Most HIV-1 Latent Reservoirs Form Primarily Within 1 Year of ART Despite Varied Clinical Histories

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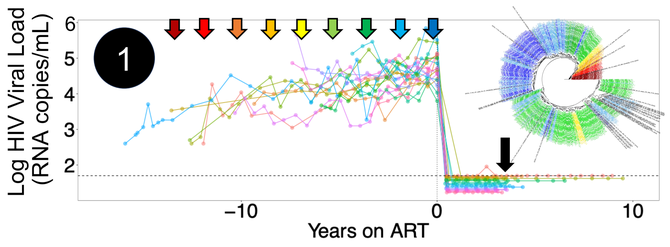
Background

- Despite continuous infection of new cells during untreated HIV-1, most cells in the long-lived proviral reservoir are infected at or near ART initiation[1,2,3], but a minority of people have their reservoir formed at a different time.
- The mechanisms that determine when the reservoir forms are unknown.
- To explore clinical factors impacting reservoir formation, we studied formation of the long-lived reservoir in a large, clinically diverse cohort of participants enrolled in the MACS & WIHS Combined Cohort Study (MWCCS).

Cohort characteristics	average (range)
Sex	M=**12** F=**7**
Age at ART initiation	**45** (33-60)
Years untreated	**7.7** (2.3-16)
Viral Load at ART (Log10 vRNA copies/mL)	**4.79** (3.8-5.7)
Years between ART initiation and reservoir sampling	**3.7** (2.9-6.5)

Methods

Figure 1: HIV vRNA levels in a cohort of 19 participants with 2.3+ years of untreated HIV-1, followed by 3+ years suppressive ART.



For each participant, we determined the pre-ART time of origin for 30+ unique reservoir proviruses.

- **Proviral sequencing**: PBMC DNA was extracted after 3+ years of suppressive ART, and proviral 3' half genomes were amplified at limiting dilution and sequenced using PacBio with barcodes[1].
- **Pre-ART vRNA sequencing:** vRNA was extracted from longitudinal pre-ART plasma samples that were collected every 6 months. Five gene regions (3 in *env,* 1 in *vif* and 1 in *nef)* were amplified and sequenced using Miseq Primer-ID[4].
- **Reservoir dating**: Longitudinal vRNA sequences were used to build phylogenetic trees mapping viral evolution for each gene region. Each reservoir provirus was genetically compared to longitudinal vRNA sequences to determine when the reservoir cell was likely infected[1].

The fraction of the HIV-1 reservoir which forms near the time of ART initiation is correlated with CD4+ T cell count and viral load at ART initiation and the degree of on-ART immune reconstruction.

Results

Figure 2: On average, 70% of the long-lived reservoir is formed within a year of ART

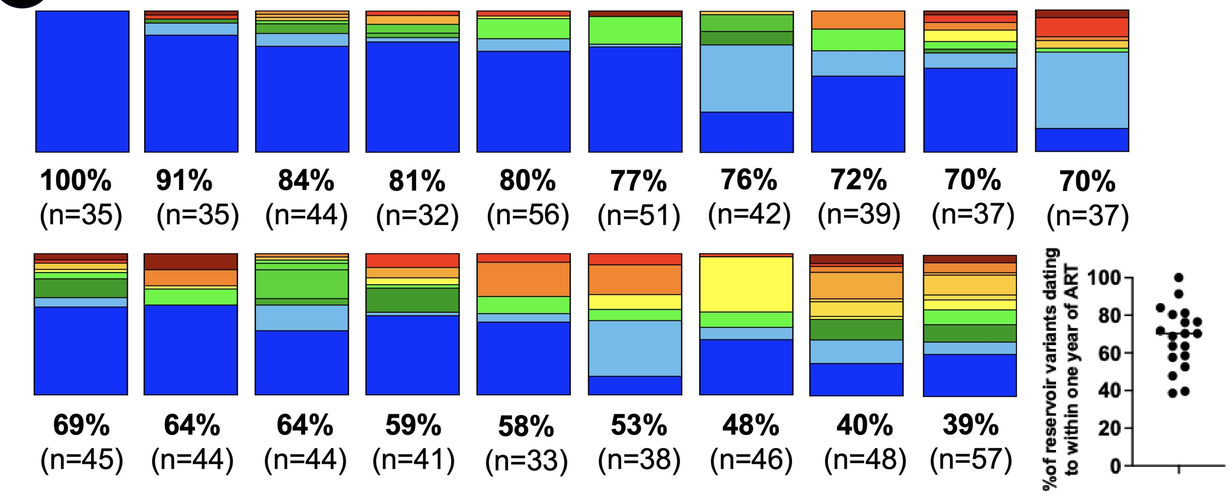
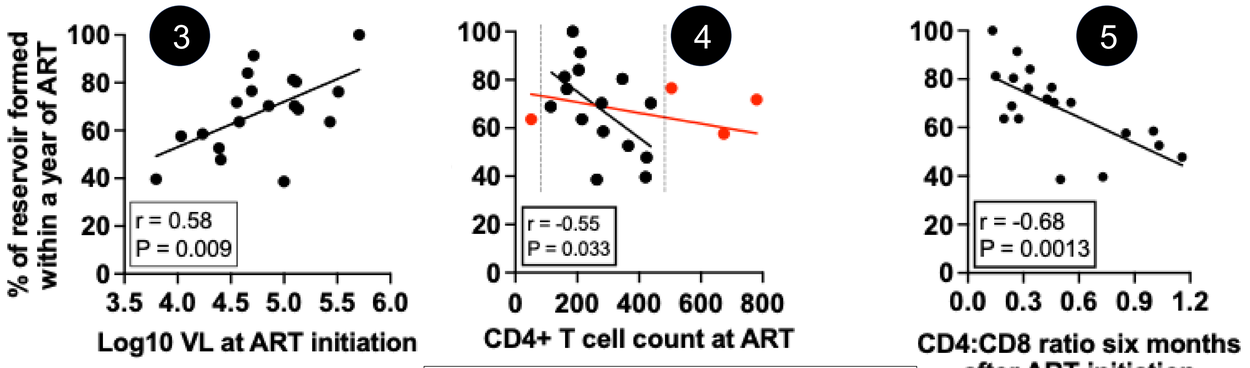


Figure 3 & 4: Viral load and immunosuppression correlate with % of the reservoir formed within a year of ART



Red dots: participants in the cohort with extremely high or low CD4+ T cell counts, who fall outside the range of CD4+T cell counts at ART initiation observed in our previous cohort.

Figure 5: Immunosuppression after starting ART correlates with % of the reservoir formed within a year of ART

Conclusions

- In this cohort of both men and women who varied greatly in immunosuppression and length of untreated infection, the long-lived HIV-1 reservoir typically formed near the time of ART initiation.
- A higher percentage of the reservoir forming within a year of ART was associated with:
  - Higher viral load at ART (Fig 3)
  - More severe immunosuppression at ART (Fig 4)
  - Worse immune reconstitution after ART (Fig 5)
- Factors not associated with time of reservoir formation include:
  - Age at ART initiation
  - Sex
  - Length of untreated infection prior to ART

Plain Language Summary

Worse immunosuppression before therapy and worse immune recovery after starting therapy are both linked to having a reservoir formed closer to the start of therapy.

This finding is exciting because it shows us a glimpse of *why* this might be happening, so we can get closer to understanding how reservoir formation is controlled, which is important for curing HIV.

Acknowledgements & Additional Key Information

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Citations:

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