Persistent HIV-1 Plasma Viremia in Patients on Therapy Caused By Macrophage-Tropic Lineage

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BACKGROUND

- Most people living with HIV-1 on antiretroviral therapy (ART), are suppressed within a few months We investigated why adherence to ART failed to suppress HIV-1 RNA levels in participants viremic for 10 months after ART initiation (Figure 1A) and another participant viremic after 16 months of ART (Figure 1B).
- Both participants were adherent to ART by observation with no evidence of drug resistance by clinical testing.

Figure 1A: Participant 1

Suppression history

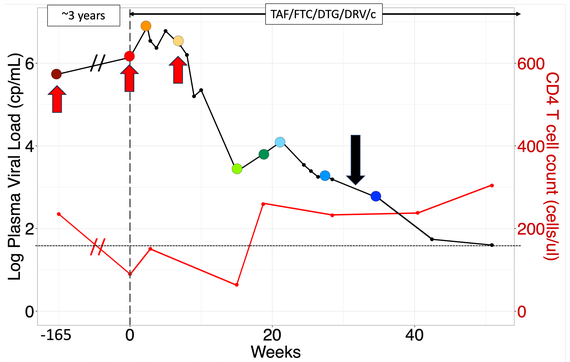
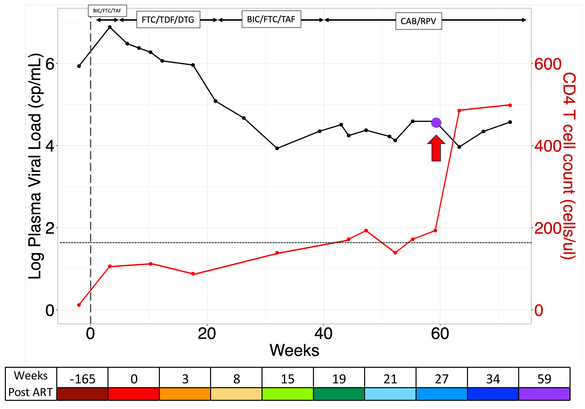


Figure 1B: Participant 2

Suppression history



METHODS

- Deep Sequencing of Viral RNA: MiSeq with PrimerID[1] was used to assess population diversity and drug resistance at multiple timepoints (colored ● timepoints in **Figures 1A and 1B**).
- 3' Half Genome Sequencing of Viral RNA and Proviral DNA: Viral RNA was isolated from plasma samples (⇧ in **Figures 1A and 1B**) and converted to cDNA. 3' Half Genomes were then amplified from cDNA and from proviral DNA (⇩ in **Figure 1A**) at limiting dilution. Amplicons were sequenced using PacBio with barcodes or FLIP-seq.
- Determining if Envelope Proteins Were Macrophage-Tropic: Expression vectors containing HIV *env* genes and a reporter virus plasmid (NL4-3-Luc.R$^-$E$^-$) were used to produce pseudoviruses. Each pseudovirus was tested for the ability to efficiently infect cells expressing a low surface CD4 density similar to that expressed on macrophages, previously shown to be a proxy for macrophage tropism[2] (**Figure 3**).

In two ART adherent participants, failure to suppress was caused by a persistent, drug-sensitive macrophage-tropic viral population.

Figure 2A: Participant 1

Partial *env* (V1-V3 region) sequencing of RNA and proviral DNA

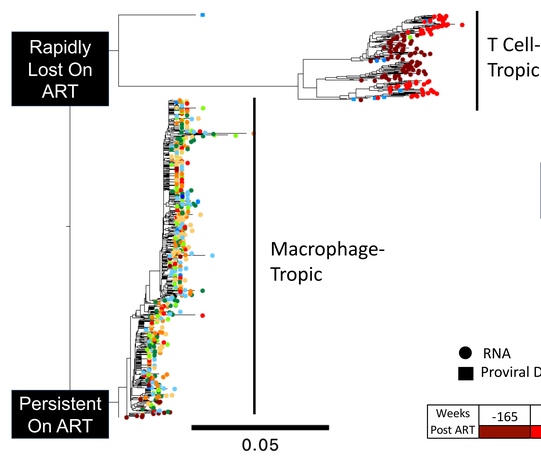
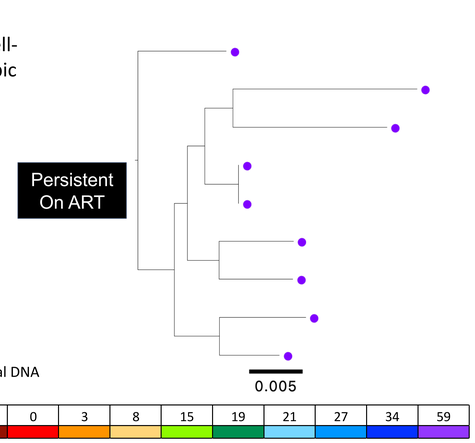


Figure 2B: Participant 2

Partial *env* (V1-V3 region) sequencing of RNA



RESULTS

- Despite being ART adherent, both Participants 1 (**Figure 1A**) and 2 (**Figure 1B**) had viral lineages that persisted in the plasma during ART.
- The persistent lineages were
  1. Drug-sensitive (Data not shown)
  2. Genetically diverse (**Figures 2A and 2B**)
  3. Macrophage tropic (**Figure 3**).
- Analyses of viral populations in plasma collected from Participant 1 (**Figure 1A**) before and after ART revealed that
  1. The T cell-tropic viral lineage decayed much faster than the macrophage-tropic lineage (**Figure 4**).
  2. The macrophage-tropic lineage independently accumulated Vpr genes with different defects (**Figure 4**).

Figure 4: Participant 1

Both macrophage- and T cell-tropic viruses are present in plasma RNA before ART, but T cell-tropic decays rapidly after ART initiation. A diverse defective Vpr population accumulates in the macrophage-tropic viruses.

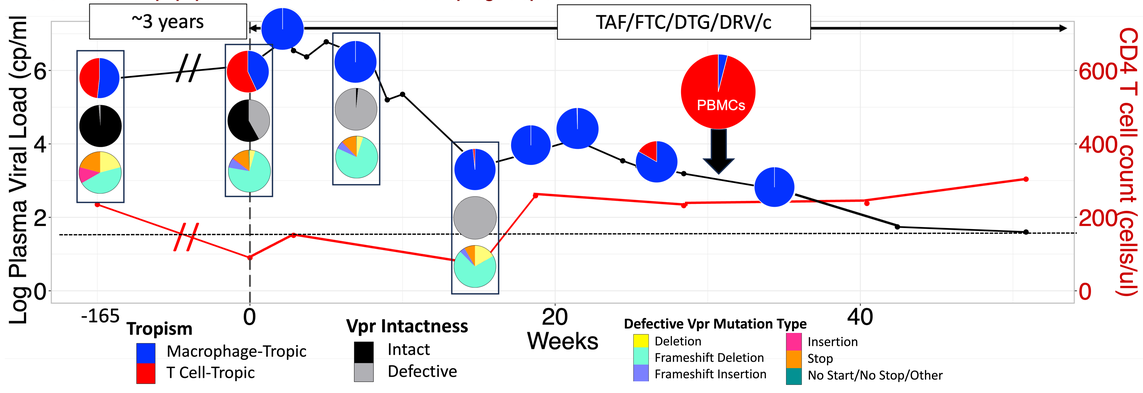
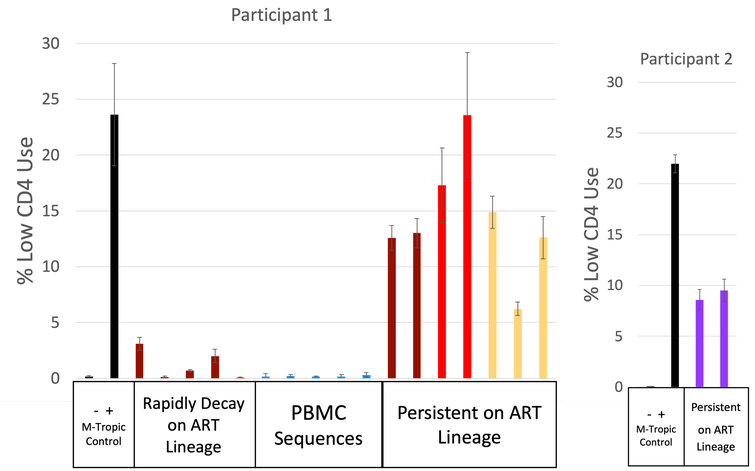


Figure 3: Analysis of Macrophage Tropism for Participants 1&2

Persistent lineages in both participants are macrophage-tropic



CONCLUSIONS

- Unlike previous studies of treatment failure or nonsuppressible viremia (NSV), the persistent viremia in these participants was not due to:
  1. Poor adherence to ART
  2. Evolution of drug resistance.
  3. Production of virus by an expanded CD4 T cell clone or clones.
- Macrophage-tropic virus persisted in the plasma during ART for both patients for an expended period of time, suggesting that it was produced by a long-lived cell such as a macrophage or microglia.
  - In Participant 1, the majority of macrophage-tropic virus lost Vpr function.
- Future Direction: Determine whether *vpr*- macrophage-tropic variants are replication competent.
- These results emphasize the need to study whether macrophage tropic viruses can persist as a latent reservoir and/or give rise to rebound virus with treatment interruption.

Citations

1. Zhou, S., C. Jones, P. Mieczkowski, and R. Swanstrom. J Virol 89, no. 16 (Aug 2015): 8540-55.
2. Joseph, S. B., K. T. Arrildt, A. E. Swanstrom, G. Schnell, B. Lee, J. A. Hoxie, and R. Swanstrom. J Virol 88, no. 4 (Feb 2014): 1858-69.

ACKNOWLEDGEMENTS

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