HIV RESERVOIRS IN ACUTE INFECTION - P-D3 # 342

THE HIV RESERVOIR DURING EARLY ANTIRETROVIRAL THERAPY AND MARAVIROC INTENSIFICATION

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Background

- Residual viremia is common among people with HIV on antiretroviral therapy (ART).
- It remains controversial if this viremia is a consequence of propagating infection or the release of viral particles from infected cells.
- Intensification of ART should impact ongoing viral propagation but not virion release.

Objective

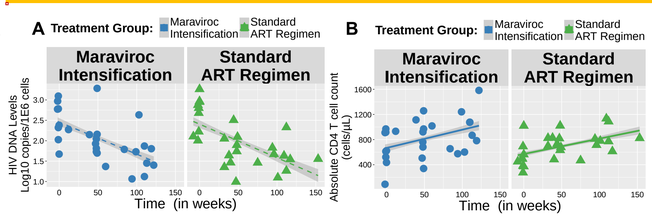
To identify HIV evolution in the context of a randomized controlled trial (RCT) of ART intensification with maraviroc (MVC) in individuals who initiated ART shortly after infection.

Methods

- Population: Eighteen acutely infected men were enrolled in a RCT, and followed for a median of 107 weeks. Whole blood was collected at three different time points: 1) within a week before the initiation of ART (median 7 days; IQR:1-12 days), 2) 48 weeks after initiation of ART (median 334 days), 3) ≥72 weeks after ART.
- Size of the reservoir: Levels of HIV DNA were quantified by droplet digital PCR in PBMC.[1]
- Sequencing: Deep sequencing of C2-V3 *env*, *gag* and *pol* (454-Roche) was performed on longitudinally collected plasma and PBMC samples. Reads were analyzed with purpose-built bioinformatics pipeline.[2]
- Molecular evolution: Sequences were analyzed for evolution by:
  1) Nucleotide diversity analysis by computing average pairwise distance between overlapping reads;
  2) Non-parametric test for panmixia (Fixation index, F_{ST});[3]
  3) Tip-date randomization technique within a Bayesian framework to assess the significance of the clock-like signal by comparing the coefficient of variation of the evolutionary rates (defined as the mean/95% highest posterior density [HPD]) obtained from the true dataset to the coefficient of variation of the 100 permutated replicates.
- Control group: A similar framework was used on 9 ART naïve HIV+ individuals with 3 serially collected blood plasma samples.

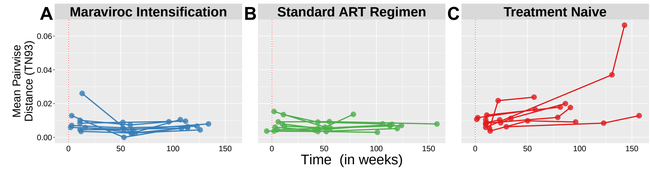
Results

Figure 1. HIV DNA decay was similar between groups (MVC +/-).



1A. HIV DNA levels decrease after starting ART (mean -0.08 log10 cp/10^6 cells/week, p<0.01), but no difference between MVC+/- groups p=0.62).
1B. The total CD4+ T cells counts increased significantly in both group(p<0.001), associated with an increase in CD4/CD8 ratio (p<0.001) with no difference between groups.

Figure 2. No significant increase in diversity in both groups (MVC +/-).

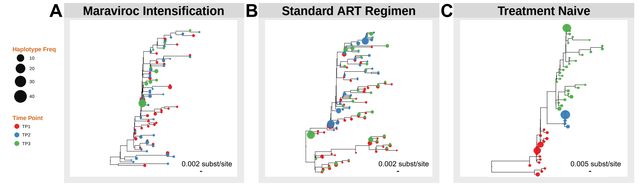


2AB. No increased diversity in treated individuals (MVC+/-).

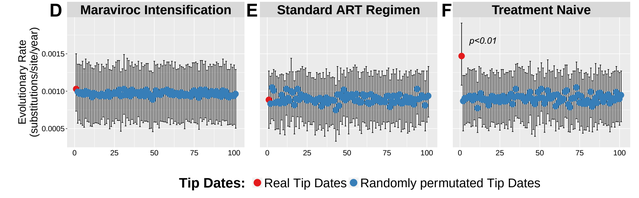
2C. Significant increased diversity (p<0.001) In the control panel (treatment naïve individuals).

Figure 3. Absence of clock-like molecular signal in both groups (MVC +/-).

Maximum Likelihood Phylogenies (C2-V3 region)



Posterior Mean and 95% HPDs of the Substitution Rates



3ABC. Maximum likelihood phylogenies showed intermingled sequences from each time-point for all but 4 regions/individuals among treated individuals (MVC+/-, panel AB) as opposed to treatment naïve individuals (panel C).
3DEF. Posterior mean and 95% highest posterior density (HPD) intervals of the substitution rates estimated from the actual data sets (far left value red dots) and the 100 permuted replicates for HIV *env* region (blue dots). The *p*-value represents the number of times the coefficient of variation (determined as $\frac{Mean\ Evolutionary\ Rate}{Variance}$ where variance = *(95%HPD upper – 95%HPD lower)* of the true dataset was smaller than the coefficient of variation in the permuted replicates.

Table 1. Population.

	All participants (n=18)	Maraviroc Intensification (n=9)	Standard ART regimen (n=9)	p value
Age (in years) - Median [IQR]	30 [24-40.3]	26 [23-42.5]	32 [26-40.5]	p=0.62
Time from EDI to ART start, in days - Median [IQR]	35 [28-85]	81 [28-91]	33 [26-85]	p=0.37
Time from ART to viral suppression, in days - Median [IQR]	84 [33-113]	84 [21-139]	77 [44-112]	p=0.98
CD4 count at baseline, cells/mm^3 - Median [IQR]	622.5 [446.8-760]	656 [481-849]	557 [443.5-644.5]	p=0.43
HIV RNA levels at baseline, log10 cp/mL - Median [IQR]	4.7 [3.7-5.5]	4.4 [3.3-6.7]	4.9 [4-5.4]	p=0.93
Duration of follow up, in days - Median [IQR]	749 [681-807]	736 [673-817]	761 [676-834]	p=0.86
HIV DNA levels at baseline, in copies/1E^6 Cells - Median [IQR]	536 [157-902]	240 [147-773]	633 [148-912]	p=0.60

Median with IQR values are shown for the entire cohort and by groups defined by Maraviroc intensification (MVC+ vs. MVC-). EDI: estimated date of infection; *Represent the p-value of a two-tailed Mann-Whitney or Fisher exact test.

- Eighteen men who started the same ART regimen plus either MVC (MVC+, n=9) or placebo (MVC-, n=9) within a median of 35 days from their estimated date of infection were included (**Table 1**).
- The coefficient of variation [mean divided by the 95% highest posterior density (HPD)] of the substitution rates of the actual and permutated estimates for each virus revealed a lack of significant support for the presence of temporal structure in the data in 45/50 (90%) of the sequenced regions among treated individuals.
- There was no difference between MVC+/- groups.

Conclusions

- Propagating viral replication is not the main mechanism of maintenance of HIV DNA populations during ART.
- Efforts aimed at eliminating the proviral reservoir should focus on other mechanisms of viral persistence.

Acknowledgments. This work was supported primarily by a grant from the National Institutes of Health, University of California, San Francisco-Gladstone Institute of Virology and Immunology Center for AIDS Research, P30-AI027763 (CNIHR), California HIV Research Program Idea awards to Sara Gianella and Joel O. Wertheim (ID15-SD-063 and ID15-SD-052), by the department of Veterans Affairs, the James B. Pendleton Charitable Trust and additional grants from the National Institutes of Health: AI100665, MH100974, MH097520, DA034978, AI007384, AI027763, AI106039, AI43638,AI074621,AI036214, MH101012, K01AI110181, UL1TR000100, CARE U19 AI096113 and AI068636-09. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the poster.

References. 1. Palmer S. et al. 2003 J Clin Microbiol 41:4531-4536.; 2. Fisher RG et al 2015 J Clin Virol 62:48-53; Hudson RR et al. 1992 Genetics 132:583-589