The paucity of clonal SIV proviral sequences during early ART treatment

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INTRODUCTION

- The HIV-1 reservoir consists of cells that contain latent but inducible HIV DNA within the host genome and is the source of viral rebound if antiretroviral therapy (ART) is interrupted
- Subjects on long-term ART exhibit latent-infected cells with identical proviral sequences
- Such HIV-1 integration sites (termed 'clones'), propagate either by antigenic or homeostatic stimuli
- Proliferation of these latent infected cells contributes to HIV persistence even during ART. Ultimately, these mechanisms lead to a skewed proviral reservoir
- Most of what we know about the make up of the reservoir derived from patients on long term ART [1,2]
- These studies have demonstrated that over time on ART the reservoir becomes more oligoclonal and concentrated into epigenetically privileged sites[2]

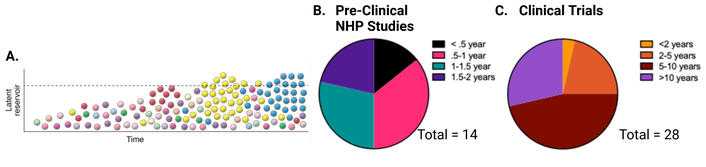


Figure 1. (A) Taken from Siliciano et.al, *Journal of Clinical Investigation*, 2020, PMID: 33016925 demonstrates the progressive skewing of the proviral reservoir. **(B-C).** Pie charts demonstrating the time periods of which (B) pre-clinical and (C) clinical trials occur in. Showing that the majority of clinical trials happen years after those of pre-clinical

HYPOTHESIS

We hypothesize that during earlier time periods of ART that are typically studied in pre-clinical trials, clonotypic makeup of the reservoir will be distinct from that of long-term suppressed people with HIV

METHODS

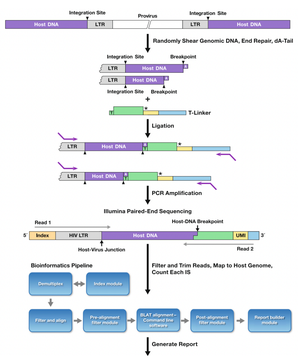


Figure 2. Adapted from Wells et all, *BMC Genomics*, 2020 PMID: 32151239 showing overview of Integration Site Assay.

- DNA was extracted from blood of SIV+ Rhesus macaques (n=3) that were ART treated for 3 and 10 months
- A linker-mediated PCR (integration site assay) coupled with next-generation sequencing was employed to determine the proviral clones within each compartment

RESULTS

Expanded clones do not accumulate within the SIV reservoir up to 10 months of therapy

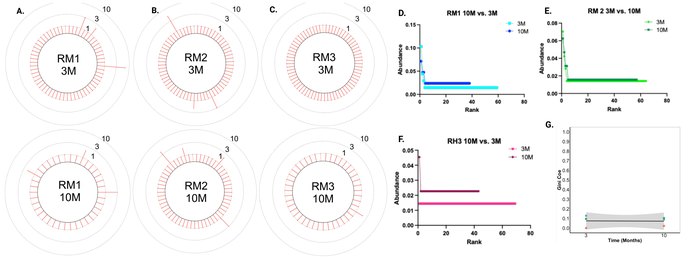


Figure 3. (A–C) Shifts in the proviral landscape of PBMCs from SIV+ Rhesus macaques (n=3) between 3 and 10 months on ART. A total of 357 integration sites were sampled, with 10.9% of the integration sites being identical. There was no statistically significant difference in the number of identical integration sites across time points (p=0.9934). (D–F) Rank abundance plots for each animal at each time point, where each point represents an individual clone ranked by abundance. These plots further demonstrate that the reservoir remains polyclonal at both 3 and 10 months. (G) The Gini coefficient was used to assess species inequality, but no significant difference was observed between 3 and 10 months (p=0.85).

There is a low degree of sharing from 3 to 10 months on ART

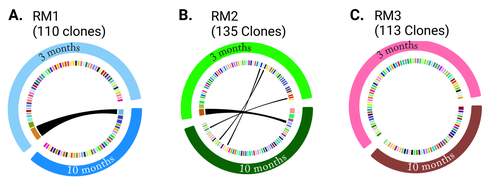


Figure 5. A-C. Circos plots demonstrating the low degree of sharing between 3 and 10 months for each animal (1.1%). Of those that were shared, there was a likelihood that they were clonal at either 3 or 10 months.

There is no preference in the integration of genic features between 3 and 10 month on ART

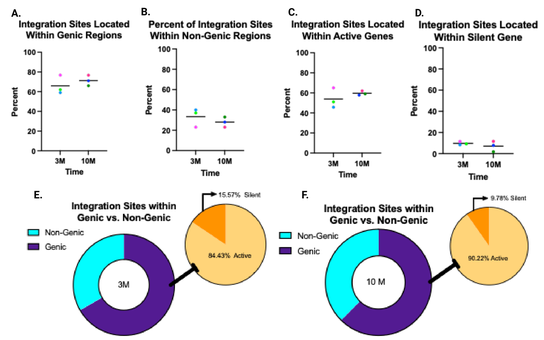


Figure 6. Integration sites were mapped to mmul_10 using RefSeq and Ensembl to determine their location in relation to genic regions. There was no significance between time points of integration sites located in **A.** genic (p=0.4483) or **B.** non-genic (p=0.4230). Additionally the genes were mapped to mRNA-seq expression data from human CD4⁺ T-cells to determine the percentage of genes that were **C.** active (p=0.3335) and **D.** silent (p=0.4234). Active gene expression was determined by a TPM > 1.0. **E-F.** Proportions of total genic vs. non-genic and active vs. silent integration sties between 3M and 10M of all animals

Results Cont.

SIV proviral clone proximity to open chromatin regions does not change from 3 to 10 months of ART

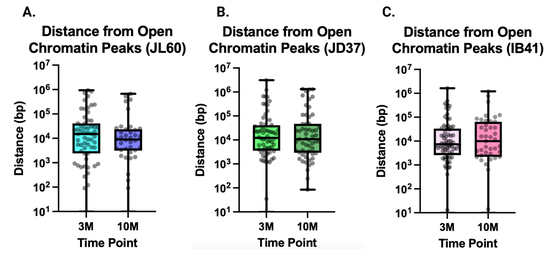


Figure 5. A-C. Basepair distance away from ATAC-seq peaks between 3 and 10 months. ATAC-Seq was taken from CD4⁺ PBMCs from Rhesus macaques. There was no significant difference between the two time points in any of the animals (p=0.5171), (p=0.8896), (p=0.7545).

DISCUSSION

- There is no significant changes in the clonal composition of the proviral reservoir between 3 and 10 months
- At 10 months, the reservoir is more polyclonal compared to long-term patients with HIV
  - Suggest that persistence during early ART may be driven more by homeostatic proliferation than antigenic clonal expansion
- Implications of this study
  - Non-human primate studies typically span 1-3 years on ART. This data demonstrates a differing landscape that occurs during these studies as compared to what is seen in clinical trials
  - Findings imply that therapies targeting homeostatic proliferation may be more effective during the early stages of ART, presenting a potential avenue for HIV cure strategies
- A limitation of this assay is that it does not report replication competence, therefore it remains unknown if these clones can reactivate and produce an active infection

ACKNOWLEDGEMENTS

JC Mudd and the rest of the Mudd Lab for their support and contribution and Lori Rowe from the TNPRC viral characterization and sequencing core. Additionally Brandon Keele from NCI-Fredrick. Funding support supplied by NIH grant numbers R01A167644 & R21OD031229

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