HIV-1 RESERVOIRS IN YOUNG ADULTS AFTER LONG-TERM ART OF CONGENITAL INFECTION.

#450

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Background

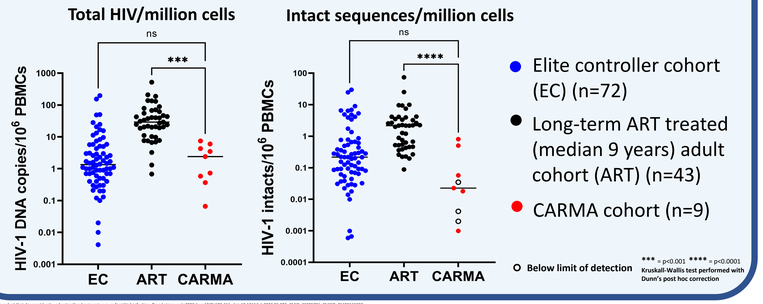
- The persistence of latently infected HIV-1 reservoir cells, with the potential to drive rebound, is a key obstacle in HIV cure strategies. The integration sites of proviruses is hypothesized to be an important factor in what drives viral rebound. HIV-1 that associates to heterochromatin and transcriptionally repressed regions may become 'blocked' from expression (1).
- Prolonged ART may allow for immune-mediated selection of proviruses with defections or intact proviruses that have become 'blocked' (2). Furthermore, this process may be enhanced when initiated during early infancy.
- **We conducted a deep analysis into the HIV-1 reservoir of a unique cohort (CARMA cohort) of 9 young adults, all of whom were infected perinatally with HIV-1 and subsequently started ART early in infancy (median: 3.9 months). They have been on uninterrupted ART for a median of 19 years (range: 9-26 years). Presented here are the reservoir data for this cohort and on three participants (LEUKOHIV 06, 05, 09).**

Methods

- **Full-length Individual Provirus sequencing (FLIP-seq).**
  - NFL sequencing assay for characterizing the HIV reservoir (3).
- **Matched Integration site and Provirus sequencing (MIP-seq).**
  - NFL assay with integration site mapping for individual HIV proviruses (2).
- **Parallel RNA, Integration site and Provirus sequencing (PRIP-seq).**
  - Simultaneous measurement of viral transcriptional activity with integration site mapping and NFL sequencing (4).
- **Quantitative Viral Outgrowth Assay (QVOA).**
  - Gold standard assay for measuring the replication competent reservoir (5). Viral outgrowth detection by Tat-dependent Luciferase assay.

Cohort reservoir overview

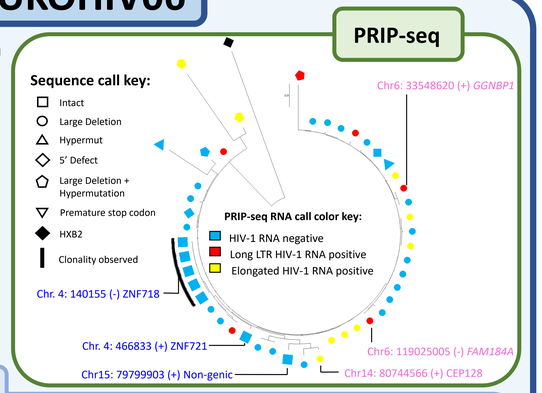
- A total of 2.23 x 10^9 PBMCs have been assayed. 2217 HIV-1 sequences detected. 73 sequences were observed to be intact and therefore replication competent.

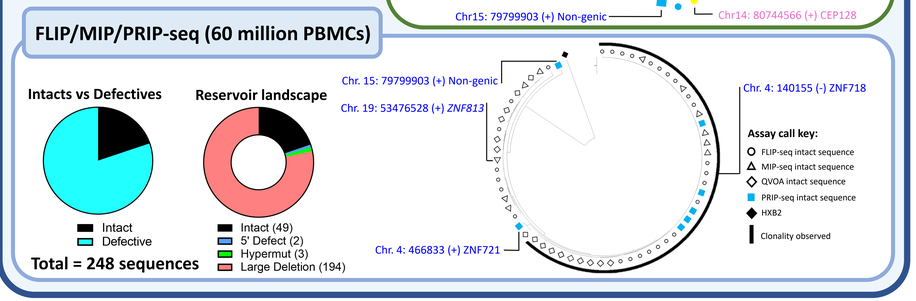


In some **young adults on LT-ART since infancy**, we observed either a **near or complete absence** of **replication competent proviruses**, or a reservoir dominated by **intact proviruses** in regions of **low transcriptional activity.**

LEUKOHIV06

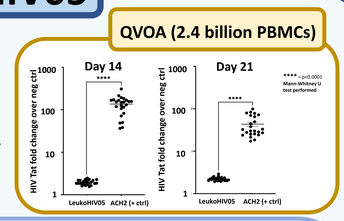
- Initiated ART 3.7 months following birth (uninterrupted treatment for 20 years). 49 intact and 159 defective HIV-1 detected (60 million PBMCs assayed).
- **A large intact clone integrates in *ZNF718*, further intact sequences also integrate in *ZNF* or non-genic regions.**
- All intact sequences detected were not transcriptionally active, but viral outgrowth was achieved by QVOA.

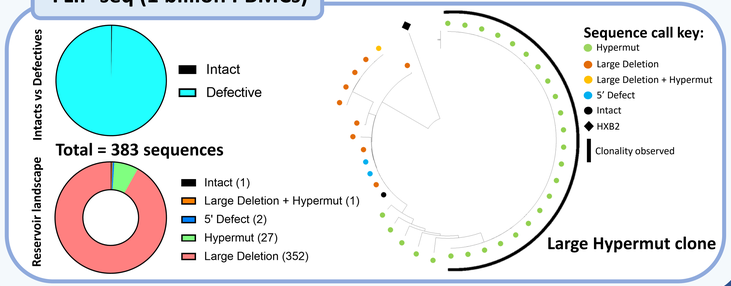




LEUKOHIV05

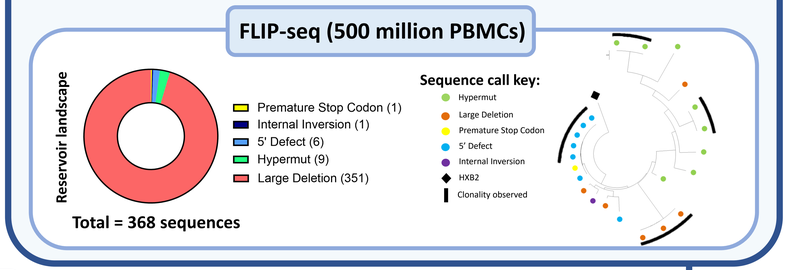
- Initiated ART 0.26 months following birth (uninterrupted treatment for 15 years).
- **No viral outgrowth across 2.4 billion PBMCs. FLIP-seq detected 1 intact and 382 defective viruses across 1 billion PBMCs.**





LEUKOHIV09

- Initiated ART 5.77 months following birth (uninterrupted treatment for 27 years).
- **FLIP-seq detected 0 intact and 368 defective HIV-1 viruses from 500 million PBMCs assayed.**
- Small defective virus clones have been observed.



Conclusions

- **Significantly less HIV-1 was detected in the CARMA cohort compared to a cohort of adults on long-term suppressive ART.** CARMA reservoirs were similar to an elite controller cohort: individuals who are capable of naturally controlling HIV-1 infection without ART (6).
- **In LEUKOHIV06: intact proviruses integrated in ZNF gene sites or non-genic regions.** ZNF genes are associated with heterochromatin and repressive features (Krüppel associated boxes) that hinder transcription. No transcriptional activity was observed in these intact proviruses when assayed by PRIP-seq. **Near absence and complete absence of HIV-1 proviruses was measured in LEUKOHIV05 and LEUKOHIV09 respectively.**
- **These data suggest that the pediatric immune system can exert effective immune selection pressure on viral reservoir cells.**

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