HIV-1 RESERVOIRS ESTABLISHED PRIOR TO THERAPY CONTRIBUTE TO REBOUND VIRUS

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BACKGROUND

Genetically-characterising rebound virus during analytical treatment interruptions (ATI) will further our understanding of the persistent HIV-1 reservoir that is the barrier to a cure. Previous studies sequencing short regions of the HIV-1 RNA genome have indicated that identical proviral sequences contribute to viral rebound during ART interruption (1,2).

METHODS

ATI study: 3 participants were treated during acute infection, continued treatment for 1 year, and interrupted ART three times. ATI plasma samples of ~1,000 copies/mL from the 1st ATI for P3, and the 2nd ATI for P4 and P5 were collected (4).

We sequenced peripheral blood samples from these participants using the full-length individual proviral sequencing (FLIPS) (3) and plasma-derived RNA using long-range sequencing (PRLS) assays (Figure 1).

All sequences were trimmed to the ~4.7kb region overlapping the *gag*-3', FLIPS and *int*-3' amplicons.

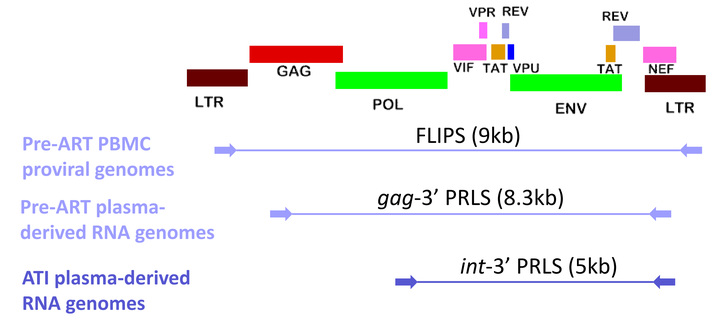

Figure 1. Peripheral blood sample timepoints were sequenced using either the FLIPS or PRLS assays. The portion and length of the HIV-1 genome sequenced by each assay is indicated.

RESULTS

1. For all 3 participants within the trimmed *int*-3' region:
   -Proportion of 100% identical sequences in the ATI plasma was higher than pre-ART plasma (Figure 2A)
   -Proportion of >99.97% identical (1 nt change between sequences) in the ATI plasma was higher than pre-ART plasma (Figure 2B)

2. We found **ATI plasma-derived sequences that were 100% identical to a pre-ART plasma sequences for all three participants**.
   - All were genetically-intact in the trimmed *int*-3' region (3rd ring, Figure 3), though one had a defect outside of this region (2nd ring, Figure 3A).

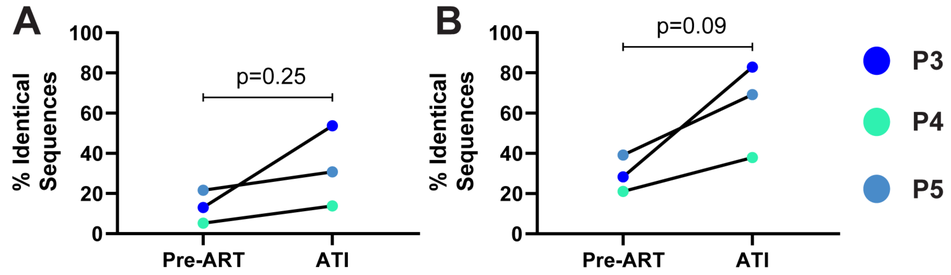

Figure 2. The proportion of sequences that were 100% (A) or >99.97% (B) identical to another sequence in the same sample within the trimmed *int*-3' region was compared between the pre-ART and ATI plasma samples.

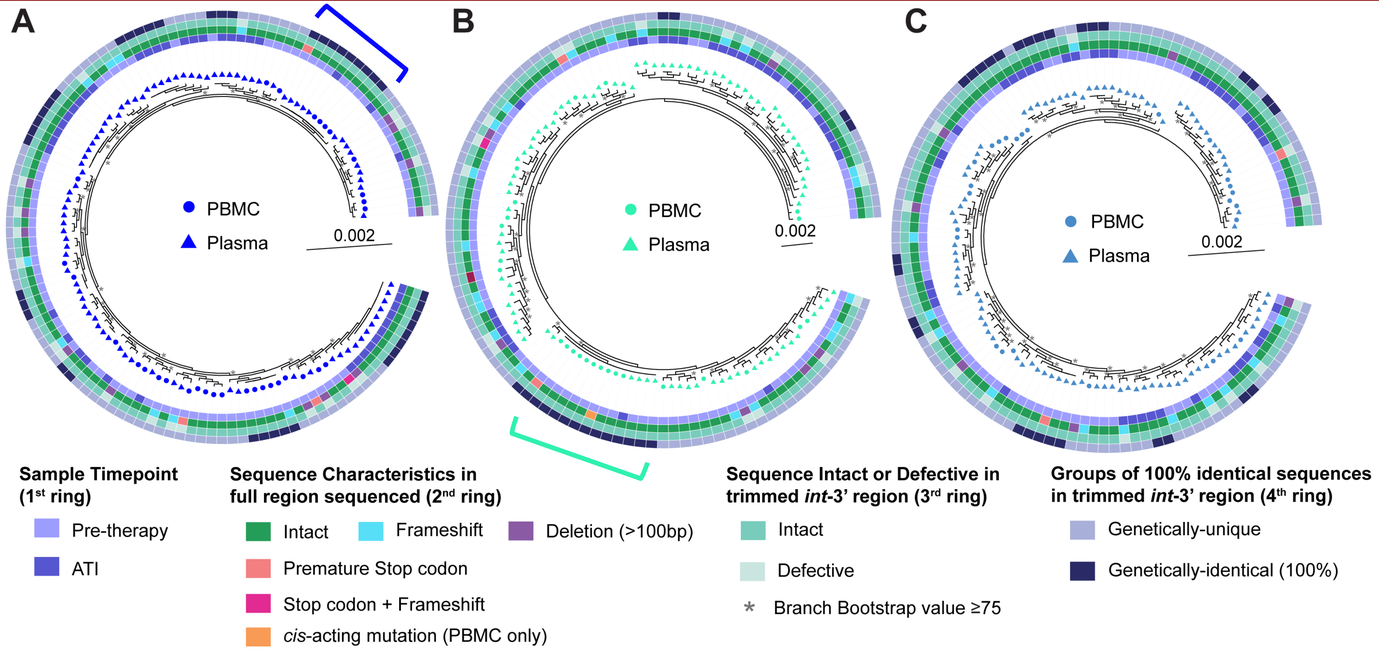

Figure 3. Phylogenetic trees of trimmed *int*-3' genomes were prepared for participants P3 (A), P4 (B) and P5 (C). 1st rings show whether the sequence is from a pre-ART or an ATI sample. 2nd rings show whether a sequence is intact, or defective with each type of defect shown, within the full region sequenced for that sample. 3rd rings show whether a sequence is intact or defective within the trimmed *int*-3' region. 4th rings show whether a sequence is 100% identical to another sequence in the tree in the trimmed *int*-3' region.

P3 and P4 (Figure 3A and B) had an **ATI plasma sequence that was 100% identical to a cluster of pre-ART plasma and PBMC sequences** in the trimmed *int*-3' region (indicated by brackets).

- For both, a subset of these pre-ART plasma and PBMC sequences were 100% identical and genetically-intact (2nd ring in figures) in the full-length sequenced region (i.e. 8.3 kb *gag*-3' for pre-ART plasma-derived RNA sequences and 9kb for FLIPS PBMC proviral sequences).
- This indicates that these proliferating cells persist during ART.

CONCLUSIONS

- A large proportion of rebounding virions present during ART interruption are genetically-identical within the sequenced region, indicating that **proliferating cells are contributing to the rebounding virus during an ATI.**
- We found evidence of proliferating cells during pre-therapy that persist during ART and contribute to viral rebound.

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