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Dynamics of HIV Clonal Expansion and Persistence During ART

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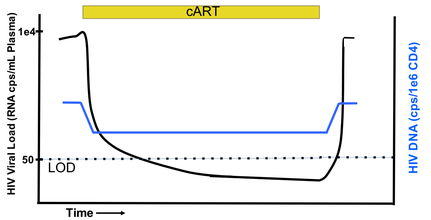
INTRODUCTION:

HIV persistence during antiretroviral therapy (ART) is a substantial obstacle to HIV cure. HIV infected cells can undergo clonal expansion. Most proviruses, including those in expanded clones, are defective, but expanded clones may include infectious HIV, indicating clonal expansion contributes to persistence. Specific clones may be highly expanded; we previously reported one provirus integrated in the *HORMAD2* gene that accounted for 20-40% of all proviruses in the patient. Mechanisms by which clones emerge and persist over time are uncertain. To investigate the dynamics of HIV clonal expansion during ART we developed multiplexed droplet digital approaches (ddPCR) to quantify HIV proviruses, including specific integrants prior to and following prolonged ART.

MODEL:

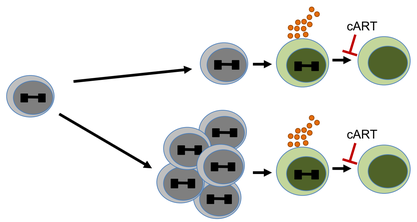
HIV Persistence:

- Despite success of cART, low level viremia persists for years after initiation.
- Viremia rebounds to pretherapy levels if cART is interrupted.
- Source of persistent viremia remains largely unknown.



Characteristics of Cells infected with HIV:

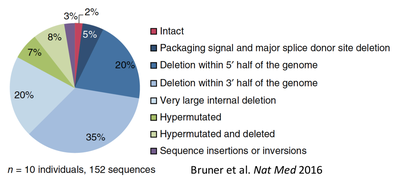
- Distributed in a number of memory and naïve CD4 subsets
- Total numbers of infected cells decline after introduction of cART
- Can undergo clonal expansion via cell proliferation
- A subset produce HIV at low levels
- Are the source of rebound Viremia
- Can be reactivated in vitro



Potential Mechanisms of Persistence:

1. Cellular
   - Cells not activated, no transcription factors
   - Integrant with unfavorable chromatin structure

2. Viral
   - Proviral structure defective or deleted
   - Cells are activated, but virus is not

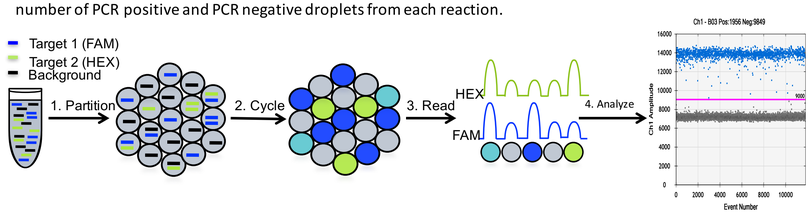


n = 10 individuals, 152 sequences Bruner et al. *Nat Med* 2016

METHODS:

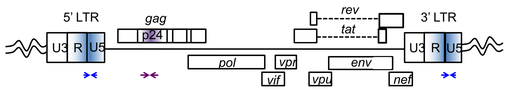
ddPCR Technology:

ddPCR quantifies DNA copy number by partitioning the PCR reactions into 20,000 nanoliter oil in water droplets (i.e., 20,000 separate reactions each containing a single molecule and a fluorescent-labeled probe), then by cycling the droplets to end point, and counting the number positive droplets with a FACS-like reader. This enables exact enumeration of amplifiable molecules in the starting sample. The readout is simply the number of PCR positive and PCR negative droplets from each reaction.



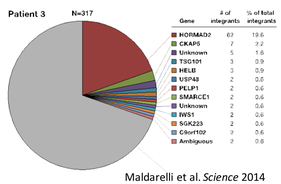
Primer Design:

We designed primers to quantify various HIV DNA targets. HIV LTRS crossing the R-U5 junction (blue), and p24 of *gag* (purple). Additionally we will quantify cell equivalents with a CCR5 set.

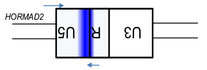


Study Population:

- HIV-1 infected individuals undergoing cART (TOTAL N=5)
- Patients suppressed with no interruptions for average 15 yrs (N=3)
- Range: 12-17 years suppressed on cART
- Integration Sites Analysis (ISA) on PID dd001 revealed a highly expanded solo LTR (PID 3, Maldarelli et al. *Science* 2014)
- Patients on cART with treatment interruptions (N=2)



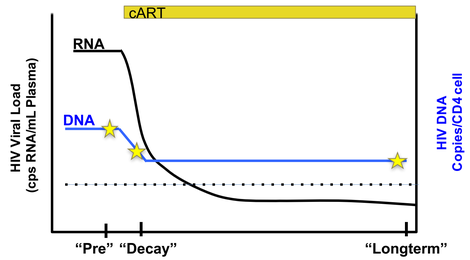
Maldarelli et al. *Science* 2014



Sampling Time points:

PBMCs collected and nucleic acids were extracted from time points during:

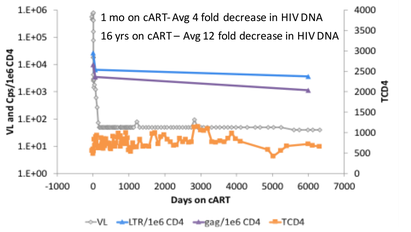
1. Pretherapy, before starting cART
2. Second phase viral decay after initiation of cART (average 1.5 mo on cART)
3. During long term cART (avg 10 yrs, range 3-16years on cART)

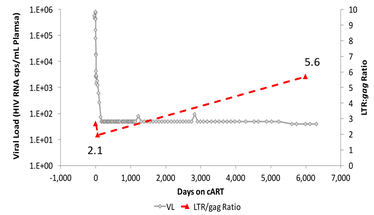


RESULTS:

HIV DNA Quantification

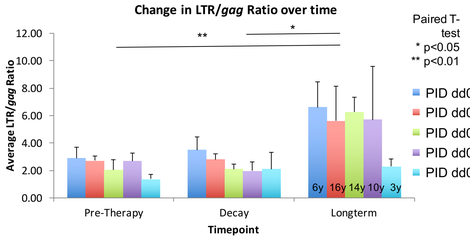
All patients had suppression of HIV RNA to <50 c/mL plasma within 5 mos on ART and HIV DNA/1e6 CD4+ cells decreased for *gag* (avg 12.6-fold) and LTR (avg 8.2-fold). PID002 DNA quantification shown below.





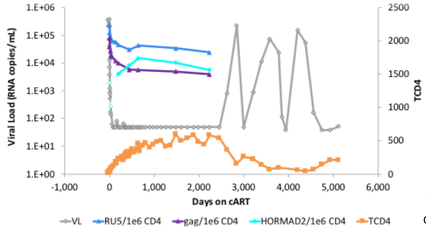
Increase in ratio of HIV LTR/*gag* DNA

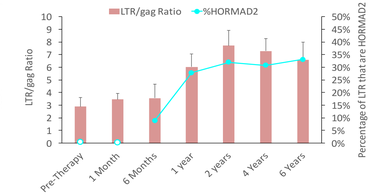
In 4 of the 5 patients, the ratio of LTR to *gag* increased between the second phase decay and prolonged time points (avg 6.5-fold, paired T-test p<0.01) demonstrating loss of full-length proviruses; in one patient, LTR/*gag* ratios remained stable.



HORMAD2 Integrant Quantification

The *HORMAD2* integrant was undetectable at pre-ART, and 1 month on cART. However, after 1yr on suppressive ART, the *HORMAD2* integrant was present at 30% of all infected cells and persisted for 6yrs.





TCD4	23	41	89	235	266	597	584
CD4%	2	3	6	9	11	19	23

CONCLUSIONS:

HIV populations change during ART with progressive *gag* deletion in most, but not all patients.
Clonal expansion of HIV infected cells can be rapid and sustained at stable levels during prolonged ART, suggesting both antigen-induced clonal expansion and homeostatic proliferation maintain HIV populations.

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