LACK OF DETECTABLE ONGOING REPLICATION ON ART IN SIV-INFECTED RHESUS MACAQUES

391

Taina T. Immonen[1], Christine M. Fennessey[1], and **Brandon F. Keele**[1]
[1]AIDS and Cancer Virus Program, Frederick National Laboratory, Frederick, MD

BACKGROUND

The question of whether HIV-1 can replicate at a low level during suppressive antiretroviral therapy (ART) remains unresolved. Detecting genetic changes associated with ongoing replication during ART is challenging in persons living with HIV-1 (PWH) due to the frequently high level of background genetic diversity prior to ART initiation, the generally unknown genetic constituents of the transmitted/founder variant(s) which differ between individuals, and limited access to tissue sites of viral replication. The NHP model provides increased sensitivity for detecting viral evolution during ART because the level of replication and genetic diversity before ART can be precisely controlled by early treatment initiation, access to tissues that are difficult to obtain from PLW, and the ability to use barcoded viruses that allow discrimination of individual lineages that are otherwise genetically identical.

METHODS

Study design: SIV DNA populations obtained near the time of ART initiation were compared to those obtained after long-term ART in 21 macaques belonging to 3 cohorts, differing in time of ART initiation and/or duration (Figure 1). All animals were infected intravenously with the barcoded SIVmac239M.

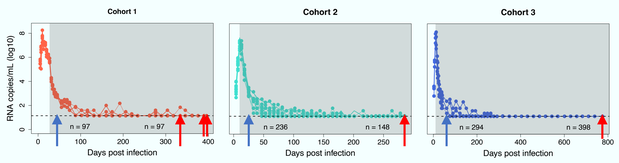


Figure 1: Plasma viral load curves. Viral RNA copies were measured in plasma using qRT-PCR with a lower limit of detection of 15 copies/mL. Cohort 1 (red) had 5 animals starting ART at 27 dpi, cohort 2 (turquoise) had 12 animals starting ART at 10 dpi, and cohort 3 (blue) had 4 animals starting ART at 10 dpi. Samples were collected and near full-length sequences generated at times indicated by arrows.

Sequence analysis: A total of 1,410 near full-length (nFL) SIV DNA sequences were obtained from peripheral mononuclear cells (PBMCs) or from axillary LN, mesenteric LN, and spleen samples. 141 (10%) sequences were obviously defective with evidence of hypermutation, frameshift mutations, stop codons or large deletions (>300 bp) and were removed from analysis. The remaining sequences were aligned in MUSCLE and neighbor-joining phylogenies rooted on SIVmac239 were constructed using the *ape* package in R.

Tests for molecular evolution: We assessed if the SIV populations at the two time points differed significantly in (i) p-distance from SIVMac239 (Mann-Whitney U test), (ii) average pairwise distance (randomization test with 10,000 simulations), (iii) population structure (test for panmixia with 10,000 simulations), and (iv) if the evolutionary rate during ART was significantly different from zero (estimated by linear regression; two-tailed F test). All statistical tests were performed in R.

All work involving research animals was conducted under a protocol approved by the Institutional Animal Care and Use Committee of the National Cancer Institute, National Institutes of Health (NIH) in NIH-Bethesda facilities. NIH-Bethesda is accredited by AAALAC International and follows the Public Health Service Policy for the Care and Use of Laboratory Animals (Animal Welfare Assurance Number D16-00602). Animal care adhered to the standards outlined in the "Guide for the Care and Use of Laboratory Animals (National Research Council; 2011), in accordance with the Animal Welfare Act.

This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. 75N91019D00024. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Longitudinal genetic analysis of 1,410 near full-length SIV DNA sequences from 21 macaques did not yield any evidence of ongoing viral replication in PBMCs or lymphatic tissues during suppressive ART.

RESULTS

No evidence for evolution on ART in PBMCs. Starting ART at d27 resulted in low baseline genetic diversity and divergence (median APD of 0.037% and median p-distance of 0.02%). Both measures decreased even further during ART (median APD of 0.028% and median p-distance of 0.014%), the SIV DNA populations at each time point were panmictic, and the evolutionary rate slopes (Figure 2) were negative in all animals. Phylogenetic analysis was consistent with evolution pre-ART, there was not evidence that any subset of the virus population continued to evolve during ART. Starting ART at 10 dpi resulted in even more homogeneous baseline populations, with median APD of 0.013%, and median p-distance of 0.0063%. None of the tests for molecular evolution yielded significant results. The combined phylogeny was star-like, with no accumulation of genetic changes consistent with a lack of detectable viral evolution on ART.

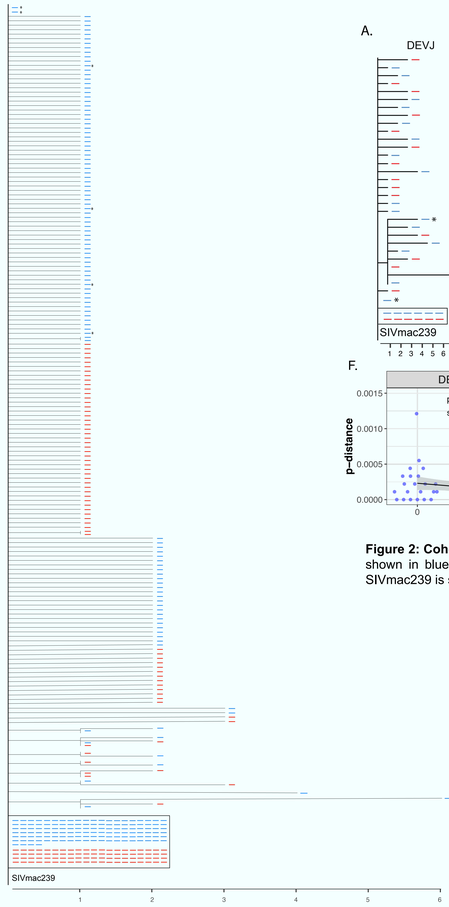


Figure 3: Cohort 2 – day 10 ART initiation tree. The NJ-tree combines all 384 sequences obtained from cohort 2 animals. Sequences from 25 and 260 dpi are shown in blue and red, respectively, with an asterisk indicating an insertion or deletion (indel).

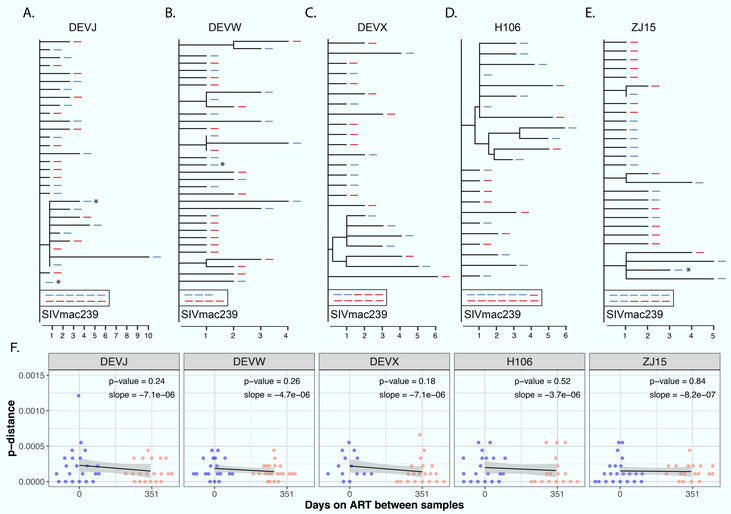


Figure 2: Cohort 1 – day 27 ART initiation trees and evolutionary rates. Sequences from 45 dpi and 285-345 dpi are shown in blue and red, respectively, with an asterisk indicating an insertion or a deletion. The p-distance (%) from SIVmac239 is shown for each sequence, with linear regression lines in black and confidence interval bands in grey.

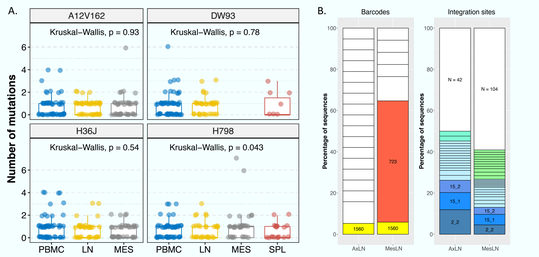


Figure 5: Compartmentalization in tissues. In (A), the number of genetic changes is shown for each sequence from PBMCs and tissues. In (B), the number of barcodes and integration sites are shown for Ax and Mes LN from one animal. The single shared barcode is yellow, and an expanded barcode in Mes LN is red. Unique integration sites are aggregated (white). Clonal sites that are not shared between LNs are shown in turquoise or green, minor shared clones are in light blue, and 3 dominant clones are in darker shades of blue.

No evidence for evolution on ART in lymphatic tissues. Population-level and phylogenetic analysis (Figure 4) did not yield any evidence of ongoing replication during ART in any tissue sample after 2 years of ART. While the mean number of mutations differed across tissues in a single animal (Figure 5A), differences were not significant in pairwise comparisons. We found 3 enriched barcodes in 3 mesenteric LN clusters from 2 animals. The most enriched barcode (58%) was not found elsewhere, while the most dominant clone (12%) at that site was found in similar proportion at an axillary LN, indicating that the barcode enrichment was not due to clonal expansion. At least 40% of infected cells were clones that were well-mixed across mesenteric and axillary LNs.

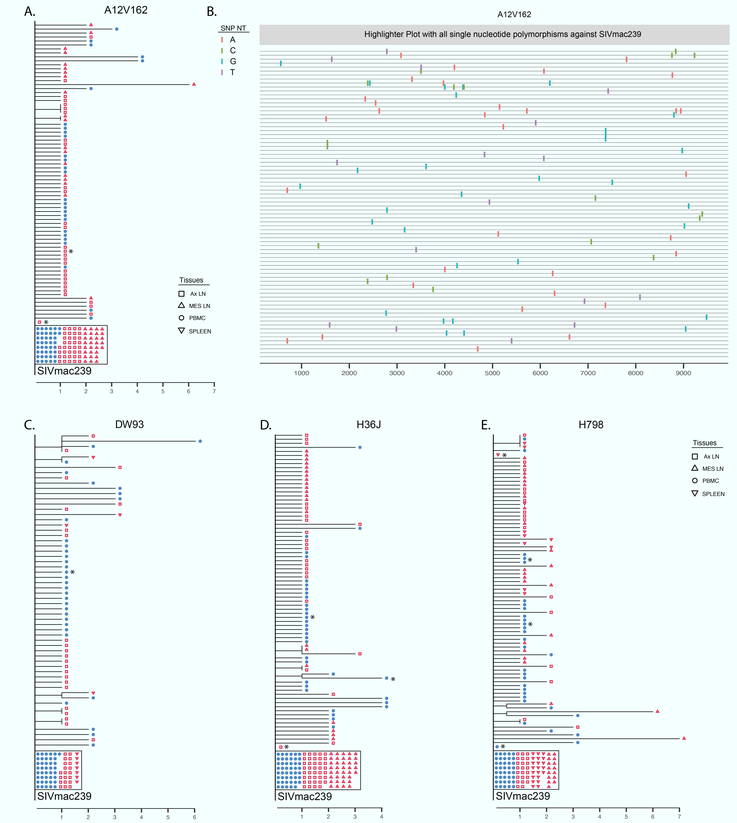


Figure 4: Cohort 3 – day 10 ART initiation trees and an example highlighter plot. Axillary and mesenteric LN, spleen, and PBMC sequences from 65 and 775 dpi are shown in blue and red, respectively, with indels indicated by an asterisk.

CONCLUSIONS

We compared over 1,000 nFL SIV DNA sequences obtained longitudinally from 21 macaques. Early ART initiation resulted in a genetically homogeneous baseline SIV DNA population, with 90% of sequences remaining intact and without obvious defects. Using calculations of divergence, diversity, overall population structure, and evolutionary rates, we did not find any evidence of ongoing replication in PBMCS after 285 to 385 days of ART, or in lymphatic tissues after 775 days of ART. While we cannot exclude the possibility of low-level ongoing viral replication in a theoretical sanctuary site, potential contribution of such a small replicating population to the rebound-competent reservoir or as a source of drug resistance escape mutations would be limited. Therefore, our results corroborating the lack of evolution on ART documented in people living with HIV and reenforce the utility of the NHP model for cure research.

ACKNOWLEDGEMENTS

We thank Drs. Jeff Lifson, Greg Del Prete and members of the Nonhuman Primate Research Support, the Quantitative Molecular Diagnostics, and the Viral Evolution Cores of the AIDS and Cancer Virus Program, Frederick National Laboratory for Cancer Research, for specimen processing, animal support, viral load analysis, and sequencing.