Persistence of inducible replication-competent HIV-1 after long-term ART

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BACKGROUND

- Antiretroviral therapy (ART) effectively inhibits HIV-1 replication but is not curative due to the persistence of a reservoir of latently infected resting CD4⁺ T cells carrying transcriptionally silent but replication-competent proviruses.
- We have previously shown that the latent reservoir in CD4⁺ T cells decays slowly with a half-life of 44 months for the first seven years of ART (Siliciano et al., *Nat Med*, 2003). The stability of the latent reservoir means these long-lived infected cells guarantee lifetime persistence.

Goal

It is unclear whether the latent reservoir continues to decay in people living with HIV (PLWH) on very long-term ART. In this study, we explore the size and composition of the reservoir in PLWH who have maintained suppressive ART for ~20 years.

METHODS

Participants: 31 PLWH maintaining full suppression of viremia for a median time of 22.8 years (Fig. 1).

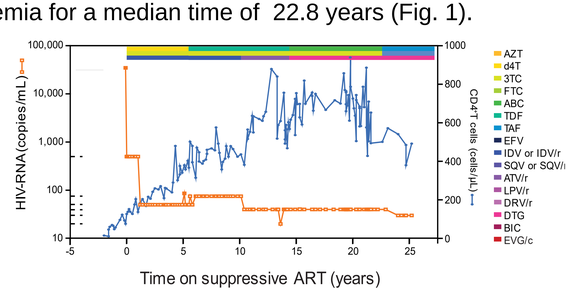

Figure 1. Clinical and virological history for a representative study participant.

We used the **quantitative viral outgrowth assay** (QVOA, Fig. 2) to determine the frequency of resting CD4⁺ T cells with inducible, replication-competent virus and the **intact proviral DNA assay** (IPDA) to measure the frequency of intact proviruses in resting CD4⁺ T cells. To determine the composition of the reservoir, **single genome sequencing** of *env* was performed on outgrowth positive QVOA supernatants and proviral DNA.

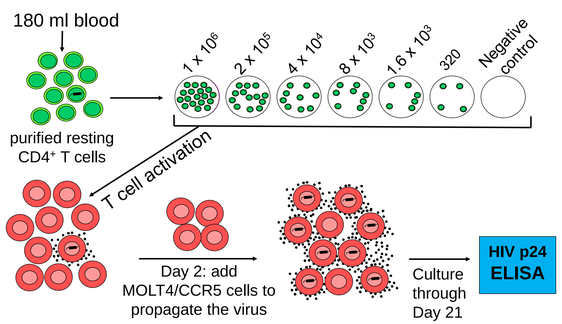

Figure 2. Quantitative viral outgrowth assay (QVOA).

The latent reservoir does *not* continue to decay in PLWH on long-term ART due to infected cell *proliferation*.

RESULTS

The QVOA and IPDA both show that reservoir decay does not continue. The frequency of resting CD4⁺ T cells with inducible, replication-competent virus increases slowly in PLWH on long-term ART.

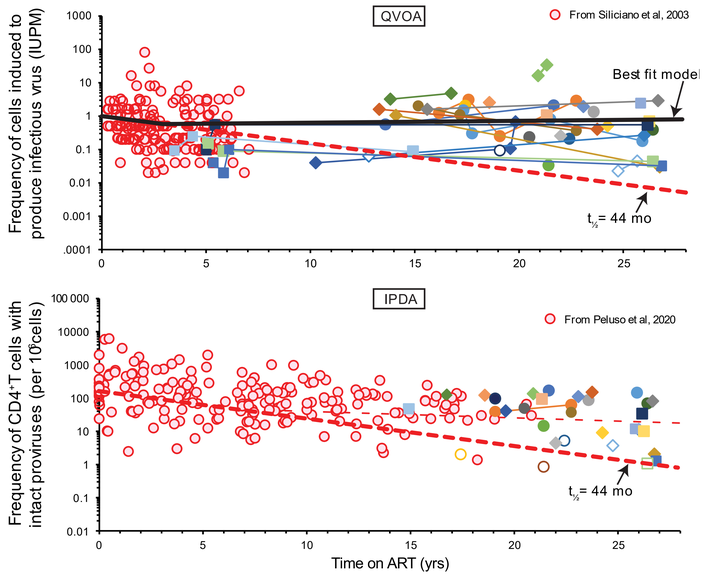

Figure 3. (*Top*) Frequency of inducible, replication-competent proviruses in resting CD4⁺ T cells over time on ART, as measured by the QVOA, for long-term ART participants (solid shapes). Prior data (pink circles) and predicted 44-month half-life (dashed red line) are replotted from Siliciano et al. (*Nat Med*, 2003). The best fit decay model (black line) incorporates cross-sectional and longitudinal measurements from repeated sampling and studies of the same participants. (*Bottom*) Frequency of intact proviruses in resting CD4⁺ T cells over time on ART, as measured by the IPDA, for long-term ART participants (solid shapes). Prior data (pink circles) from Peluso et al. (*JCI Insight*, 2020) and predicted 44-month half-life (dashed red line) from Siliciano et al. (*Nat Med*, 2003) are graphed.

Positive correlation between QVOA and IPDA measurements.

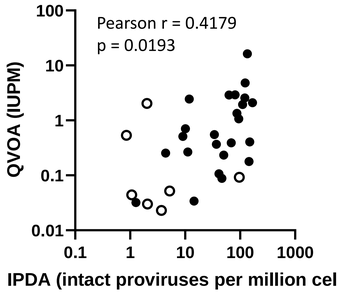

Figure 4. Correlation plot between QVOA and IPDA measurements after long-term ART.

The inducibility index (QVOA/IPDA) remains unchanged in PLWH on long-term ART.

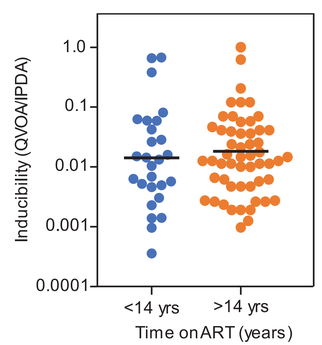

Figure 5. Inducibility plotted as a ratio of QVOA/IPDA measurements from PLWH on ART for <14 years (Bruner et al., *Nature*, 2019) and after long-term ART.

Large clones dominate the latent reservoir after very long-term ART.

- Inducible, replication-competent QVOA isolates (red) are largely clonal and make up a small proportion of the total diversity of the reservoir.

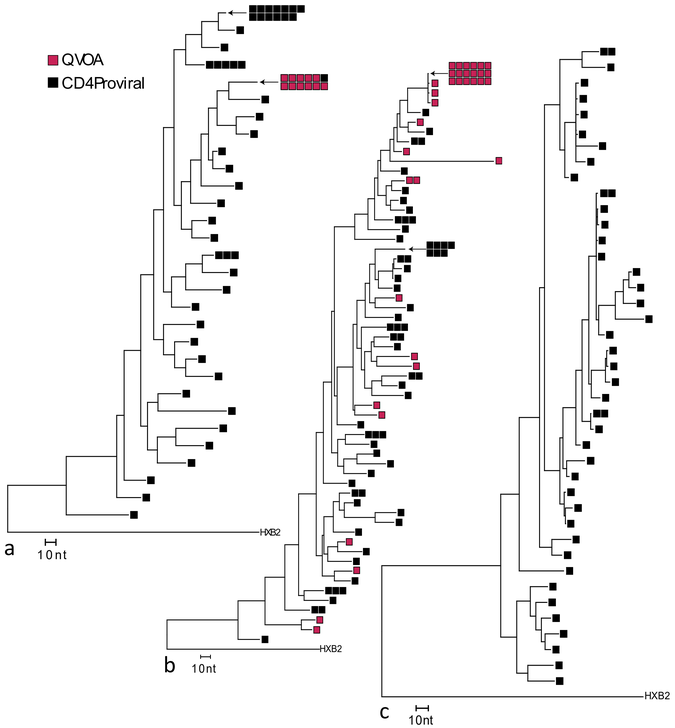

Figure 6. Phylogenic comparison of *env* sequences between QVOA viral outgrowth (red) and proviruses from resting CD4⁺ T cells (black) of representative participants with high (a, b) to no (c) viral outgrowth.

CONCLUSIONS

- After seven years of ART, reservoir decay does not continue and the frequency of cells with inducible, replication-competent proviruses begins to increase slowly. This is likely due to the proliferation of infected cells.
- Inducibility of proviruses is not reduced in resting CD4⁺ T cells of PLWH on very long-term ART.
- Large expanded T cell clones play a major role in contributing to long-term reservoir persistence.
- Due to the persistence of inducible, replication-competent proviruses in resting CD4⁺ T cells, ART should not be discontinued in PLWH on long-term ART.

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