890	75%	78% (1,709,867)	4% (80,873)	19% (409,150)	12% (272,836)
Eastern Cape	307,288	246,452	80%	73% (180,293)	3% (8,518)	23% (57,642)	11% (40,400)
Free State	163,073	127,318	78%	82% (104,938)	2% (2,382)	16% (19,998)	11% (13,579)
Gauteng	660,849	484,233	73%	80% (385,673)	4% (21,502)	16% (77,058)	10% (49,145)
KwaZulu-Natal	906,783	655,937	72%	82% (540,553)	3% (18,122)	15% (97,262)	10% (63,042)
Limpopo	214,066	161,926	76%	70% (113,193)	5% (8,357)	25% (40,376)	17% (28,241)
Mpumalanga	282,750	200,903	71%	73% (146,749)	6% (11,550)	21% (42,604)	14% (27,445)
Northern Cape	41,511	32,874	79%	69% (22,695)	3% (867)	29% (9,212)	20% (6,655)
North West	200,833	148,091	74%	70% (103,415)	4% (5,450)	15% (39,226)	18% (27,395)
Western Cape	174,008	142,156	82%	80% (113,498)	3% (3,835)	17% (24,823)	11% (16,191)

**1029 Comparing Adherence Methods: Which Best Predicts Virological and Resistance Outcome?**

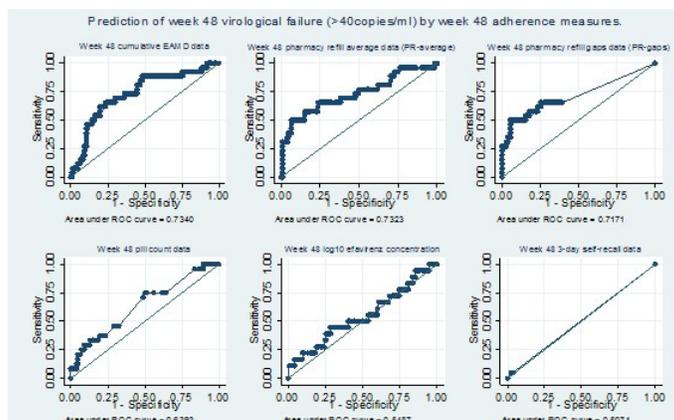
**Catherine Orrell<sup>1</sup>**; Karen Cohen<sup>1</sup>; Rory Leisegang<sup>1</sup>; David R. Bangsberg<sup>2</sup>; Gary Maartens<sup>1</sup>; Robin Wood<sup>1</sup>  
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**Background:** Novel approaches to identify incomplete adherence are necessary to realise the full benefits of HIV treatment. We compared real-time electronic methods with patient-reported and objective adherence measures in an ART-naïve cohort in South Africa.

**Methods:** We recruited ART-naïve participants from a community ART clinic. We collected demographic and disease data, CD4 count and HIV-RNA at weeks 0, 16 and 48. HIV-RNA >500 copies/ml triggered a genotype. We quantified adherence using self-report (SR), tablet return (TR), average adherence by pharmacy refill (PR-average), calculation of medication-free days (PR-gaps), therapeutic drug monitoring (TDM) and an electronic adherence monitoring device (EAMD). We modelled associations between adherence measures and virologic and genotypic outcomes using logistic regression, and constructed receiver operator curves (ROC) to assess performance of adherence measures in predicting outcomes.

**Results:** The 230 participants had median (IQR) adherence: SR 100% (100-100), TR 100% (95-107), PR-average 103% (95-105), PR-gaps 100% (95-100) and EAMD 86% (59-94) at week 48. Efavirenz concentrations were therapeutic (>1mg/ml) in 92%. Retention in care was 81% (186/230), with 83% (155/186) achieving HIV-RNA <40 copies/ml. EAMD, PR-average and PR-gaps best predicted virological outcome at week 48 with area under the ROC (AUC ROC) of 0.73 (95%CI 0.61-0.83), 0.73 (95%CI 0.61-0.85) and 0.72 (95%CI 0.59-0.84) respectively. EAMD, PR-gaps and PR-average were all highly predictive of resistance at week 48, with AUC ROC of 0.92 (95%CI 0.87-0.97), 0.86 (0.67-1.0) and 0.83 (95%CI 0.65-1.0) respectively. SR, TR and EFV concentrations were poorly predictive of virological or resistance outcomes.

**Conclusions:** Adherence data from EAMD and pharmacy refill measures predicted resistance and virological failure similarly. Pharmacy refill data is a reasonable option for monitoring adherence in resource-limited settings where electronic monitoring is unavailable.



### 1030 Adherence Predicts Failure on PI-Based Second-Line ART in Rural South Africa

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**Background:** Rollout of ART in sub-Saharan Africa has been accompanied by high prevalence of virological failure (VF) and resistance on NNRTI-based first line ART. This has led to increasing numbers being switched onto PI-based second line ART (SLART). The limited data on outcomes on SLART in sub-Saharan Africa are predominantly from urban settings. This study determined the incidence and risk factors for VF amongst second line patients receiving routine HIV care and treatment in a government program in a poor and rural setting in Kwazulu-Natal.

**Methods:** Participants were HIV-1 positive adults >15 years initiating SLART between April 2007 and January 2014. They were identified from the Africa Centre's ART Evaluation and Monitoring System. Their clinical data were anonymised and linked to the Africa Centre's demographic information system. Exposures were adherence measured as a medication possession percentage (MPP); the duration on failing first line regimen; non-ownership of a refrigerator for storing soft gel lopinavir tablets. We defined VF as viral load (VL) >1000 copies/ml after 6 months of commencing SLART, or death or loss to follow up within 12 months without evidence of suppression (VL<1000). We used competing risk regression for analysis with other death as a competing risk.

**Results:** Three hundred and fifteen adults started SLART. The median age was 33 (IQR 28-38) years and 72% were female. Unemployment was high (89.8%). Median CD4 at switch was 220 (IQR 113-342) cells/mm<sup>3</sup>. Nine patients with < 6 months follow up at January 2014 were excluded from analysis. The overall incidence rate of VF on SLART was 21.4 (95%CI 17.9-25.7) per 100 person-years. The cumulative incidence function of failure by 5 years was 45%. A higher MPP in the first 12 months of SLART was strongly associated with a lower risk of VF (sHR=0.67, 95%CI 0.45-0.99, and sHR=0.22, 95% CI 0.09-0.51, comparing 60-94% and ≥95% MPP, respectively, with <60% MPP, p<0.01). This association remained after adjusting for confounders. There was no evidence of an association with refrigerator ownership or duration on failing first line ART.

**Conclusions:** There is a high incidence of VF on PI-based SLART in this rural setting with high unemployment. The level of adherence to treatment predicts VF. This has implications for the move to near universal, early ART in resource limited settings particularly as there is a lack of availability of third line ART.

### 1031 Outcomes of Patients Enrolled in ART Adherence Clubs After Viral Resuppression

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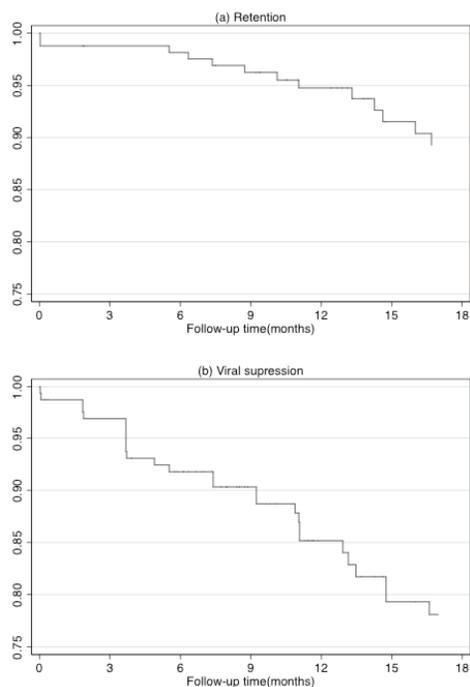
**Background:** Eligibility for simplified models of antiretroviral therapy (ART) care and delivery have to date been limited to low-risk stable patients. There is no evidence whether such models also provide retention and adherence benefits for patients who have struggled to achieve or maintain viral suppression.

**Methods:** Beginning in February 2012, a "Risk of Treatment Failure" (ROTF) intervention was implemented for patients with consecutive viral loads (VL) above 400 copies/mL at a high-burden ART clinic in Khayelitsha, South Africa. On their ART refill dates, ROTF patients attended a lay healthcare worker led group support session followed by a consultation with a nurse trained to provide integrated adherence and clinical management for patients failing ART. Patients who re-suppressed (VL<400 copies/mL) were enrolled in an Adherence Club (AC). ACs were comprised of ~30 stable patients who met 5 times per year and were facilitated by a lay healthcare worker who conducted a brief symptom screening and distributed pre-packed ART. We conducted a retrospective cohort analysis of patients who re-suppressed following the ROTF intervention and joined an AC. We describe patient characteristics and outcomes [mortality, loss to follow-up (LTFU) and viral rebound] using Kaplan-Meier methods with follow-up to mid-June 2015.

**Results:** A total of 165 patients were enrolled in an AC following the ROTF intervention (81.8% female, median age 36.2 years). Seventy-nine percent (79.0%) were on second-line ART at AC enrolment. The median time from ART initiation to ROTF intervention was 3.4 years [inter-quartile range (IQR): 2.1-5.5 years] and from ART initiation to AC enrollment-4.7 years (IQR: 3.4-7.2). Over the study period, two patients died (1.2%). Six-, 12- and 18-months after AC enrollment, retention in any form of care was 98%, 95% and 89%, respectively (Figure 1A). Thirty-six patients experienced viral rebound and 92%, 85% and 78% maintained viral suppression 6-, 12- and 18-months after AC enrollment (Figure 1B).

**Conclusions:** Our findings suggest that patients who struggled to achieve or maintain viral suppression in routine clinic care can have good outcomes in simplified models of ART care and delivery following re-suppression. These simplified models may remove barriers imposed by clinician-led models such as transport cost and time. Further research is necessary to understand how models of care can better prevent viral rebound and support previously non-adherent patients.

**Figure 1.** Kaplan Meier plots of outcomes in an Adherence Club after viral resuppression: (a) 18-month retention in any form of care, and (b) 18-month viral suppression.



**1032 Substance Use Is a Major Barrier to Viral Suppression Among Key Populations in India**

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**Background:** HIV viral suppression is critical to achieving benefits of antiretroviral therapy (ART) not only for individual health, but also for prevention of onward HIV transmission. Limited data on viral suppression derive from key populations in low-and-middle-income countries where these groups continue to experience disproportionately high rates of HIV transmission.

**Methods:** We recruited 12,022 men who have sex with men (MSM) and 14,481 people who inject drugs (PWID) across 27 cities in India (target=1000 per site) using respondent-driven sampling from 10/2012 – 9/2013. Participants had to be ≥18 years old and self-identify as male and report sex with a man in the prior year (MSM) or report injection drug use in the prior 2 years (PWID). 1,146 MSM and 2906 PWID were HIV positive. We characterize barriers and facilitators of viral suppression (HIV RNA<150 copies/ml) among ART initiators using multi-level logistic regression.

**Results:** Of HIV positive MSM and PWID, 347 (25%) and 595 (24%) initiated ART, respectively. Median age was 35 years, 33% had primary school education or less and 44% of PWID were female. 67% of PWID reported active drug injection; 16% and 21% of MSM and PWID, respectively, had evidence of alcohol dependence. Of those who initiated ART, 268 (78%) of MSM and 443 (77%) of PWID were virally suppressed. Barriers to viral suppression among both groups included homelessness and harmful/hazardous alcohol use or alcohol dependence (Table). Among PWID, active drug injection was an additional barrier to suppression and women were significantly more likely to be suppressed than men. Among MSM, higher educational attainment was significantly associated with viral suppression. Neither needle exchange nor opioid substitution was associated with suppression among PWID. Adjustment for adherence (visual analogue scale) or premature discontinuation did not substantially attenuate these associations.

**Conclusions:** Levels of viral suppression among those on ART in this population were high, but are still short of new UNAIDS targets. Moreover, while viral suppression among those on ART was high, overall viral suppression (19% among MSM and 18% among PWID) was suboptimal such that population viral suppression will only be achieved through broad-based interventions that simultaneously support testing, linkage and adherence. Interventions need to be targeted towards those with active substance use, as they may also be most likely to transmit HIV to others.

**Table. Correlates of HIV viral suppression among MSM and PWID**

	Adjusted odds ratio (95% confidence interval)*	
	MSM (n=347)	PWID (n=595)
Age (per 10 years)	1.17 (0.94, 1.44)	1.42 (0.86, 2.35)
Female sex		2.43 (1.20, 4.93)
Education		
Primary school or less	1	
Secondary school	0.71 (0.45, 1.13)	
High school graduate	2.43 (1.16, 5.10)	
Homelessness	0.51 (0.35, 0.74)	0.11 (0.01, 0.89)
Injection in prior 6 months		0.51 (0.31, 0.85)
Alcohol use (AUDIT)		
Low alcohol use	1	1
Harmful/hazardous use	0.55 (0.12, 2.47)	0.54 (0.31, 0.96)
Dependence	0.54 (0.32, 0.93)	0.26 (0.12, 0.57)

\*also adjusted for region of India; models incorporate Respondent-Driven Sampling-II weights; MSM: men who have sex with men; PWID: people who inject drugs.

### 1033 Durable Viral Suppression Among HIV-Diagnosed Persons United States, 2012-2013

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**Background:** Viral suppression is associated with reduced morbidity, mortality, and risk of transmitting HIV. Estimating the percentage of persons with HIV who have durably suppressed viral loads (DSVL), plasma HIV burden, and time without viral load (VL) suppression can help in monitoring disease burden and HIV transmission risk potential and guide interventions.

**Methods:** We used data from the National HIV Surveillance System reported from 17 jurisdictions to determine viral burden among persons aged  $\geq 13$  years who received a diagnosis of HIV infection before 2011 and were alive through 2013. DSVL was defined as all viral loads  $< 200$  copies/mL during 2012-2013. Viremia copy-years were estimated to determine plasma HIV burden. HIV transmission risk potential was estimated by time viral load was above 200 copies/mL over the 2-year period.

**Results:** Of 264,865 persons engaged in care (with at least 1 VL) in 2011, 251,649 (95%) had VL tests between 2012 and 2013. The median number of VL tests during the 2-year period was 5. Of the 251,649 persons, 62% had DSVL. The percentages of persons with DSVL were lower among females (vs. males: 55% & 64%), black/African American (vs. white: 53% & 74%), and persons aged 13-24 years old (vs.  $\geq 55$  years: 39% & 73%). The geometric mean viremia copy-years in the 2-year period among those without DSVL was higher than the geometric mean averaged across all persons (7,730 vs. 356 copies/mL). Among those without DSVL, the average number of days a person spent above 200 copies/mL was 452 days, corresponding to 62% of the 2-year observation time. Among those without DSVL, female, black/African American, and persons aged 13-24 also had substantially higher geometric means of viremia copy-years and higher numbers of days with VL above 200 copies/mL compared to their respective counterparts (female vs. male: 8,360 & 7,494 copy-years; 478 & 442 days; black/African American vs. white: 9,341 & 5,851 copy-years; 483 & 412 days; aged 13-24 vs.  $\geq 55$  years: 13,971 vs. 3,356 copy-years; 550 & 377 days).

**Conclusions:** It is encouraging that about two-thirds of HIV-diagnosed persons in care had suppressed VL over a 2-year period. The remaining one-third had high plasma burden and spent substantial time without VL suppression, which increases the risk of HIV transmission. Greater disparities in disease burden and transmission risk potential were seen in several subgroups. Targeted care and treatment efforts are needed to address the disparities.

Viral Load Burden, 2012-2013	Persons living with HIV N=251,649	Persons living with HIV without DSVL N=95,186
Percentage with DSVL	62.2%	37.8%
Geometric mean viremia copy-years	356 <sup>a</sup>	7,730 <sup>b</sup>
Mean number of days a person spent above 200 copies/mL	179 <sup>a</sup>	452 <sup>b</sup>

<sup>a</sup>Due to some missing data, results are based on 251,611 persons living with HIV

<sup>b</sup>Due to some missing data, results are based on 95,148 persons living with HIV

### 1034 Changes in Viral Load Across US Clinics Over Time

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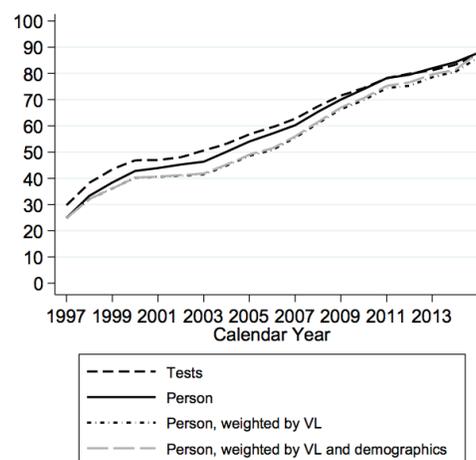
**Background:** Individual HIV clinics have reported increasing viral suppression rates. We evaluated changes in viral load (VL) over time and identified associated factors in HIV care settings. We were particularly interested in the impact of changing demographic and clinical characteristics such as shifting substance use patterns, adherence, and use of integrase inhibitors.

**Methods:** Data are from 29,467 participants at 8 HIV clinics in the national CFAR Network of Integrated Clinical Systems (CNICS) cohort who had VL values between 1997-2014. We determined the annual percentage of participants with an undetectable VL ( $< 400$  copies/mL) each year including all tests and limiting them to 1/person/year. We then accounted for differential loss to follow-up by inverse probability of censoring weights based on prior VL and demographic factors. We used logistic mixed models to estimate associations with undetectable VL. Models were restricted to 2010-2014 and individuals on antiretroviral therapy (ART) to minimize impact of changes in treatment initiation guidelines.

**Results:** The number of VL tests per year ranged from 9,180-39,540. Participants with undetectable VL values increased from 30% in 1997 to 87% in 2014 (see Figure) and results were similar when correcting for loss to follow-up. Undetectable VL percentages were higher for each decade of older age and in men vs. women (p values  $< 0.05$ ). For example, while the undetectable VL percentage increased over time for both men and women, men consistently averaged  $\sim 3\%$  higher than women. Neither mean adherence increased nor current substance use decreased over time, and some drugs (e.g. marijuana, methamphetamines) increased. In multivariate models of individuals on ART after 2010, demographic factors including older age, white race, male sex and better medication adherence remained associated with undetectable VL (p values  $< 0.05$ ), as were integrase inhibitor use (OR 2.4, 95% CI 2.2-2.7, p $< 0.001$ ).

**Conclusions:** VL suppression rates at clinics across the US have improved dramatically in recent years. Adherence and substance use remained key predictors of absolute VL values but did not explain the improved percentage of viral suppression over time. Increased use of integrase inhibitors is likely one contributing factor. Findings suggest the need for increased evaluation and support for at-risk subgroups such as women and younger patients, and the need for a better understanding of factors that are driving changes in VL.

**Figure.** Percentage of suppressed viral load values over time



**1035 Differences in HIV Viral Suppression by Frequency and Type of Healthcare Visits**

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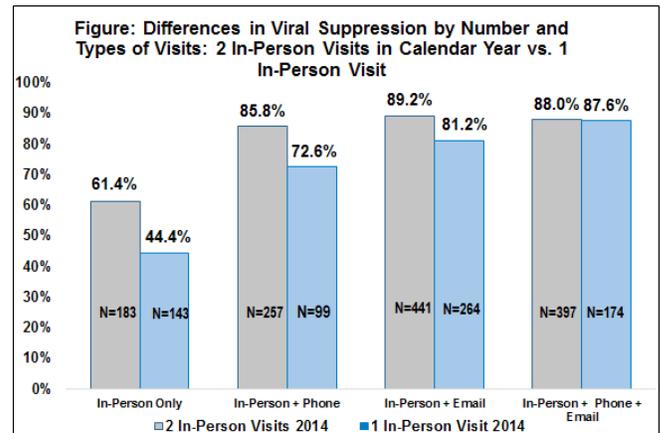
**Background:** HIV care typically involves two in-person visits per year, but there is increasing use of alternative patient-provider “visits” (telephone and/or email). We explored differences in HIV viral suppression by frequency and type of visits.

**Methods:** The study population consisted of adult HIV+ patients in Kaiser Permanente Mid-Atlantic States (KP), providing comprehensive HIV care to health plan members in District of Columbia, Maryland, and Virginia. We restricted to members with ≥6 months membership and ≥1 viral load (VL) measurement in 2014. HIV viral suppression (“BLQ”; HIV RNA <200 copies/mL) was measured at last VL measurement during 2014. We compared HIV viral suppression by number of in-person visits to an HIV specialist or primary care (1 or ≥2), and among those with 1 in-person visit by additional use of phone and/or email visits to an HIV specialist (none, phone only, email only, and “both” phone and email). The reference group was those with ≥2 in-person visits (with or without phone or email).

We also compared those with ≥2 in-person visits by visit type, with ≥2 in-person visits only as reference. Adjusted odds ratios (OR) for BLQ by frequency and type of visits were obtained from multivariable logistic regression adjusting for age, sex, race/ethnicity, and HIV risk.

**Results:** Among 1958 subjects, there were 1278 (65%) with ≥2 in-person visits and 680 (35%) with 1 in-person visit. Among those with 1 visit, 15% also had a phone visit, 39% had an email visit, 21% had neither, and 26% had both. As shown in the Figure, %BLQ was greater for ≥2 visits compared with 1 visit, except if both phone and email visits were used. In adjusted models, BLQ was reduced compared with ≥2 in-person visits for 1 in-person visit only (OR=0.48 [95% CI: 0.30, 0.74]) or 1 in-person + phone (OR=0.46 [0.28, 0.78]). However, differences were not statistically significant comparing ≥2 in-person visits with 1 in-person + email (OR=0.81 [0.54, 1.21]) or both (OR=1.06 [0.63, 1.80]). Among patients with ≥2 in-person visits only, there was no significant difference in the adjusted odds BLQ among 2 in-person plus email or plus phone or plus both compared with 2 in-person visits only, although there is a trend toward greater odds of BLQ with 2 in-person plus email (OR=1.65 [0.99, 2.76]).

**Conclusions:** One in-person visit per year was not statistically different with respect to HIV viral suppression compared with 2 in-person visits, if supplemented by alternative communications such as email with or without phone.



**1036 Community Viral Load: Measure Validation and Public Health Utility**

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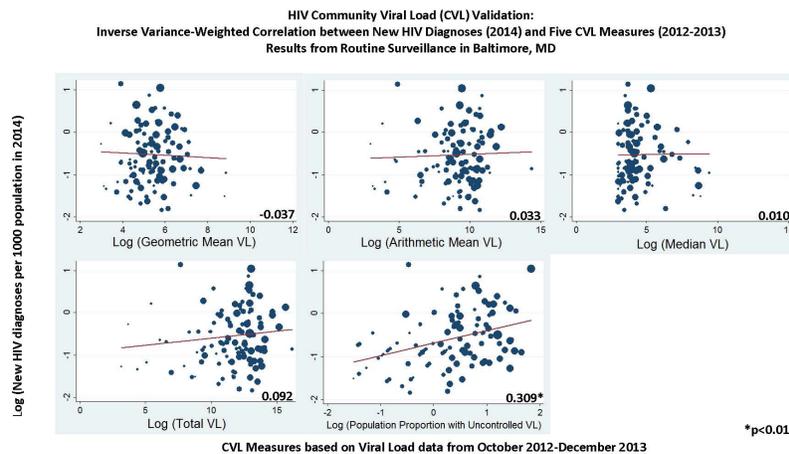
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**Background:** Reductions in HIV Community Viral Load (CVL) are associated with decreased HIV infections. In an environment of constrained public health resources, HIV control activities may be best targeted to high CVL areas or CVL “hotspots.” There is a lack of consensus, however, among HIV experts regarding how best to calculate CVL, i.e. how to aggregate individual-level viral loads to community areas in order to best reflect community-level transmission dynamics. The objective of this analysis was to determine the extent to which each of five CVL metrics in the past year predicts new HIV cases in the subsequent year in one mid-Atlantic U.S. city with an established HIV epidemic. The goal of this analysis was to inform HIV control activities in the local jurisdiction.

**Methods:** We calculated 5 different CVL measures at the census tract level, using viral load data between October 2012 and December 2013 in Baltimore, MD via routine surveillance. Data includes point-of-diagnosis as well as in-care viral loads. The CVL measures derived from the HIV public health literature included arithmetic mean VL, geometric mean VL, median VL, total VL, and population proportion with unsuppressed VL (VL>400 copies/mL). We then used census tract level rates of new HIV diagnoses in 2014 to gauge the predictive value of each of the CVL measures via inverse variance weighted modeling of the relationships between log-transformed new diagnoses (2014) and log-transformed estimates of CVL (2012-13).

**Results:** The 2012-13 surveillance data was comprised of 2,542 HIV-infected individuals living in 96.5% (193/200) Baltimore City census tracts. The study population was 65.0% male and 84.5% African American, with a mean age of 47.1 years. 9.1% of individuals were newly diagnosed and 33.5% had an unsuppressed viral load. Of the CVL metrics analyzed, proportion with unsuppressed VL was most strongly associated with new cases in the subsequent year (correlation=0.309; p=0.001). The remaining CVL measures were not significantly associated with new cases (see Figure).

**Conclusions:** Population proportion with unsuppressed VL in the past year was the CVL measure that was most predictive of new HIV diagnoses in the subsequent year. In this one mid-Atlantic U.S. city, population proportion with unsuppressed VL may be the most appropriate measure for utilization in targeted HIV control activities.



**1037 HIV Viral Suppression Among Adults Diagnosed With Depression in the United States**

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 CDC, Atlanta, GA, USA

**Background:** Reducing new HIV infections and improving health outcomes for people living with HIV are 2020 National HIV/AIDS Strategy (NHAS) goals achievable through viral suppression induced by antiretroviral therapy (ART). HIV patients' depression may play a role in providers' decisions to initiate ART and in HIV patients' ability to adhere to ART and achieve viral suppression.

**Methods:** Data were collected during 06/2009–05/2013 from 18,095 Medical Monitoring Project (MMP) respondents. MMP is a surveillance system that produces nationally representative estimates of behavioral and clinical characteristics of HIV-infected adults receiving medical care in the United States. Using medical record data, we estimated the percentage of HIV-infected persons with diagnosed depression. We examined associations between diagnosed depression and sociodemographic and clinical characteristics, including ART prescription during the past year, ART adherence, and sustained viral suppression (all viral load measurements <200 copies/mL in past year). Multivariate logistic regression was used to estimate adjusted prevalence ratios for viral suppression among patients prescribed ART, with and without diagnosed depression.

**Results:** Overall, 25% (95% Confidence Interval [CI]: 23–28) of HIV-infected adults in care were diagnosed with depression. Of all HIV patients, 91% (CI: 91–92) were prescribed ART; among those prescribed ART, 69% (CI: 67–70) had sustained viral suppression. Compared to those without depression, patients with depression were more likely to be prescribed ART and, among those prescribed ART, less likely to be adherent and achieve viral suppression (Table 1). After adjustment for ART adherence and race, those with depression were less likely than those without depression to achieve viral suppression (adjusted Prevalence Ratio [PR]=0.93, [CI: 0.91–0.96]). Adjustment for other factors associated with both depression and viral suppression, such as age and injection drug use, did not change this association.

**Conclusions:** We did not find evidence that providers were less likely to prescribe ART to patients with depression. While ART adherence was lower among patients with depression, adherence alone did not account for the lower likelihood of viral suppression among patients with depression. Support of ART adherence in persons with depression and further exploration of reasons for lower viral suppression in these individuals will be instrumental in achieving NHAS 2020 goals.

**Table 1. ART Prescription, ART Adherence, and Viral Suppression among Adult HIV Patients Diagnosed with Depression: Medical Monitoring Project, 2009-2012, United States.**

	Prescribed ART <sup>1</sup> Weighted % (95% CI)	P for modified Rao-Scott chi-square test	ART Adherent <sup>2,4</sup> Weighted % (95% CI)	P for modified Rao-Scott chi-square test	Achieved Viral Suppression <sup>3,4</sup> Weighted % (95% CI)	P for modified Rao-Scott chi-square test
<b>Depression</b>						
<b>Yes</b>	93 (92-94)	0.0001	84 (83-86)	< 0.0001	66 (63-68)	< 0.0001
<b>No</b>	90 (90-91)		88 (86-89)		70 (68-71)	

<sup>1</sup> ART prescribed within the last 12 months

<sup>2</sup> 100% dose adherent over past 3 days

<sup>3</sup> All viral loads in last 12 months <200 copies/mL

<sup>4</sup> Calculated only among those prescribed ART

**1038 Clinician and Patient Attitudes Toward Financial Incentives for HIV Care (HPTN 065)**

**Jennifer H. Farris**<sup>1</sup>; Allison Zerbe<sup>2</sup>; Ann Kurth<sup>3</sup>; Brett Hanscom<sup>4</sup>; Laura McKinstry<sup>5</sup>; Barry Zingman<sup>6</sup>; Fred Gordin<sup>6</sup>; Deborah Donnell<sup>4</sup>; Bernard Branson<sup>7</sup>; Wafaa M. El-Sadr<sup>2</sup>

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**Background:** HPTN 065 examined the feasibility of an enhanced test, link-to-care, plus treat approach for HIV prevention in Bronx, NY and Washington, DC. Financial incentives (FIs) were assessed for effectiveness of enhancing linkage-to-care of HIV-infected persons and viral suppression for patients on antiretroviral therapy (ART). We surveyed ART-prescribing clinicians and HIV-infected patients at care sites in the two jurisdictions to assess attitudes about the use of FIs to enhance these HIV care outcomes.

**Methods:** All ART-prescribing clinicians at 37 participating care sites were asked by email to complete an anonymous web-based survey, administered 5/2013-12/2013, with a nominal incentive upon survey completion.

During an ACASI computer-based survey, patients enrolled in HIV care at 10 clinics (4 in Bronx, 6 in DC; 6 randomized to FI, 4 to standard of care) participating in HPTN 065 were asked similar questions from 12/2013-12/2014, to those asked of clinicians. Both surveys were conducted before FI effectiveness data were analyzed.

**Results:** We analyzed data from 141 clinicians (response rate of 53%) and from 725 patients, 479 (66%) from FI sites. Clinicians were female (57%), white (62%), 47 years (median) and physicians (67%) who reported caring for a median of 105 (interquartile range (IQR) 50-240) HIV-infected patients. Patient respondents were mostly male (69%); 62% African-American; median age 52 years (18-77); 42% were men who have sex with men, and 95% were on ART.

Sixty nine percent of clinicians and 78% of patients agreed or strongly agreed that it is a "good idea" to provide "rewards" to get patients to link to care, and 80% of clinicians and 72% of patients agreed or strongly agreed that monetary "rewards" will encourage linkage more quickly. Both clinicians and patients suggested a median of \$50 (clinician IQR \$25-\$75; patient IQR \$25-\$100) as a worthwhile FI for linkage. Seventy eight percent of clinicians and 69% of patients agreed or strongly agreed that "rewards" will help patients maintain ART adherence. Clinicians suggested a median of \$40 (IQR \$20-\$50) and patients \$50 (IQR \$25-\$100) as a worthwhile FI for an undetectable viral load. Of note, the suggested FIs were less than FI amounts used in the HPTN study.

**Conclusions:** The majority of both clinicians and patients indicated that the use of FIs would likely improve linkage-to-care and ART adherence. Clinicians and patients suggested similar dollar amounts for incentives.

**1039 A Commitment Contract for Virologic Suppression in Poorly Adherent HIV+ Individuals**

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<sup>1</sup>Stanford Univ, Stanford, CA, USA; <sup>2</sup>Harvard Univ, Boston, MA, USA; <sup>3</sup>Emory Univ, Atlanta, GA, USA; <sup>4</sup>Emory Univ Sch of Med, Atlanta, GA, USA

**Background:** Effective interventions to improve adherence have remained an elusive goal. The goal of this clinical trial was to determine whether a commitment contract informed by behavioral economics principles would lead to persistent virologic suppression (VS) among HIV+ patients with poor antiretroviral therapy (ART) adherence.

**Methods:** This study was a randomized controlled trial enrolling patients failing ART, combined with a non-randomized passive control group in a publicly funded HIV clinic serving inner-city Atlanta, GA, USA. Patients had to be ≥18 years and have evidence of virologic failure (plasma viral load (pVL) >200 copies/mL) while receiving ART. 19 individuals were randomized to a commitment contract arm (behavioral economics, BE, arm), and 21 individuals were randomized to a conditional cash transfer arm (active control, AC, arm). In addition, 92 individuals served as passive controls (PC arm). The PC arm received routine care and no financial incentives. The AC arm received conditional cash transfers of \$30 if they attended their regularly scheduled provider visits. The BE arm was offered a commitment contract developed using behavioral economics insights. BE participants had

a choice either to receive \$30 conditional on attending their provider visit or to receive \$30 conditional on both attending their provider visit and surpassing an ART adherence threshold. 6 of the 19 individuals in the BE arm chose the conditional contract. The primary endpoints were VS (pVL<=200 copies/mL) at the end of the incentive period and at an unanticipated post-incentive study visit that occurred approximately 6 months after incentives ended.

**Results:** Overall median age was 41.49 years, 77.5% were male, and 87.5% were Black. Final results showed the odds of VS at the end of the incentive period were higher in the BE arm compared to the AC arm and compared to the PC arm [adjusted odds ratio (AOR) 1.336, 95%CI 0.325 to 5.498, p=0.688 and AOR 5.223, 95%CI 1.630 to 16.776, p=0.005, respectively]. Importantly, the differences were larger at the unanticipated post-incentive study visit [AOR 3.088, 95%CI 0.764 to 12.484, p=0.114 and AOR 5.791, 95%CI 2.031 to 16.508, p=0.001, respectively].

**Conclusions:** This research demonstrated the feasibility of using commitment contracts in routine HIV care to persistently improve ART adherence and VS. Financial rewards coupled with individual choice might sustain behaviors that would otherwise dissipate when incentives are removed.

#### 1040 Quantifying Viral Load Distribution in a Clinic Population Using the Lorenz Curve

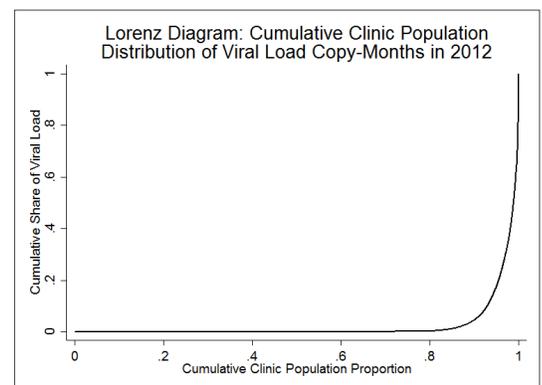
**Katerina Christopoulos;** Wendy Hartogensis; David V. Glidden; Christopher Pilcher; Monica Gandhi; Elvin H. Geng  
*Univ of California San Francisco, San Francisco, CA, USA*

**Background:** Quantifying the extent to which viremia is concentrated within groups or equally distributed in a population can inform engagement efforts. We apply the Lorenz curve, which plots cumulative population viral burden against cumulative patient population, to characterize the distribution of viremia in a safety-net HIV clinic.

**Methods:** We extracted demographic and clinical data from the electronic medical record of patients who had a primary care visit to a safety-net HIV clinic in San Francisco in 2012. We excluded patients not in care for at least 6 months at the first 2012 visit. We calculated viral copy-months for 2012 using a published trapezoidal method in which the months in an interval were multiplied by the average of two measurements defining the interval. Lorenz curves were generated in Stata 13 using the population percentiles for the cumulative distribution of total viral load copy-months in 2012. We then calculated unadjusted and adjusted odds ratios for being in the top 10<sup>th</sup> percentile of viral load copy-months. The main multivariate model included age, gender, race/ethnicity, HIV risk factor, perfect retention in care (no missed visits and no gap in care >180 days), and ART initiation.

**Results:** In 2012, there were 1,917 established patients, of whom 40 had no viral load results. Of the remaining 1,877 patients, the median age was 47 (range 18,78), most were men (87%), and just over half were men who have sex with men (53%). Whites comprised 47%; blacks 22% and Latinos 24%. ART initiation had occurred in 97% of patients and 28% had perfect retention. Median CD4 cell count was 499 (IQR 330-694) and median viral load was 20 (range 20 – 2.6 x 10<sup>6</sup>). The total number of viral copy-months was 2.33 x 10<sup>8</sup>, and 10% of patients held 94% of the virus. In a multivariate model controlling for the expected protective effects of ART initiation (OR 0.49, p=0.04) and perfect retention (OR 0.29, p<0.001), factors that increased the odds of being in the top 10<sup>th</sup> percentile of viral load copy-months were age 18-29 years and 30-49 years vs. age 50 and above (OR=6.3, p<0.0001 and OR=2.6, p<0.0001), black vs. all other races/ethnicities (OR 1.7, p=0.01), or history of IDU vs. MSM or heterosexual (OR 1.5, p=0.04).<

**Conclusions:** In a public HIV clinic, younger age, black race, and history of IDU were associated with a greater share of viral copy-months. The cumulative distribution function of the Lorenz curve offers a novel method to identify populations that could benefit from additional resources.



#### 1041 Recent Increases in Virologic Suppression Among HIV-Positive MSM in Vancouver, Canada

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*<sup>1</sup>Univ of British Columbia, Vancouver, BC, Canada; <sup>2</sup>BC Cntr for Excellence in HIV/AIDS, Vancouver, BC, Canada; <sup>3</sup>Univ of California San Francisco, San Francisco, CA, USA; <sup>4</sup>Univ of Victoria, Victoria, BC, Canada; <sup>5</sup>Vancouver Coastal Hlth, Vancouver, BC, Canada; <sup>6</sup>Simon Fraser Univ, Burnaby, BC, Canada*

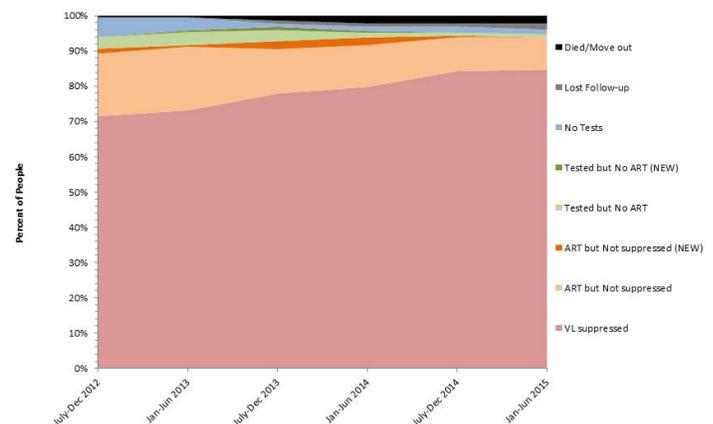
**Background:** MSM account for 60% of new HIV diagnoses in British Columbia (BC). We examined trends in virologic suppression and the determinants of significant viremia among HIV-positive participants in a cohort of MSM in Vancouver.

**Methods:** Male participants aged ≥16 years and reporting sex with a man in the past six months were recruited between February 2012 and January 2015 using respondent driven sampling. Participants completed a self-administered computer-based survey every six months and a nurse administered rapid HIV test. Data included sexual and drug-use behaviour, and mental health using the Hospital Anxiety and Depression Scale (HADS). Attitudes towards HIV treatment were measured using the HAART Optimism Scale. We linked study participant information to VL and HIV treatment data in the BC HIV Drug Treatment Program to determine the proportion at each care cascade step during each six-month period from July 2012 to June 2015. We used generalized estimating equation modelling to identify factors associated with VL ≥200 copies/mL in each period.

**Results:** We recruited 774 participants, of whom 134 (17.3%) were seeds. Median age was 33 years (IQR: 26-47). Of these, 210 participants were HIV positive prior to July 2012; an additional 19 seroconverted by the final time period. We observed a significant trend towards increased levels of virologic suppression (VL<200 copies/mL) from 79% of HIV positive participants in the first six month period to 85% in the final period (p<0.001 for trend). An average of 14% of HIV positive participants were receiving ART, but were not VL suppressed, with another 1% newly diagnosed, and 7% either moved, died, lost to follow-up, not on ART or with no VL result in each six month period. Unsuppressed VL was independently associated with ecstasy use (adjusted OR [AOR]=2.00; 95% CI 1.22-3.26), crystal methamphetamine use (AOR=1.58; 95% CI 1.01-2.45), and mild symptoms of depression (HADS-depression subscale score >8) (AOR=1.61; 95% CI 1.00-2.58). Older participants (AOR=0.97 per year; 95% CI 0.94-1.00) and those with greater HAART Optimism scores (AOR=0.91 per unit; 95% CI 0.87-0.95) were less likely to have episodes of unsuppressed VL.

**Conclusions:** Our results demonstrate a significant trend towards increased VL suppression among HIV infected MSM, reaching 85% in early 2015. Most individuals with unsuppressed VL were diagnosed and receiving ART. Improved clinical management of depression and reducing drug-use should be promoted as a means to optimize virological con

Cascade of care for HIV positive MSM in Vancouver, Canada: 2012 - 2015



**1042 State-Space Models for Engagement, Retention, and Reentry in the HIV Care Cascade**

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**Background:** We propose a state space representation of the HIV care cascade and corresponding statistical framework that can describe the longitudinal dynamics along the phases in the cascade. For this representation, each phase in the HIV care cascade is viewed as a 'state'. Then various types of patient behaviors such as the cyclical process of engagement, disengagement, and re-entry into care (a.k.a. 'churn') in the cascade are described in terms of transition from one state to another. We illustrate the proposed framework using data on 57,596 patients enrolled in AMPATH (a partnership between Kenya and North American institutions) between 2008 and 2012. As testing, linkage, and viral load data was highly limited, we focused on retention aspects of care.

**Methods:** We operationalized a 5-state care cascade including engaged in care, disengaged from care, transferred-out, lost-to-follow-up (LTFU), and deceased. Transition probabilities were defined by  $P_{jk}(t) = \Pr(S(t)=k | S(t-1)=j)$  where  $S(t)$  denotes the state at time  $t$ . For example,  $P_{11}(t)$  represents probability of transition from engaged to engaged, which is retention;  $P_{21}(t)$  is probability of transition from disengaged to engaged, which is re-entry into care. We used a multinomial regression for longitudinal data to capture the effects of covariates on state transitions.

**Results:** On average, in a given 200-day interval, 78% of individuals were engaged in care, 16% disengaged, 0.4% transferred, 5% LTFU, and 1% died. Among those engaged at a given time, probability of retention, disengagement, transfer-out, and death were .84, .13, .001, and .01. Once disengaged, probability of return to care, continued disengagement, LTFU, and death were .10, .54, .35, and .004. Regression modeling identified that among those engaged, patients with  $CD4 > 350$ , age  $> 35$  and patients on ARV were more likely to remain engaged in care, while male patients and those whose CD4 was not recently measured were substantially less likely. Among those disengaged, older patients, those previously on ART, and those with  $CD4 < 350$  were more likely to return to care, and less likely to be permanently LTFU.

**Conclusions:** Our representation of the HIV care cascade is a novel application of state space models, and includes regression formulations. It provides a unified approach to modeling individual-level longitudinal data from a clinical cohort. A simple version of the model is illustrated here, but an extended version includes testing, initial linkage, and viral suppression.

Table. Overall state transition rates (STR) from engaged (or disengaged) state and relative risk ratio (RRR) for effect of covariates on the STR. (\*Note: transition from disengaged to transfer-out was removed from the model due to the small sample size (n=44))

State at t-1	Engaged in care (S(t-1)=1)				Disengaged from care (S(t-1)=2)			
	Engaged (S(t)=1)	Disengaged (S(t)=2)	Transfer-out (S(t)=3)	Death (S(t)=5)	Engaged (S(t)=1)	Disengaged (S(t)=2)	LTFU (S(t)=4)	Death (S(t)=5)
<b>Rate of transition from 'engaged' at time t - 1 to state at t</b>				<b>Rate of transition from 'disengaged' at time t - 1</b>				
	0.84	0.13	0.01	0.01	0.1	0.54	0.35	0.04
<b>Relative risk ratio (RRR) for effect of covariates on state transition rates (relative to disengagement at t)</b>								
On ARV	2.90 (2.82, 2.97)	—	2.62 (2.29, 3.01)	1.59 (1.47, 1.73)	2.34 (2.19, 2.50)	—	0.76 (0.74, 0.78)	1.15 (0.85, 1.56)
Age $\geq 35$ at enrollment	1.37 (0.86, 0.90)	—	0.81 (0.73, 0.91)	1.40 (1.30, 1.51)	1.13 (1.07, 1.20)	—	1.05 (1.02, 1.08)	1.45 (1.07, 1.96)
Male	0.88 (0.86, 0.90)	—	0.78 (0.69, 0.88)	1.55 (1.44, 1.66)	1.03 (0.97, 1.09)	—	0.96 (0.94, 0.99)	1.09 (0.81, 1.47)
Most recent CD4 < 350	ref	ref	ref	ref	ref	ref	ref	ref
Most recent CD4 $\geq 350$	1.69 (1.65, 1.73)	—	0.98 (0.87, 1.11)	0.27 (0.24, 0.30)	0.84 (0.78, 0.90)	—	1.14 (1.11, 1.17)	3.04 (1.99, 4.65)
CD4 not measured in last 200 days	0.34 (0.33, 0.36)	—	0.41 (0.32, 0.51)	0.46 (0.42, 0.52)	0.72 (0.65, 0.79)	—	0.91 (0.87, 0.96)	1.73 (1.02, 2.96)

**1043 How Far Are We From Early cART for All? A Nationwide Population-Based Study in France**

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**Background:** Ensuring early universal access to combination antiretroviral treatment (cART), and especially within the first year of HIV infection, is critical to reach the end of AIDS and control the HIV epidemic. However, how far or how close we are from early universal cART initiation remains unknown.

**Methods:** We estimated the timing of HIV care in France in 2010 using statistical modeling and two large data sources: the national HIV surveillance system and the French Hospital Database on HIV (FHDH). To estimate the distribution of times from infection to diagnosis, we fitted a back-calculation model to the annual numbers of new HIV diagnoses. To estimate the distribution of times from HIV diagnosis to care entry, from care entry to cART initiation and from cART initiation to reaching undetectable viral load, we used survival methods and data on the dates of HIV diagnosis, care entry, cART initiation and viral suppression of the 6268 HIV-infected individuals who newly engaged in care between 2008 and 2010 and were enrolled onto the FHDH cohort. We summed up the distributions to obtain the distributions of time intervals from HIV infection to cART access. Figures were computed overall and by HIV exposure group.

**Results:** We found that only 8.3% of HIV-infected individuals accessed cART within the first year of infection (see Table). This proportion reached 10.1% among men who have sex with men (MSM). The estimated median time interval from HIV infection to cART initiation was 5.0 years (IQR: 2.7-7.9). MSM had the shortest median time to cART initiation (4.4 years) and injecting drug users (IDUs) the longest (8.1 years). Time lost in accessing cART was mainly due to delays in HIV testing (overall median: 3.2 years), except for IDUs where it was also due to delayed care entry once diagnosed (median of ~1 year versus <1 month for other groups). Times to access cART once in care and times to reaching viral suppression once on cART were short (<6 months in median).

**Conclusions:** Our study shows that even in a country like France, where the health care system offers one of the best environments for HIV care, we are far from early ART for all. Similar gap is likely to exist in other settings and should be investigated. To close this gap, evaluating patient flow-time through the continuum of care will be key to identify what kind of actions is needed to accelerate cART access.

**Table: Estimated distribution of times (in months) between selected stages of the HIV care cascade in France in 2010**

	Time from HIV infection to diagnosis	Time from HIV diagnosis to entry in HIV care	Time from care entry to cART initiation	Time from HIV infection to cART		Time from cART initiation to viral suppression (<50 copies/mL)
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	% accessing cART within first year of infection	Median (IQR)
Overall	38.2 (14.6-66.4)	0.5 (0.2-2.3)	5.4 (1.3-22.9)	60.1 (32.9-94.5)	8.3%	5.5 (3.0-9.8)
MSM	26.8 (7.4-55.6)	0.6 (0.2-2.4)	10.3 (1.7-26.2)	52.2 (27.3-84.1)	10.1%	5.6 (3.0-9.3)
IDUs	38.3 (14.9-68.9)	11.1 (0.3-135.0)	3.5 (1.2-21.3)	96.8 (47.9-201.8)	5.5%	6.5 (3.2-14.4)
French heterosexual women	50.0 (28.2-73.7)	0.7 (0.2-2.8)	6.1 (1.2-25.0)	70.3 (44.5-101.8)	5.0%	4.9 (2.7-8.2)
Non French-national heterosexual women	36.2 (16.5-60.8)	0.7 (0.2-2.3)	3.5 (1.1-15.7)	56.1 (30.1-85.0)	8.3%	4.4 (2.6-7.2)
French heterosexual men	46.8 (19.0-79.9)	0.4 (0.1-1.4)	5.4 (1.1-21.8)	66.3 (36.4-103.4)	6.7%	6.0 (3.3-10.9)
Non French-national heterosexual men	48.0 (22.0-77.5)	0.5 (0.1-1.7)	3.2 (0.9-13.8)	65.9 (35.1-99.4)	7.2%	5.9 (3.7-11.6)

IQR: inter-quartile range

**1044 Using Treatment As Prevention Could Eliminate the HIV Epidemic in MSM in Copenhagen**

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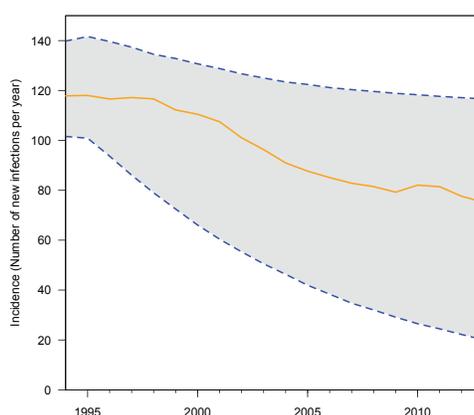
**Background:** The WHO has proposed using “treatment as prevention” (TasP) to eliminate HIV, and UNAIDS has proposed treatment targets to be met by 2021. However, the effectiveness of TasP in the “real-world” remains unknown. We determine the impact that TasP has had on the HIV epidemic in the MSM community in Copenhagen over the past ~20 years. UNAIDS has identified Copenhagen as a priority city, and the MSM community as a priority risk group, for HIV elimination. The WHO HIV elimination threshold is one new infection per 1,000 individuals per year.

**Methods:** We use a Bayesian CD4-staged back-calculation approach to analyze historical treatment and diagnosis data from the Danish HIV Cohort Study (DHCS): we begin in 1996 when effective therapies were introduced. We then use a predictive model that simulates transmission dynamics from 2013 to 2025. The model is parameterized to reflect the epidemiological conditions in the MSM community in Copenhagen. The back-calculation model and DHCS treatment data provide initial conditions for the predictive model.

**Results:** Our results show, between 1996 and 2013, the number of MSM in Copenhagen capable of transmitting HIV decreased by ~63%: from 2,218 (median, 95% Bayesian credible interval, BCI: 1,955-2,381) to only 819 (median, 95% BCI: 463-1,065). In addition, the annual number of new infections decreased by ~36%: from 117 (median, 95% BCI: 94-140) to 75 (median, 95% BCI: 20-117), see Figure. We estimate by 2013 treatment coverage had reached 73% (median, 95% BCI: 67-83%). We found coverage increased as incidence decreased. Using our transmission model we predict the WHO elimination threshold will be reached by 2021. We predict the annual incidence in 2021 will be 0.9 (median, BCI: 0.6-1.1) new HIV infections per 1,000 MSM. This will result in 51 (median, BCI: 39-64) new infections.

**Conclusions:** Our study provides a proof-of-concept that TasP could be effective in eliminating HIV in resource-rich settings. Importantly, our results show that the HIV epidemic in the MSM community in Copenhagen is very close to the WHO elimination threshold. Notably, the conditions in Copenhagen have been optimal for TasP to have had a significant impact: high treatment coverage, high viral suppression rates, and high retention. Even under these optimal conditions, it has taken several decades for TasP to have a population-level effect. This implies that it will be essential to use other interventions, such as pre-exposure prophylaxis, in combination with TasP.

Fig. 3A:



**1045 Estimated HIV Transmissions to Female Partners of HIV-Infected Men**

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**Background:** The majority of HIV-positive females acquired HIV through heterosexual contact. To better understand the dynamics of male-to-female HIV transmission, we used data on behaviors and viral suppression among HIV-positive males to model HIV transmissions to their female partners.

**Methods:** Using weighted respondent-level data on risk behaviors and viral load of HIV-positive persons in the United States from CDC’s National HIV Behavioral Surveillance and Medical Monitoring Project, and population-size estimates from the National HIV Surveillance System, we developed a static, deterministic model to estimate the number of HIV

transmissions from HIV-positive males to their female partners in 2009 stratifying by the attributes of HIV-positive men, including their primary risk factor for acquiring HIV: male-to-male sex (MSM), heterosexual sex, and injection drug use (IDU).

**Results:** An estimated 864,301 HIV-positive men had 950,178 female sex partners, of whom 873,508 were of HIV-negative or unknown sero-status (discordant). We estimated that, on average, HIV-positive MSM had 0.3 HIV-discordant female sex partners, heterosexual men had 3.0 female partners, and persons who inject drugs (PWID) had 2.3 partners. Most transmissions to females were from main partners (82%). Of estimated HIV transmissions to females, 27% of the males had MSM as their primary risk factor for acquiring HIV, 35% heterosexual sex, and 31% IDU. Transmissions to females varied by male partner's race and primary risk factor. White males transmitting HIV to females mostly acquired HIV through heterosexual sex (73%); among Hispanic males, IDU was the most common primary risk factor (50%); the primary risk factor of black males varied: for an estimated 34% it was MSM, 38% heterosexual sex, 23% IDU, and 5% both MSM and IDU.

**Conclusions:** Reducing HIV among high-prevalence populations, especially MSM and IDU, can have important benefits for other populations. Most males transmitting HIV to female had MSM or IDU risk factors. Although a small percentage of HIV-positive MSM reported female partners, MSM make up a large portion of the prevalent HIV infections and consequently comprise a substantial proportion of transmissions to females.

**Estimated percentage of HIV-positive males, mean number of female sex partners, and percentage of HIV transmissions from males to females by primary risk factor for acquiring HIV among males**

Primary risk factor for acquiring HIV among males	HIV-positive males	Mean number of female partners <sup>2</sup>		HIV transmissions to females
		All	Discordant <sup>2</sup>	
Male-to-male sexual contact (MSM)	67%	0.30	0.29	27%
Heterosexual contact	11%	3.16	3.02	35%
Injection drug use (IDU)	14%	2.68	2.34	31%
MSM-IDU	8%	2.06	1.88	7%

<sup>1</sup>Estimated mean number of female partners per HIV-infected male

<sup>2</sup>Limited to females who are HIV-negative or of unknown HIV status

**1046 Utility of the 1% HIV Prevalence Threshold in Defining Concentrated HIV Epidemics**

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**Background:** Regions with HIV prevalence < 1% are defined as experiencing 'concentrated' HIV epidemics, with larger epidemics labeled as 'generalized'. The surveillance and prevention implications include prioritizing resources on key populations (e.g. persons engaged in sex work [SW]) in 'concentrated' epidemics or on the general population otherwise. We hypothesize that the 1% threshold does not distinguish HIV epidemics by the underlying risk factors that sustain HIV spread, such as unprotected SW.

**Methods:** We developed synthetic HIV epidemics using deterministic mathematical models of heterosexual HIV transmission, using sub-national demographic, behavior, and biological data in Sub-Saharan Africa synthesized from a systematic review of these data across subpopulations. We generated 9000 plausible HIV epidemics of three types, according to risk factors that sustain HIV spread: concentrated (which we define as where unprotected SW was necessary and sufficient for HIV spread), generalized (defined as unprotected SW insufficient on its own for HIV spread), and mixed (defined as sustained epidemics that were neither concentrated nor generalized). We estimated the diagnostic performance of the general population HIV prevalence to distinguish a concentrated HIV epidemic from other types.

**Results:** The sensitivity, specificity, positive predictive value, and negative predictive value of the 1% prevalence threshold was 65%, 92%, 80%, and 84% respectively early in the HIV epidemics (circa 1990), indicating that 35% of the time the 1% threshold would fail to identify an epidemic exclusively sustained by sex work. If epidemic types were equally likely in real-life, then there is an 80% probability that an epidemic with <1% HIV prevalence is driven solely by sex work, and a 16% probability that an epidemic called 'generalized' because HIV prevalence is ≥1%, is in fact driven only by sex work. Sensitivity increased and specificity decreased later in the epidemics because rising condom use during sex work had reduced HIV prevalence in concentrated HIV epidemics. The findings were similar for differentiating between concentrated/mixed from generalized epidemics.

**Conclusions:** The 1% HIV prevalence threshold is neither sensitive nor specific enough to adequately define HIV epidemics, and hence should not form the basis of how surveillance and interventions should be prioritized. The 1% threshold should be replaced with approaches which distinguish epidemic types based on risk factors that sustain HIV spread.

**Table 1. Diagnostic utility of the <1% threshold based on synthetic heterosexual HIV epidemics using parameter inputs from sub-national data across Sub-Saharan Africa**

Epidemic Type	Year (of outputs from synthetic epidemics)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Concentrated <sup>1</sup> vs. Non-concentrated (Mixed and Generalized)	1990	65%	92%	80%	84%
	2008	89%	75%	65%	94%
(Concentrated and Mixed) vs. Generalized	1990	60%	90%	93%	53%
	2008	83%	63%	85%	68%

<sup>1</sup>Exclusively sustained by unprotected sex work  
PPV (positive predictive value); NPV (negative predictive value)

**1047 The Role of Young Women in the HIV Epidemic in Benin, West Africa**

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**Background:** Young women are particularly vulnerable to acquiring HIV in Sub-Saharan Africa. But their contribution to onward HIV transmission has not been explored. In Cotonou, Benin, HIV prevalence declined from 3% to 0.5% (1998 to 2008) among men but remained stable at 3.6% among women. The prevalence decline in men followed a

decline in female sex workers (FSW) prevalence during the SIDA1/2/3 FSW HIV prevention intervention. We aimed to estimate the population attributable fraction (PAF) of young women to overall HIV incidence during the HIV epidemic compared to FSW.

**Methods:** We used a deterministic age-stratified model of HIV transmission informed and calibrated to Cotonou demographic, sexual behaviour and HIV prevalence data by subgroups and time. The model was used to derive the fraction of all HIV infections acquired (acquisition PAF, A-PAF) or transmitted (transmission PAF, T-PAF) by young women aged 15-24 (including/excluding FSW) over different periods (epidemic onset-1993, 1993-2012, 2005-2015). We compared these to PAFs for FSW of all ages, defined as the relative difference in number of incident HIV infections between the baseline scenario and a counterfactual with HIV acquisition risk or transmission risk set to 0 in the relevant subgroup.

**Results:** Young women contributed more to HIV acquisition than transmission, but FSW contributed more to transmission over each time period. Young women's T-PAF and A-PAF increased over time, and their acquisition of infection was estimated to have contributed to 22% (12-36%) of all HIV infections between the epidemic onset and 1993, increasing to 47% (28-67%) between 1993-2012, and 29% (9-50%) between 2005-2015, and slightly more when young FSW were included (Figure, A-PAF). Young women alone may have contributed to less than 25% (12-43%) of all HIV transmissions over the different periods (Figure, T-PAF). The FSW T-PAF declined over time (T-PAF= 92% onset-2013, but 7% between 2005-15), to the same order as young women's T-PAF.

**Conclusions:** Although the contribution of FSW to HIV transmission has declined over time, their role in driving onward HIV transmission remains as important as the role of young women, suggesting that HIV prevention, especially HIV treatment for FSW, must be maintained and enhanced. Young women remain highly vulnerable to HIV acquisition, highlighting the need for prevention interventions focused on them, but their risk alone cannot sustain the HIV epidemic in Benin, nor can interventions focused only on young women end it.

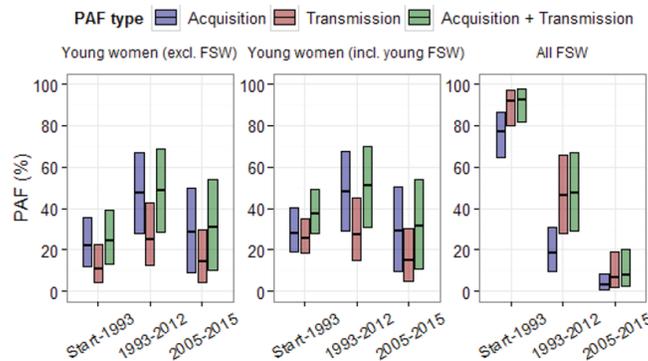


Figure 1 shows the acquisition and transmission PAF (5<sup>th</sup>, 50<sup>th</sup> and 95<sup>th</sup> percentiles) over various time periods of young women (aged 15-24), both excluding and including young FSW, compared to the corresponding PAF for all FSW (aged 15-59).

**1048 Effect of Eliminating CD4 Thresholds on Number of New ART Initiators in South Africa**

Jacob Bor<sup>1</sup>; Shahira Ahmed<sup>2</sup>; Matthew P. Fox<sup>2</sup>; Sydney Rosen<sup>2</sup>; Frank Tanser<sup>3</sup>; Deenan Pillay<sup>3</sup>; Till Bärnighausen<sup>4</sup>

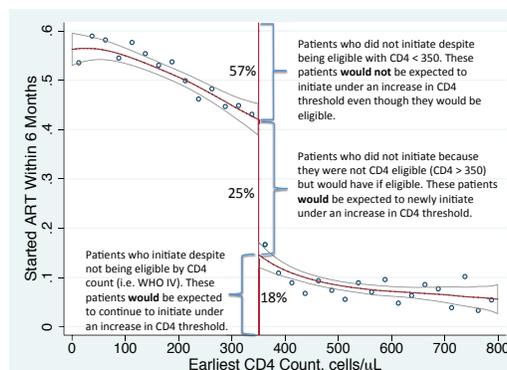
<sup>1</sup>Boston Univ Sch of PH, Boston, MA, USA; <sup>2</sup>Boston Univ, Boston, MA, USA; <sup>3</sup>Africa Cntr for Hlth and Pop Studies, Mtubatuba, South Africa; <sup>4</sup>Harvard Sch of PH, Boston, MA, USA

**Background:** WHO now recommends eliminating CD4 count eligibility criteria for ART, with the goal of expanding the numbers of HIV-infected persons on therapy. Using a novel quasi-experimental method to obtain empirical estimates of the effect of CD4 eligibility on ART uptake, we predict the total number of new ART initiators in South Africa that would result from eliminating CD4 thresholds.

**Methods:** We analyzed clinical records from all patients (n=11,307) in the Hlabisa sub-district public sector ART program with a first CD4 count between August 2011 and December 2012. Using a regression discontinuity design and the 350-cell threshold, we estimated the proportions of patients: initiating ART within 6 months due to a Stage 3 or 4 condition, initiating ART due solely to CD4 count, and not initiating ART despite being eligible. We also estimated the distribution of first CD4 counts: <350, 350-500, and >500. Using national (NDoH) data on the number of ART initiators in 2013 (n=614,000), we estimated the number of new initiators per year if CD4 criteria were eliminated.

**Results:** In our study, 18% of patients initiated ART due to condition and would initiate under any threshold (Fig, bottom). An additional 25% would initiate if the threshold was increased (Fig, middle) and 57% would not initiate despite having an eligible CD4 count (Fig, top). Under a policy extending eligibility to all patients regardless of CD4 count, just 30% {25/(25+57)} of patients newly eligible would be expected to initiate ART within 6 months. Of all patients seeking care, 55% (6256) had a first CD4 <350 cells; 20% (2223) CD4 350-500 cells; and 25% (2858) CD4 >500 cells. Relative to the proportion of all patients initiating within 6 months (32%, 3658), raising the threshold to 500-cells is expected to have increased the number of initiators by 16% {20\*25/32}. Eliminating CD4 criteria will increase new initiators by an additional 17% {25\*25/(32\*1.16)}. If these numbers hold nationally, then South Africa can expect 98,000 additional initiators per year from raising the threshold to 500 and a further 121,000 initiators per year from eliminating CD4 criteria.

**Conclusions:** Eliminating CD4 thresholds would lead to timely ART initiation by an additional 121,000 South Africans per year (a 4% increase in the total number on ART). Twice that number will enter care and not initiate. Removing CD4 criteria alone, without improving HIV testing, linkage to care, and ART initiation procedures, will not achieve the country's 90-90-90 targets.



**1049 Optimizing Resource Allocation to Reduce HIV Incidence Across Sub-Saharan Africa**

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Imperial Coll London, London, UK

**Background:** New prevention interventions offer substantial promise for capitalizing on previous progress against the HIV pandemic; but it has been unclear how these can be deployed jointly to best effect and this is complicated by geospatial heterogeneities in the epidemic and its drivers. We hypothesize that a strategy which confronts epidemic heterogeneity and considers available prevention interventions holistically—by allocating resources optimally between countries, subnational regions, population groups, and interventions—can achieve significantly greater impact than maintaining current patterns and bring us closer to the ambitious goal of ending AIDS as a public health threat by 2030.

**Methods:** We have developed a mathematical model that captures 80% of the HIV burden across sub-Saharan Africa, describing HIV transmission dynamics among adults in low-risk and key populations at the subnational (administrative level 1) scale in 18 countries. In each subnational region we have calibrated the model against antenatal clinic, survey, census, key population, and treatment data. We use this modeling framework to interrogate the performance of combination prevention strategies that exploit epidemic heterogeneity to varying degrees.

**Results:** The models find that, given our assumptions, optimizing combinations of available prevention interventions according to subnational epidemiology can reduce overall HIV incidence by 75% from 2010 levels by 2030, exceeding the 50% reduction achievable under current allocation patterns for the same total expenditure. Moreover, we confirm that single-value thresholds (for example based on incidence) are insufficient metrics for determining when a particular intervention should be used; instead a holistic 'allocation' perspective should be taken. Broadly, a transition from current allocation patterns to the optimal topography would shift funds out of regions with declining epidemics and into those with emerging epidemics and high disease burdens.

**Conclusions:** While details of future funding allocation depend on economic and political factors beyond our scope, this work leverages both the powerful suite of available prevention strategies and our understanding of the geospatial heterogeneity of HIV to set out the direction of travel toward the most cost-effective and impactful overall pattern of funding for sub-Saharan Africa.

**1050 New ARVs Could Represent Over USD 3 Billion in Cost Savings Through 2025**

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**Background:** Several new antiretrovirals (ARVs) are likely to be introduced in low- and middle-income countries (LMICs) by 2019 that represent a range of clinical and cost advantages to current products. These include tenofovir alafenamide fumarate (TAF), low dose efavirenz (EFV400), and dolutegravir (DTG). As programs evaluate adoption of these products, it is important that they understand the cost savings implications of doing so.

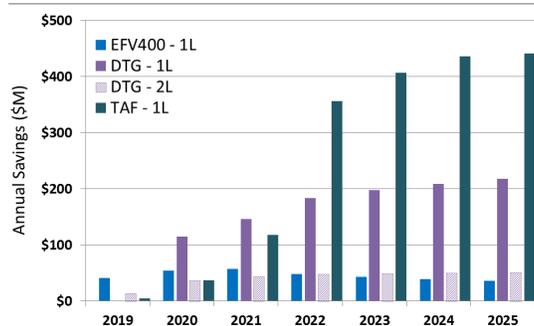
**Methods:** CHAI's forecast for currently available products was used as baseline. Historical uptake analogs as well as theoretical curves that fit a Gompertz function were used to model competition between new and current products based on anticipated clinical and price differentiation. Competitive sets were as follows: TAF displacing tenofovir disoproxil fumarate (TDF) and zidovudine (AZT) in first-line, EFV400 and DTG displacing EFV600 and nevirapine (NVP) in first-line, and DTG replacing TDF and AZT-based backbones in second-line. Prices over time were estimated based on market intelligence using raw material costs, formulation costs, threshold volumes required for economies of scale, and manufacturer profit margins. Each product's annual cost savings was calculated by multiplying the price differential between new and current products by the number of patients projected to be on the relevant new product each year.

**Results:** TAF was projected to aggressively replace TDF and AZT in first-line. By 2025, TAF would represent over 95% of that market and savings of over USD 1.5 billion. For first-line treatment, DTG was projected to aggressively replace EFV600 and NVP, representing 80–90% of that market by 2025, with EFV400 representing most of the remainder. Collectively, DTG and EFV400 may represent total savings of over USD 1 billion through 2025. Lastly, DTG was projected to replace over 90% of TDF and AZT-based backbone use in second-line, with associated cost savings of USD 300 million through 2025.

Current pricing methodologies and plans to advance approach will be shared.

**Conclusions:** TAF, EFV400, and DTG will enable programs in LMICs to put more patients on treatment due to lower per capita spend. Our findings support concerted efforts by national programs and donors to advocate for accelerated availability and strongly encourage uptake of new ARVs to realize their savings potential. Clear commitments for rapid adoption of these products would encourage more manufacturers to produce larger "at-scale" volumes at competitive prices, and help increase patients on care.

Estimated Savings from New ARVs 2019–2025\* (USD millions)



\*With higher launch prices, expect minimal to negative savings for first 1–3 years of DTG launch (2017–2019)

**1051 Impact of Improving HIV Care and Treatment and Initiating PrEP in the United States, 2015–2020**

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**Background:** Key strategies to reduce HIV incidence in the US are 1) improving diagnosis, care and treatment of people living with HIV (PLWH), and 2) delivering pre-exposure prophylaxis (PrEP) to people at risk for HIV. National HIV/AIDS Strategy (NHAS) 2020 goals provide targets for the first strategy, including increasing to 90% the proportion of PLWH who are diagnosed, to 85% the proportion linked to care (LTC), and to 80% the proportion diagnosed who achieve VLS. Clinical trials have established a large reduction in HIV transmission risk among PLWH who achieve VLS and the efficacy of PrEP in preventing HIV among men who have sex with men (MSM), people who inject drugs (PWID), and high-risk heterosexuals (HRH). However, the effectiveness of PrEP when layered onto improvements in the diagnosis, care and treatment of PLWH has not been well established.

**Methods:** We developed a dynamic, compartmental model of HIV transmission. In the base case, we estimated that in 2015, 87% of PLWH were diagnosed, 80% LTC and 36% VLS among diagnosed. In one scenario, we increased diagnosis to 90%, LTC to 85% and VLS to 60% by 2020. We repeated that scenario with VLS increased to 80%. For the base case and scenarios, we assessed the effect on HIV incidence from 2015 to 2020 of improvements in diagnosis, LTC and VLS alone. We then assessed the marginal benefit of initiating PrEP

in 2015 among 40% of high-risk MSM, 10% of PWID and 10% of HRH and maintaining constant coverage through 2020. We applied a 96% reduction in HIV sexual transmission to PLWH who achieved VLS and assumed PrEP efficacy of 73% for MSM, 75% for HRH and 49% for PWID.

**Results:** In the base case, PrEP reduced HIV incidence by 18% (48,221 cases) over 5 years. Compared with the base case, improving to 90% diagnosed, 85% LTC and 60% VLS among diagnosed reduced incidence by 34% (88,908 fewer cases); the marginal benefit of adding PrEP was an additional 12% (31,988 cases) reduction. Compared with the base case, the scenario with 80% VLS reduced incidence by 63% (168,132 cases); the marginal benefit of PrEP was an additional 7% (16,929 cases).

**Conclusions:** Increasing diagnosis, care and treatment of PLWH resulted in large decreases in HIV incidence by 2020. The marginal benefit of PrEP decreased as diagnosis, care and treatment improved. However, even at high levels of viral load suppression, PrEP continued to achieve reductions in HIV incidence over 5 years.

Table 1: The impact of improving diagnosis, care and treatment among PLWH and delivering PrEP to people at risk of HIV, United States., 2015-2020

	Total Number of New Infections, 2015-2020			
	HET	MSM	IDU	Total
Current continuum of care, No PrEP (Base Case)	72,069	166,448	26,813	265,330
Current continuum of care, PrEP	67,224	124,531	25,354	217,109
HIV infections prevented, n (%)*	4,845 (7%)	41,917 (25%)	1,459 (5%)	48,221 (18%)
Improved continuum of care (60% VLS among diagnosed), No PrEP	45,619	111,509	19,293	176,422
HIV infections prevented, n (%)*	26,450 (37%)	54,938 (33%)	7,520 (28%)	88,908 (34%)
Improved continuum of care (60% VLS among diagnosed), PrEP	42,646	83,539	18,248	144,434
HIV infections prevented, n (%)*	29,423 (41%)	82,909 (50%)	8,565 (32%)	120,896 (46%)
Improved continuum of care (80% VLS among diagnosed), No PrEP	25,346	59,522	12,331	97,198
HIV infections prevented, n (%)*	46,723 (65%)	106,926 (64%)	14,482 (54%)	168,132 (63%)
Improved continuum of care (80% VLS among diagnosed), PrEP	23,702	44,906	11,661	80,269
HIV infections prevented, n (%)*	48,367 (67%)	121,541 (73%)	15,152 (57%)	185,060 (70%)

\* Compared to the base case (current continuum of care, no PrEP)

## 1052 PrEP Is Only Cost-Effective Among MSM in the Netherlands When Used on Demand

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**Background:** Pre-exposure prophylaxis (PrEP) with tenofovir and emtricitabine has been demonstrated to effectively prevent new HIV infections among men who have sex with men (MSM). It has recently been shown that on demand PrEP use, in which individuals take PrEP just before and after sexual contact, can be just as effective as daily PrEP in preventing HIV infections. The high cost of PrEP is still a primary limitation in its use for HIV prevention. The aim of this study was to compare the epidemiological impact and cost-effectiveness of both daily and on demand PrEP compared to no PrEP.

**Methods:** A deterministic mathematical model was calibrated to the well-defined Dutch HIV epidemic among MSM. We aimed to predict the impact of PrEP targeted to 10% of the most sexually active individuals (median 4,500 MSM/year) over the coming 12 years, including two years of scale-up. Cost-effectiveness ratios were calculated to predict the cost-effectiveness of daily (€7,099/year) and on demand (€3,550/year) PrEP, and prevented infections were calculated to predict the epidemic impact. A decrease of 50% in the price of PrEP was considered in a sensitivity analysis, given that generic PrEP may soon become available. Cost-effectiveness ratios below €20,000 were considered to be cost-effective in this analysis.

**Results:** Targeting PrEP to 10% of the most sexually active individuals was predicted to prevent 14.0% (interquartile range [IQR] 11.9%-16.3%) of new infections over the coming 12 years compared to no PrEP usage. Daily PrEP was predicted to cost €36,300 per quality adjusted life year (QALY) gained (IQR €34,000-€45,100). On demand dosing of PrEP has the potential to cut the cost per QALY gained by more than half to a cost-effective €17,700 (IQR €16,500-€22,200) per QALY gained over 12 years compared to no PrEP usage. This cost per QALY can be further reduced to €8,400 (IQR €7,700-€10,700) if the cost of on demand PrEP is reduced by 50%. If increasing numbers of patients are diagnosed and placed on treatment early, however, PrEP will have a diminished impact on the epidemic. This can result in the cost per QALY gained of on demand PrEP to increase to as much as €32,400 with full-price PrEP, and €16,200 with reduced-price PrEP.

**Conclusions:** The use of PrEP is only cost-effective when used on demand, and can become far more cost-effective when generic PrEP becomes available. The precise cost-effectiveness estimate is, however, dependent upon the impact of earlier treatment initiation on the future HIV epidemic.

## 1053 Linkage of UK HIV and Tuberculosis Data Using Probabilistic and Deterministic Methods

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**Background:** Tuberculosis (TB)-HIV co-infection is a major cause of death worldwide. Understanding the impact of HIV and TB prevention and treatment programs requires high quality surveillance data of both diseases as well as co-infected persons. Internationally there is a lack of routine surveillance data on the proportion of cases with HIV who have had TB, and many TB surveillance systems do not consistently collect HIV data. Databases containing highly confidential information (e.g. HIV status) typically contain no unique identifiers and little identifying information, making record linkage difficult. The two key approaches to record linkage, probabilistic and deterministic, both have significant limitations, particularly when few common variables are available.

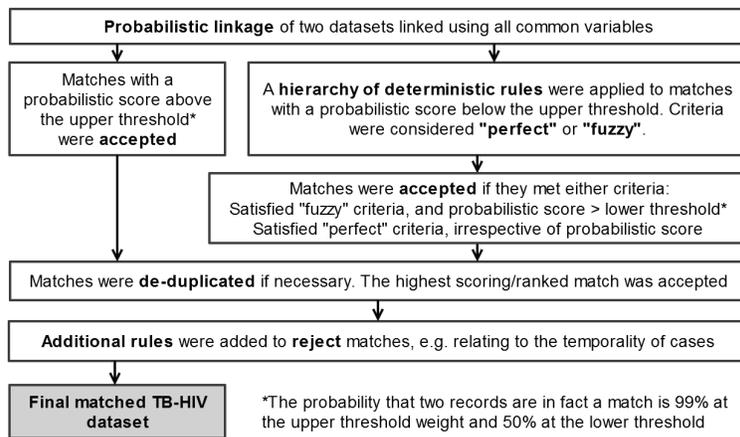
We developed a novel approach to link national HIV and TB surveillance datasets by trialling probabilistic and deterministic linkage methods, plus combined approaches.

**Methods:** A deterministic matching algorithm, using a hierarchy of matching criteria, was developed using Public Health England surveillance data; for 2001-2011 the TB database had 87,907 case reports and the HIV database 105,078. This was compared to an existing probabilistic method which utilised two thresholds of match weight for accepting/rejecting matches and manual review of records between these thresholds. With no unique identifiers for validation, we evaluated deterministic criteria by calculating the mean probabilistic weight of the matches they identified. Criteria with a mean weight greater than the upper threshold were included in the algorithm. We compared algorithms based on probabilistic, deterministic, and combined approaches.

**Results:** The novel, combined method accepts the above-threshold results from the probabilistic method, and the deterministic algorithm is then run in place of manual review to accept or reject intermediate-weight matches (Figure). Additional rules were added to accept/reject matches. The new method found 4308 (94.2%) and 4509 (85.5%) of the cases identified through probabilistic (n=4574) and deterministic matching (n=5277) respectively, and additional high-quality matches not identified by probabilistic (n=583) and deterministic (n=382) methods.

**Conclusions:** Our novel approach combines probabilistic and deterministic methods and is quicker and more repeatable with less potential bias than a method requiring manual review. We propose that this approach is considered when linking large datasets with minimal common variables.

Figure: The final hybrid method, a combination of probabilistic and deterministic methods



1054 Optimal Rollout of Treatment As Prevention in Africa: Efficiency Versus Equity

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**Background:** WHO, and UNAIDS, have proposed using “treatment as prevention” (TasP) to eliminate HIV in sub-Saharan Africa (SSA). We design treatment allocation strategies for TasP based on different optimization criteria: maximizing efficiency in prevention versus ensuring equity in access to treatment. Strategies are calculated in terms of the division of a supply of treatment among, and within, healthcare districts (HCDs). We focus on Lesotho, where HIV prevalence in the general population is 40% and treatment coverage ~30%.

**Methods:** We use kriging and adaptive bandwidth kernel density estimation to construct a concentration of infection (Col) country-level map of the HIV epidemic. We use georeferenced HIV-testing data from ~7,000 individuals, and high-resolution demographic data. We then use the Col map and optimization techniques to calculate, and compare, treatment allocation strategies that maximize either efficiency or equity.

**Results:** Our Col map shows the geographic location of all HIV-infected individuals (15 to 49 years old) in Lesotho. We estimate there are ~188,000 infected individuals; ~70% live in rural areas where the average Col is 4-10 infected individuals/km<sup>2</sup>, 30% live in urban areas where there are more than 400 infected individuals/km<sup>2</sup>. We found significant differences in allocation strategies depending upon whether the objective was to maximize efficiency in prevention or to ensure equity in access to treatment. More treatment is needed to maximize efficiency than to achieve equity in access in HCDs with a high Col. The converse occurs in HCDs with a low Col: more treatment is needed for equity than efficiency.

**Conclusions:** Our results have significant implications for rolling out TasP, and eliminating HIV in SSA. Our results apply to other countries in SSA with generalized HIV epidemics and a large rural population. Our results clearly show it will not be possible to maximize the efficiency of TasP and to ensure equity in access to treatment. Choosing to maximize efficiency will be more beneficial for uninfected individuals in urban areas: their risk of infection would be reduced. Choosing to ensure equity in access will be more beneficial for HIV-infected individuals in rural areas: their mortality risk would be reduced. Choosing to maximize efficiency would increase the probability of eliminating HIV, but would exacerbate the already significant health disparities between urban and rural communities.

1055 Modeled Effectiveness of Nondaily PrEP Based on Sex Coverage Data From HPTN067 ADAPT

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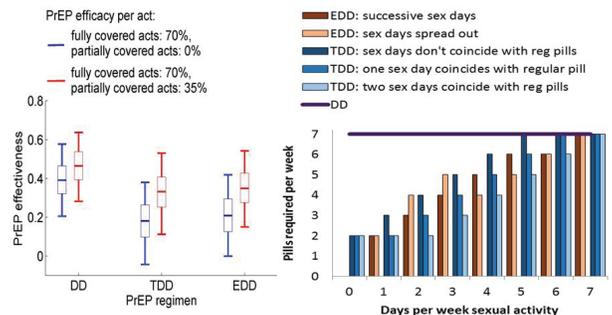
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**Background:** Non-daily PrEP dosing is a strategy that may be effective if sufficient PrEP doses correspond with sexual exposure. HPTN 067 ADAPT compared the feasibility of non-daily PrEP dosing regimens in populations at high risk. We modeled the reduction in HIV incidence and the number of pills that would be needed under different dosing regimens.

**Methods:** We used a stochastic mathematical model informed by South African data to simulate one year of sexual behavior of a female cohort (average 1.2 sex-days/week) under three PrEP regimens from the trial: daily (DD), time-driven (TDD, two regular pills/week 3-4 days apart plus one pill within 2h after sex) and event-driven (EDD, pills taken within 2 days before and 2h after sex) dosing. We explored a wide range of PrEP efficacy per sex act defined as fully covered if pills were taken within 2 days before and 1 day after an act and partially covered if only one of these pills were taken. Regimen effectiveness was estimated as 1 minus the ratio of HIV incidence when PrEP is used vs not used. As a proxy for costs saved, the number of pills required for each regimen was compared across different frequencies and distribution of sexual intercourse assuming perfect adherence.

**Results:** Data from the South African site suggest that 72% (21%), 36% (53%) and 42% (46%) of sex acts were fully (partially) covered with DD, TDD and EDD, respectively. At reported coverage, predicted PrEP effectiveness was 39%, 18%, 21% with DD, TDD and EDD, respectively, assuming 70% PrEP efficacy only for fully covered sex acts (see Figure). Regimens’ effectiveness increased to 47% (DD), 33% (TDD) and 35% (EDD) assuming 35% PrEP efficacy for partially covered sex acts. Assuming perfect adherence, 2, 3-4, and 4-5 pills/week are required with EDD for 1, 2 and 3 sex-days/week, respectively compared to 2-3, 2-4 or 3-5 pills/week with TDD and 7 pills/week for DD. Fewer pills are needed with EDD if sex-days are successive and with TDD if sex-days coincide with regular pill-taking days.

**Conclusions:** Non-daily PrEP may substantially reduce the number of pills required for the level of sexual activity observed in the HPTN 067 ADAPT trial. However, non-daily PrEP is unlikely to be as effective as daily PrEP in reducing HIV incidence among women in South Africa due to higher sex act coverage observed in the daily use arm. The significant proportion of sex acts partially covered by PrEP implies that the effectiveness of non-daily PrEP depends on the protection provided with partial dosing.



Left: Reduction in HIV incidence due to PrEP use based on 1000 simulations per scenario - interquartile range (box), 90% uncertainty interval (whiskers). Right: Pills needed by PrEP regimen for different frequency of sexual activity assuming perfect adherence

**1056 Scale-up of Antiretroviral Therapy and Preexposure Prophylaxis in Swaziland**

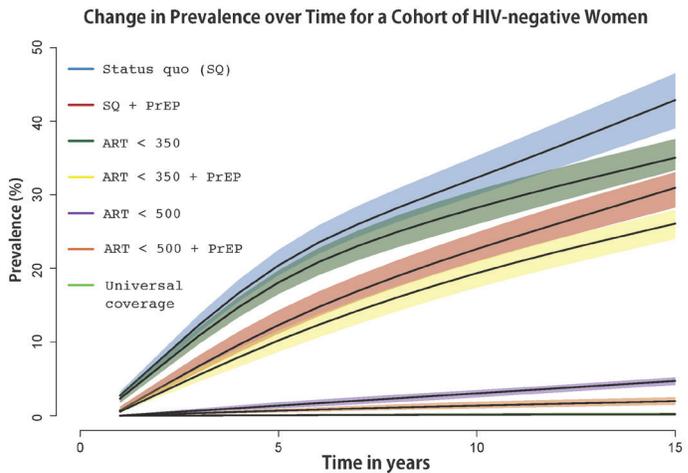
**Eugene T. Richardson<sup>1</sup>**; Futhi Dennis<sup>2</sup>; Nokwazi Mathabela<sup>2</sup>; Khanya Mabuza<sup>2</sup>; Allen Waligo<sup>2</sup>; Eran Bendavid<sup>1</sup>; Sabina Alistar<sup>1</sup>; Marelize Gorgens<sup>3</sup>; Francois Venter<sup>4</sup>  
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**Background:** With an adult prevalence of 31%, Swaziland has a severe, generalized HIV epidemic. Despite behavior change and other prevention programs, including scale-up of antiretroviral therapy (ART), new infections continue to be a problem, especially in young women (where incidence is 4.2%). The importance of population-specific combination prevention approaches to HIV has made mathematical modeling a necessary tool for planning efforts. As part of the evaluation of policy measures to end Swaziland's HIV epidemic by 2030, we modeled the efficacy and cost effectiveness of various treatment and prevention strategies.

**Methods:** Using demographic and epidemiological data from Swaziland, we constructed dynamic compartmental models as well as network models to assess the impact of ART scale-up as well as PrEP offered to 10% of the highest risk population over the next 15 years.

**Results:** Continuing the status quo—where ~\$110 million is spent yearly on HIV programs and the median CD4 at initiation is 234—will yield 10.5 million quality-adjusted life years (QALYs) between 2015–30. For an added \$110 million over 15 years, another 300,000 QALYs can be gained by offering PrEP to 10% of the highest risk population. This represents a cost of \$366 per QALY gained. Compared to status quo, scale up of ART to CD4 < 350 yields an additional 800,000 QALYs at \$288 per QALY gained, while universal ART coverage yields an additional 1.5 million QALYs at \$327 per QALY gained. Figure 1 shows the potential benefit of PrEP delivered to one high-risk group in particular—young women—over the next 15 years.

**Conclusions:** In the current setting of low median CD4 at ART initiation, immediate role-out of PrEP to 10% of the highest risk population is very cost-effective at \$366 per QALY gained. As the country gets to 100% test and treat, however, PrEP is no longer cost effective. Since scale up of ART to universal coverage will take many years, there is impetus to roll out PrEP to populations where both risk and PrEP adherence are deemed to be highest. This strategy is also supported by the most recent WHO guidelines, which recommend offering PrEP “to people at substantial risk of HIV infection,” specified as >3% incidence. Given the significant preventive benefit of ART scale-up, however, an important caveat for PrEP programs is that they should be rolled out only if they do not detract from existing ART programs or future ART scale-up.



**1057 Dapivirine Vaginal Ring Preexposure Prophylaxis for HIV Prevention in South Africa**

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**Background:** A vaginal ring (VR) containing dapivirine (DPV) is under evaluation for pre-exposure prophylaxis (PrEP) for HIV prevention among women. However, the potential impact and cost-effectiveness of DPV PrEP scale-up is unknown. Further, cross-resistance is common between DPV and first-line antiretroviral therapy (ART) in resource-limited settings.

**Methods:** We modeled the HIV epidemic in KwaZulu-Natal, South Africa and compared the combined scale-up of ART, male medical circumcision (MMC) and DPV VR PrEP to a baseline scenario of just ART and MMC. We simulated four strategies of PrEP scale-up among women during 2017–2027: unprioritized (to 15–54 year-olds) or age-prioritized (to 15–24 or 20–29 year-olds) reaching 15% overall population-level coverage; or prioritized to female sex workers (FSWs) (~0.1% overall coverage). We examined scenarios of low (50%) or high (95%) average adherence, assuming 90% PrEP efficacy against wild-type and drug-resistant HIV, and 80% cross-resistance between ART and PrEP, and modeled HIV drug resistance dynamics in genital and blood compartments. We examined health outcomes and drug resistance consequences relative to baseline and calculated cost-effectiveness ratios while discounting healthcare and intervention costs (PrEP costs: \$95/person-year) and health outcomes by 3% annually.

**Results:** At low (50%) adherence, unprioritized DPV VR PrEP scale-up prevented 8.8% of (undiscounted) new infections over ten years at \$8,678 per infection prevented. Impact and costs improved modestly with scale-up among women aged 15–24 (9.4% infections prevented, \$8,059 per infection prevented) but more substantially when focused to women aged 20–29 (14.1%, \$5,052). Scale-up among FSWs prevented the fewest infections overall (4.6%; given their small group size), but at lower cost, reducing the cumulative total costs by \$21.4 million. At high (95%) compared to low adherence, HIV prevention increased by 86%–106% and cost-effectiveness ratios decreased by 52%–57% (Table). PrEP scale-up decreased prevalent drug-resistant cases at 2027 by 1.6%–7.4% and 4.4%–14.8% in the low and high adherence scenarios respectively; however, these decreases diminished by relative 2%–12% when in addition to blood, resistance was also tracked in the genital compartment.

**Conclusions:** DPV VR PrEP could have considerable impact on HIV prevention at compelling economic value when prioritized to women by age and could decrease drug resistance, even if adherence is modest.

Scenario	New HIV infections	Discounted total cost, million \$	\$/infection prevented	Drug-resistant cases, blood	Drug-resistant cases, genital
Baseline (no PrEP)	661 017	20 165.4		476 019	476 019
<b>50% average PrEP adherence (outcomes relative to baseline)</b>					
PrEP to women 15–54	-8.8%	+420.8	8 678	-4.75%	-4.17%
PrEP to women 15–24	-9.4%	+414.7	8 059	-4.90%	-4.35%
PrEP to women 20–29	-14.1%	+390.5	5 052	-7.37%	-6.53%
PrEP to FSWs	-4.6%	-21.4	cost-saving	-1.64%	-1.54%
<b>95% average PrEP adherence (outcomes relative to baseline)</b>					
PrEP to women 15–54	-16.4%	+375.5	4 170	-9.33%	-8.95%
PrEP to women 15–24	-17.6%	+365.5	3 790	-9.81%	-9.44%
PrEP to women 20–29	-26.5%	+316.8	2 179	-14.85%	-14.29%
PrEP to FSWs	-9.5%	-45.3	cost-saving	-4.45%	-4.36%

**1058 Cost-Effectiveness of the Intravaginal Dapivirine Ring: A Modeling Analysis**

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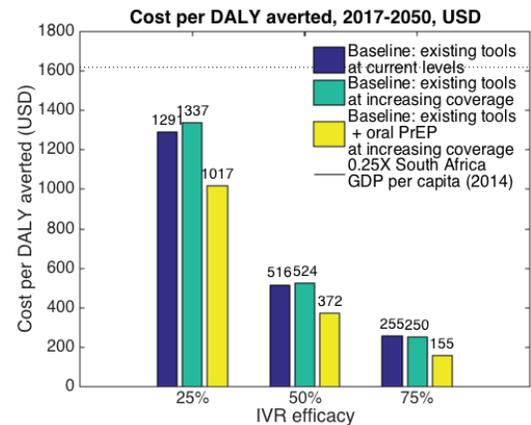
<sup>1</sup>Imperial Coll London, London, UK; <sup>2</sup>Bill and Melinda Gates Fndn, Seattle, WA, USA

**Background:** New female-controlled products are urgently needed for HIV prevention and intravaginal rings (IVRs) that release antiretroviral drugs such as dapivirine (DPV) are one technology being developed. Two phase III trials of a monthly DPV ring are underway and expect to report efficacy results in late 2015 and early 2016.

**Methods:** We modeled the introduction of the DPV ring in South Africa from 2017, assuming a range of efficacy estimates (25%, 50%, 75%). The intervention was highly prioritized to high-risk women (30% coverage among sex workers and 10% among other women with multiple sexual partners) and introduced under different assumptions about the counterfactual scenario: (1) current levels of existing HIV prevention methods (condom use, male circumcision, early ART) are maintained over time; (2) existing prevention methods increase over time; (3) as (2) with the addition of oral PrEP. We assumed a one-off fixed cost of 10 million USD for the introduction of the DPV ring plus 5 million USD per year for mass media. The variable cost ranged from 107-115 USD per person per year depending on the population sub-group, and we assume that prevention and treatment interventions call on the same overall 'HIV budget'. We estimated the health impact and cost-effectiveness of the DPV ring relative to the three counterfactual scenarios per disability adjusted life year (DALY) averted. All costs are discounted at 3% per year.

**Results:** The DPV ring could avert 125-175 thousand, 265-364 thousand or 427-588 thousand infections at 25%, 50% and 75% efficacy, respectively, from 2017-2050 under the different counterfactual scenarios. This represents 1.1-1.9%, 2.5-4.2% and 4.0-7.0% of total HIV infections in this period at corresponding cost-effectiveness of 1000-1300, 370-520 and 160-260 USD per DALY averted (Figure 1). All cost-effectiveness estimates are below 25% of South African GDP per capita.

**Conclusions:** The DPV ring could substantially and cost-effectively generate health among women in South Africa even under the lowest efficacy estimates, provided it can be successfully prioritised to those at greatest risk. However cost-effectiveness does not necessarily imply the intervention is affordable and in other settings the ring may be less likely to be cost-effective. The success of the DPV ring will also be determined by user demand and adherence, and new and forthcoming data on women's preferences will be critical for determining its use across different settings.



**1059 Determinants of Economic Efficiency in HIV Prevention: Evidence From ORPHEA Kenya**

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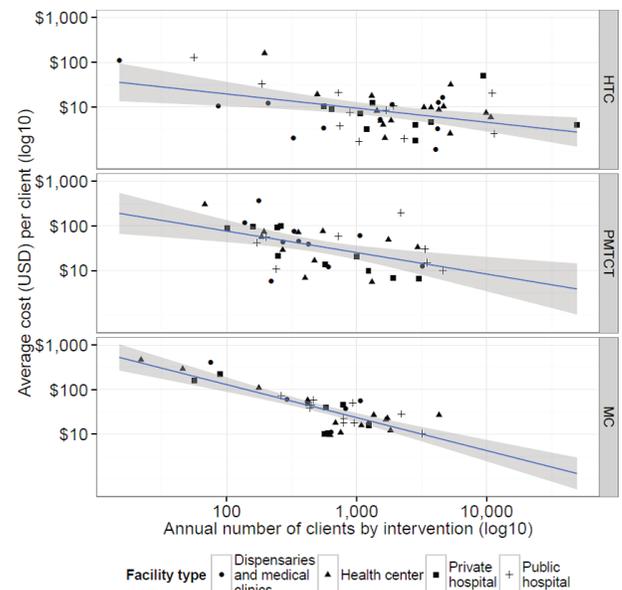
<sup>1</sup>Brown Univ Sch of PH, Providence, RI, USA; <sup>2</sup>Northeastern Univ, Boston, MA, USA; <sup>3</sup>INSP, Cuernavaca, Mexico; <sup>4</sup>Univ of Nairobi, Nairobi, Kenya

**Background:** We analyze determinants of economic efficiency for three HIV prevention interventions in Kenya: HIV testing & counselling (HTC), prevention of mother-to-child transmission (PMTCT), and male circumcision (MC). As part of the "Optimizing the Response of Prevention: HIV Efficiency in Africa" (ORPHEA) project, input data were collected retrospectively from sample of government and non-governmental health facilities for 2011-12.

**Methods:** Multi-stage sampling was used to determine the sample of health facilities by type, ownership, size, and interventions offered totaling 175 sites in 78 health facilities in 33 districts across Kenya. Data sources included key informants, registers and time-motion methods. Total costs of production were computed using both quantity and unit price of each input. Average cost was estimated by dividing total cost per intervention by number of clients accessing the intervention. Forward-selection stepwise regression methods were used to identify and analyze significant determinants of log-transformed average costs (p<0.1).

**Results:** Results show evidence of economies of scale for all three interventions: doubling the number of clients per year was associated with average cost reductions of 39% for HTC, 49% for PMTCT, and 69% for MC. Moreover, task shifting was associated with reduced costs for both PMTCT (47%) and MC (44%), but not for HTC. Costs in hospitals were higher for both HTC (56%) and PMTCT (60%) in comparison to non-hospitals, but this was not the case for MC. Performance incentives for staff were associated with increased costs in both HTC (50%) and PMTCT (64%), but not in MC. Facilities that performed community-based testing had higher HTC average costs (49%); and lower MC costs were associated with availability of male reproductive health services (81%) and presence of community advisory board (58%).

**Conclusions:** Aside from increasing production scale, HIV prevention costs may be contained by using task shifting, non-hospital sites, service integration and community supervision. The extant results have implications for HIV prevention programs in Kenya, and sub-Saharan Africa more generally.



**1060 Global Variation in the Impact of Male Circumcision in Preventing HIV Among MSM**

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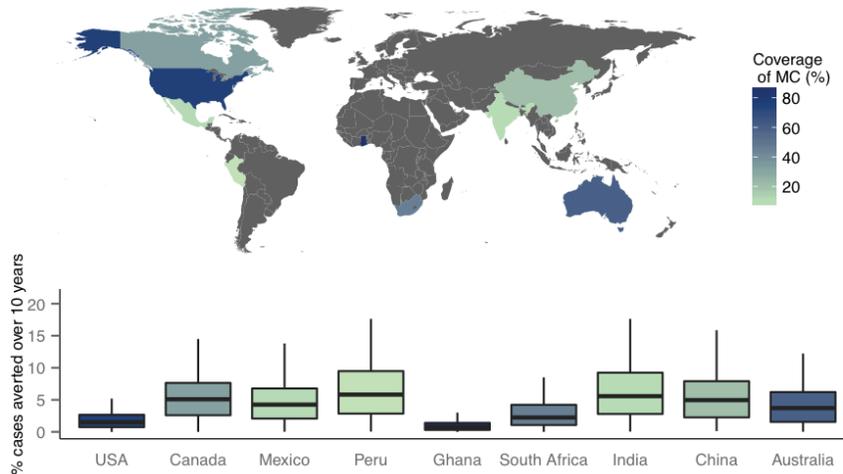
**Background:** The effectiveness of male circumcision (MC) as an HIV prevention measure among heterosexual men has been demonstrated in clinical trials. However, the efficacy and population-level effectiveness of MC among men who have sex with men (MSM) remains uncertain, and is likely to depend on the behavioral and demographic characteristics of specific MSM populations. We assessed the potential impact of MC among MSM in different settings worldwide to help determine the conditions in which it could be an effective HIV prevention measure for MSM.

**Methods:** We developed a deterministic compartmental model of HIV transmission among MSM and simulated the HIV epidemic in nine selected countries. The model incorporates infectivity by type of sex act, sexual mixing by role preference, condom use, three stages of HIV with varying infectivity, and assumes a 40%–67% MC efficacy during insertive anal sex. The model was calibrated to country-specific HIV prevalence and current coverage of MC (Figure), and accounted for the uncertainty in other parameter values based on literature review. We compared intervention strategies of MC scale-up where 25%, 50% and 100% of uncircumcised, uninfected MSM engaging in insertive anal intercourse more than 50% of the time were circumcised over the course of 5 years. Impact was measured as cumulative fraction of new HIV infections averted over 10 years.

**Results:** The predicted impact of MC varied substantially across settings (0%–17%). Countries with high existing levels of MC (e.g. USA or Ghana) would see a minimal impact (<3%/6% when circumcising 25%/50% of insertive MSM). The maximum impact of 8%/16% was observed in countries with low existing levels of MC (e.g. Peru and India). An upper bound of 38% impact is predicted using the 100% coverage intervention. The impact was most pronounced among MSM receiving MC, although herd effects were also observed among their partners. In uncertainty analysis, the intervention impact was positively correlated with role segregation in each setting (greater impact was seen when MSM had a strong preference for either insertive or receptive intercourse).

**Conclusions:** MC among MSM is likely to have the greatest impact in highly role-segregated settings with low MC coverage, such as Peru or India. However, our results suggest that the public health benefits among MSM would likely be modest, with the intervention unlikely to avert more than 17% of infections even in the most favorable of settings under realistically achievable coverage.

**Figure:** Top: World map with modelled countries highlighted. Bottom: Boxplots (medians, quartiles and 95% confidence intervals), colour-coded by current MC coverage as indicated on the map, showing the % of new HIV cases averted over 10 years among all MSM achieved by circumcising 50% of uninfected, predominantly insertive MSM over the course of five years.



**1061 What Does Community HIV Testing Really Cost in South Africa?**

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<sup>1</sup>Médecins Sans Frontières, South Africa & Lesotho, Eshowe, South Africa; <sup>2</sup>Médecins Sans Frontières, Roma, Lesotho; <sup>3</sup>Médecins Sans Frontières, Cape Town, South Africa; <sup>4</sup>Médecins Sans Frontières, South Africa & Lesotho, Cape Town, South Africa

**Background:** The World Health Organization 2015 Consolidated HIV Testing Guidelines recommends community testing but this strategy is not widely used in the South African public sector due to cost concerns. Médecins Sans Frontières has rolled out three innovative models in rural KwaZulu Natal; mobile testing units (MTU) and fixed-testing sites (FTS) manned by counselors, and door-to-door testing (D2D) delivered by community health workers. These models reach more children and adolescents than clinics, and create opportunities to link negative people to preventive services and to link positive people to care. To illustrate the financial cost of realizing such benefits we estimated the costs related to performing an integrated “Test & Link” per model.

**Methods:** This cross-sectional cost analysis from a service provider perspective used an ingredients approach. Costs were stratified: diagnostics; staff time; sensitization; infrastructure; communication; and, transport and equipment. We used programme statistics, and financial and procurement data to calculate the monetary value of all resources. The cost in USD of one “Test & Link” per model in 2014 was our primary outcome. Up-front and running costs were calculated. Assumptions were that: “Test & Link” providers worked 18 days/month in average and used 20-25 minutes per negative test, and 40-50 minutes per positive test.

**Results:** The cost of one “Test & Link” was \$7,82 per positive test for D2D testing, \$7,97 for clinic-based testing, \$11,12 for FTS and \$14,93 for MTU. For FTS and MTU the main driver of cost difference was staff time, which comprised 63,5% (FTS) and 45,7% (MTU) of the cost of each positive test. D2D testing was the least expensive, and the only community-based model of testing that was delivered more cheaply than clinic-based testing. Diagnostics comprised 40,8% of the cost of each positive test at the D2D.

**Conclusions:** Cost per positive “Test & Link” was lower for D2D testing than clinic-based testing. Community testing needs not cost more than clinic-based testing if lay cadres can be engaged. This evidence supports wider adoption of community testing in South Africa, especially door to door testing, to reach populations such as children (at high risk of morbidity), young adults and men (at high risk of acquiring as well as transmitting HIV).

Table1. Cost per “Test & Link” broken down by ingredient and by model

Ingredient	FTS (7065) Test & Link 2014 (Tests per day=32; Proportion HIV+ 3.3%)		MTU (11554) Test & Link 2014 (Tests per day =53; Proportion HIV+6.9%)		D2D (15112) Test & Link 2014 (Tests per day =69; Proportion HIV+3.3%)		Clinic-Based (Test & Link 2014)	
	HIV-	HIV+	HIV-	HIV+	HIV-	HIV+	HIV-	HIV+
Diagnostics	\$ 1,52	\$ 3,13	\$ 1,52	\$ 3,13	\$ 1,52	\$ 3,13	—*	—*
Staff	\$ 5,38 (68.5%)	\$ 7,05 (63.3%)	\$ 5,13 (43.9%)	\$ 6,80 (45.5%)	\$ 2,09 (26.1%)	\$ 2,49 (31.8%)	—*	—*
Sensitization	\$ 0,09	\$ 0,06	\$ 0,06	\$ 0,06	\$ 0,04	\$ 0,04	—*	—*
Infrastructure	\$ 0,30	\$ 0,30	-	-	-	-	—*	—*
Transport	-	-	\$ 4,85 (41.8%)	\$ 4,85 (32.5%)	\$ 1,13	\$ 1,13	—*	—*
Communication	\$ 0,04	\$ 0,04	\$ 0,04	\$ 0,04	\$ 0,73	\$ 0,73	—*	—*
Equipment	\$ 0,48	\$ 0,48	\$ 0,03	\$ 0,03	\$ 0,27	\$ 0,27	—*	—*
<b>TOTAL per Model</b>	<b>\$ 7,85</b>	<b>\$ 11,12</b>	<b>\$ 11,66</b>	<b>\$ 14,93</b>	<b>\$ 5,81</b>	<b>\$ 7,82</b>	<b>\$ 7,07</b>	<b>\$ 7,97</b>
Cost per test at DoH	\$ 7,07	\$ 7,97	\$ 7,07	\$ 7,97	\$ 7,07	\$ 7,97	\$ 7,07	\$ 7,97
Balance compared with DoH*	+\$ 0,78	+\$ 3,15	+\$ 4,59	+\$ 6,96	-\$ 1,26	-\$ 0,15	\$ 0,00	\$ 0,00

\* Department of Health: Data obtained from the 2015 South African HIV and TB Investment Case (Source: SANAC). Disaggregated data not available.

**1062 Costs of Hybrid Mobile Multi-Disease Testing With High HIV Test Coverage, East Africa**

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**Background:** In 2013-14, we achieved 89% adult HIV testing coverage using a hybrid mobile HIV testing approach of 2-week multi-disease community health campaigns (CHC) followed by home-based testing (HBT) of CHC non-attendees in 32 communities in Uganda and Kenya (SEARCH: NCT01864603). To inform scalability, we sought to determine: 1) overall costs of our hybrid testing approach; and 2) costs associated with including point-of-care (POC) CD4 testing and multi-disease services (hypertension, diabetes, and malaria) - elements crucial to our hybrid approach, but absent in most community-based testing strategies.

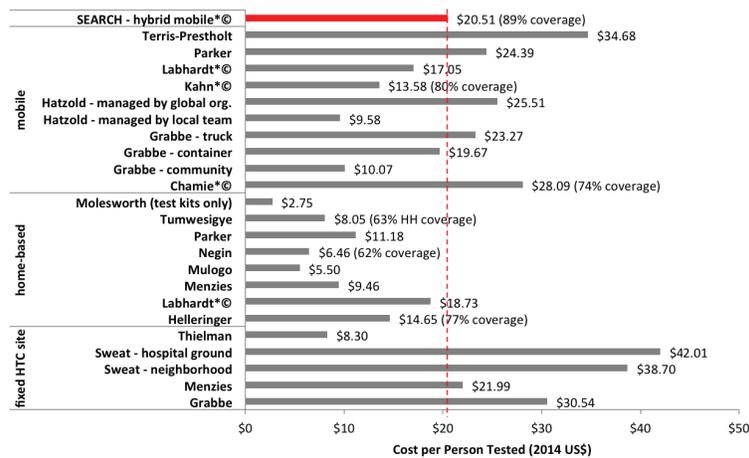
**Methods:** We applied micro-costing methods to quantify resources used for population-wide HIV testing using our hybrid mobile approach in 12 of 32 SEARCH Trial communities. Key intervention components are door-to-door baseline census enumeration, multi-disease CHCs, and HBT of CHC non-attendees. POC CD4 tests were done for all HIV+ participants. Data were obtained from expenditure records and study logs (e.g., costs for salaries, rapid HIV antibody and POC CD4 tests, transportation, and mobilization). Interviews with study staff and time and motion exercises were conducted to determine staff time allocation to various activities.

**Results:** The average cost per adult (≥15 years) tested for HIV was \$20.51 (range: \$17.06 – \$32.08 [SD = \$3.84]) across communities [2014 US\$], including POC CD4 at \$16 per test which represents 5-13% of total testing costs. Cost per adult tested at CHCs was \$13.83 vs. \$31.71 via HBT. The costs per adult tested were consistent across sites, except for an island community where staff transportation costs were high. The cost per HIV+ adult identified was \$231 (range: \$87 – \$1,245 [SD=\$336]); the variability in this measure was mainly due to differences in HIV prevalence (e.g., HIV prevalence of 23.56% vs. 1.62%). The marginal cost attributable to multi-disease services at CHCs was \$1.16 per person for hypertension and diabetes screening, and \$0.90 per person tested for malaria. Figure 1 compares costs of our hybrid mobile approach vs. mobile testing alone, HBT alone, and fixed venue based testing.

**Conclusions:** While achieving high HIV testing coverage with added intervention components including census, POC CD4 testing, and multi-disease services, the cost of this hybrid mobile testing approach is in the range of previously reported mobile, home-based, and fixed venue based HIV testing implementations.

**Cost Comparison of HIV Testing Approaches in Sub-Saharan Africa**

Excl. studies in South Africa



\* Multi-disease services  
 © Included point-of-care CD4 testing

**1063 Targeting Serodiscordant Couples Within Home HIV Testing Campaigns: A Modeling Study**

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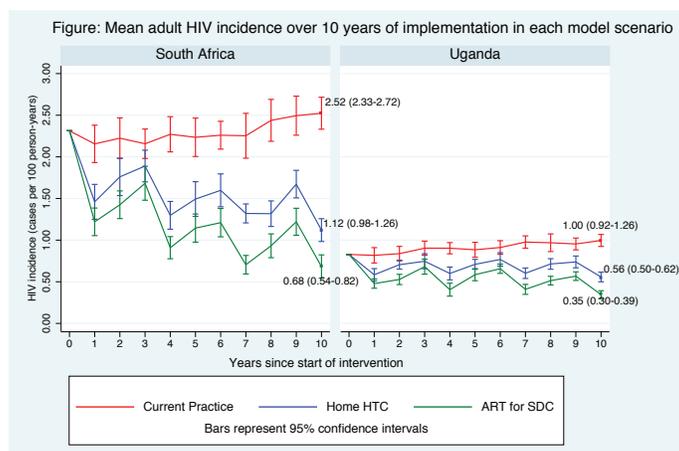
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**Background:** The World Health Organization recommends immediate antiretroviral therapy (ART) for HIV-infected members of HIV serodiscordant couples (SDC) to reduce the risk of HIV transmission. Despite the proven efficacy of this intervention, slow implementation and low uptake have limited its impact on population-level HIV incidence. Home HIV testing and counseling (HTC) campaigns have the potential to increase ART uptake among SDC by incorporating couples' testing and ART referral. Alternatively, because these campaigns have achieved high rates of testing and linkage to care in the general population, there may be little gained by adding activities to target SDC. We estimated the reduction in adult HIV incidence achieved by incorporating ART for SDC into home HTC campaigns in KwaZulu Natal, South Africa (KZN) and southwestern (SW) Uganda.

**Methods:** We constructed dynamic, stochastic, agent-based network models of HIV transmission for each setting, parameterized with HTC and linkage data from 2011-2012. We compared adult HIV incidence after 10 years under 3 scenarios: 1) baseline rates of HIV testing and linkage to care without a home HTC intervention (Current Practice), 2) home HTC campaigns delivered every 3 years, with linkage to ART for eligible persons (Home HTC), and 3) immediate ART for SDC, regardless of CD4, delivered during home HTC campaigns every 3 years, with 90% ART uptake among couples who tested (ART for SDC). In all scenarios, ART eligibility criteria for the general population was based on a CD4 threshold of <350 cells/ul (Ugandan and South African guidelines at the time of data collection).

**Results:** ART for SDC reduced adult HIV incidence by 38% compared to Home HTC: from 1.12 (95% CI: 0.98-1.26) to 0.68 (0.54-0.82) cases per 100 person-years (py) in KZN, and from 0.56 (0.50-0.62) to 0.35 (0.30-0.39) cases per 100 py in SW Uganda (Figure); 1/4 of incident HIV infections were averted over 10 years compared to the Home HTC scenario. The proportion of virally suppressed HIV-infected persons increased by approximately 15% with ART for SDC.

**Conclusions:** Our analyses suggest that using home HTC to identify SDC and deliver immediate ART could avert substantially more new HIV infections than home HTC alone, with a smaller number needed to treat to prevent new HIV infections. Scale-up of home HTC will not diminish the effectiveness of targeting SDC for treatment. Home HTC programs should invest resources to increase rates of couples' testing, disclosure, and linkage to care.



**1120 Pharmacokinetics, Safety, and Efficacy of Maraviroc in Pediatric Patients With R5 HIV**

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**Background:** Maraviroc (MVC) is a CCR5 antagonist approved to treat adults infected with CCR5-tropic (R5) HIV-1. Study A4001031 was conducted to evaluate the pharmacokinetics (PK), safety and efficacy of MVC in treatment-experienced (TE) pediatric patients.

**Methods:** This is an open-label, two-stage (stage 1: dose-finding; stage 2: safety/efficacy), age-stratified, non-comparative, multicenter study to evaluate the PK, safety and efficacy of MVC plus optimized background therapy (OBT) in TE children infected with R5 HIV-1. A total of 103 participants were enrolled into one of four age/formulation cohorts (Table) and dosed twice daily. Initial doses were determined by body surface area (BSA) and OBT, based on interactions with MVC in adults. Dose adjustment and PK re-evaluation occurred if average concentrations ( $C_{avg}$ ) at Week 2 were <100 ng/mL (stage 1).

**Results:** The majority of participants (90/103) included in the Week 48 primary analysis received OBT containing potent CYP3A4 inhibitors. The dosing strategy resulted in 49/50 stage 1 participants rolling over into stage 2 achieving  $C_{avg}$  concentrations  $\geq 100$  ng/mL irrespective of age, BSA or OBT, and an exposure range similar to that seen in adults. MVC was well-tolerated with a safety profile comparable to that of adults. The majority of treatment-emergent adverse events (TEAEs) were of Grade 1 severity. There were no deaths. None of the Grade 3 or 4 TEAEs or serious adverse events was considered to be related to MVC. Fourteen subjects had Grade 3 or 4 laboratory abnormalities with Grade 3 neutropenia the most common (n=8). All cohorts had a median decrease from baseline in HIV-1 RNA of >1 log<sub>10</sub>, while 67/103 subjects (65.0%) achieved HIV-1 RNA <400 copies/mL using the FDA snapshot (MSDF) algorithm, and 49/103 (47.5%) achieved HIV-1 RNA <48 copies/mL. An increase from baseline in median CD4+ cell count and percentage was seen for all age-groups. A total of 23 (22.3%) patients experienced protocol-defined virologic failure with few instances of non-R5 tropism (n=5), MVC resistance (n=1) or clinically relevant resistance-associated mutations (n=3).

**Conclusions:** Participants achieved the target  $C_{avg}$  with exposure ranges similar to that observed in adults. MVC's safety profile in this population was comparable to that seen in adults with no new safety concerns identified. Virologic efficacy was comparable to what was reported in a similarly treatment-experienced adult population. These data support dosing recommendations for TE patients 2- <18 years old.

	Cohort 1 ≥2 - <6 years Liquid MVC (N=16)	Cohort 2 ≥6 - <12 years Tablet MVC (N=31)	Cohort 3 ≥6 - <12 years Liquid MVC (N=13)	Cohort 4 ≥12 - <18 years Tablet MVC (N=43)	Total (N=103)
Race (White/Black/Asian/Other)	1/11/2/2	5/21/3/2	1/12/0/0	9/27/6/1	16/71/11/5
Median baseline log <sub>10</sub> HIV-1 RNA (copies/mL)	4.9	4.3	4.6	4.4	4.4
Median baseline CD4+ count (cells/μL)	977	471	438	406	471
Median treatment duration (days)	958	1093	969	714	914
Week 2 - MVC $C_{avg}$ geometric mean (ng/mL) for stage 1 subjects enrolled in stage 2	237 (N=12)	261 (N=11)	264 (N=10)	240 (N=17)	248 (N=50)
Week 48 - MVC $C_{avg}$ geometric mean (ng/mL)	164 (N=9)	290 (N=8)	169 (N=8)	199 (N=12)	199 (N=37)
Subjects with Serious Adverse Events	2	2	2	6	12
Subjects with Grade 3 or 4 adverse events	2	1	1	2	6
Discontinuations due to Adverse Events	0	0	1	1	2
Median Change from baseline in log <sub>10</sub> HIV-1 RNA at Week 48 (copies/mL)	-2.7	-2.1	-2.3	-1.2	-2.2
Median Change from baseline in CD4 count at Week 48 (cells/μL)	266	286	143	130	192

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**Bold numbers indicate a presenting author role for that abstract.**

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