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BACKGROUND

The goal is to identify the source of viral rebound during a monitored analytical treatment interruption (ATI) in the STAR cohort by **examining the genetic composition of proviral DNA in different subsets** from participants on antiretroviral therapy and compare this to rebounding virus after ATI.

METHODS

- Eleven participants were sampled from different anatomical sites (peripheral blood, lymph nodes (LN) and gut-associated lymphoid tissue (GALT)) prior to and during ATI.
- Single-genome sequencing (SGS) of V1V3 *env* region of plasma-derived RNA was performed (De Scheerder et al., CELL HOST & MICROBE, 2019).

In an ongoing study, Full-Length Individual Proviral Sequencing (FLIPS), Integration Site Loop Amplification (ISLA) and modified matched integration site and proviral sequencing (MIP-Seq) assays were performed on the T cell subsets from 3 participants.

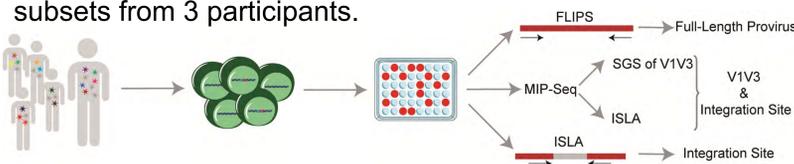


Figure 1: Schematic overview of used methods.

RESULTS

Participant STAR 9: Identical hypermutated provirus found across different memory T cell subsets

- 126 IS and 127 proviral genomes were sequenced, only 3 unique intact proviruses (2%) were identified.
- No link with integration site (IS) due to failure of V1V3 SGS caused by primer mismatching.
- 15 (52%) of the 29 hypermutated sequences are identical and distributed over different memory subsets: 4 in T_{CM} (27%), 5 in T_{TM} (33%) and 6 in T_{EM} (40%).

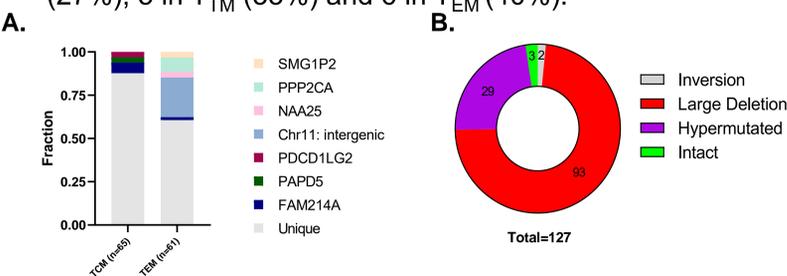


Figure 2: A. Integration Sites recovered via ISLA. B. Schematic overview of the FLIPS sequences.

Participant STAR 11: Intact proviruses integrated in genes regulating transcription and tumor growth

- 99 IS and 107 proviral genomes were sequenced yielding 14 intact proviruses (13%) with the highest proportion found in the T_{EM} subset (n=13, 45%).
- Four different clusters of identical sequences could be identified of which 2 consisted of intact T_{EM} sequences respectively linked to an IS in **ZNF274** (8% of all IS) and **GGNBP2** (3% of all IS).

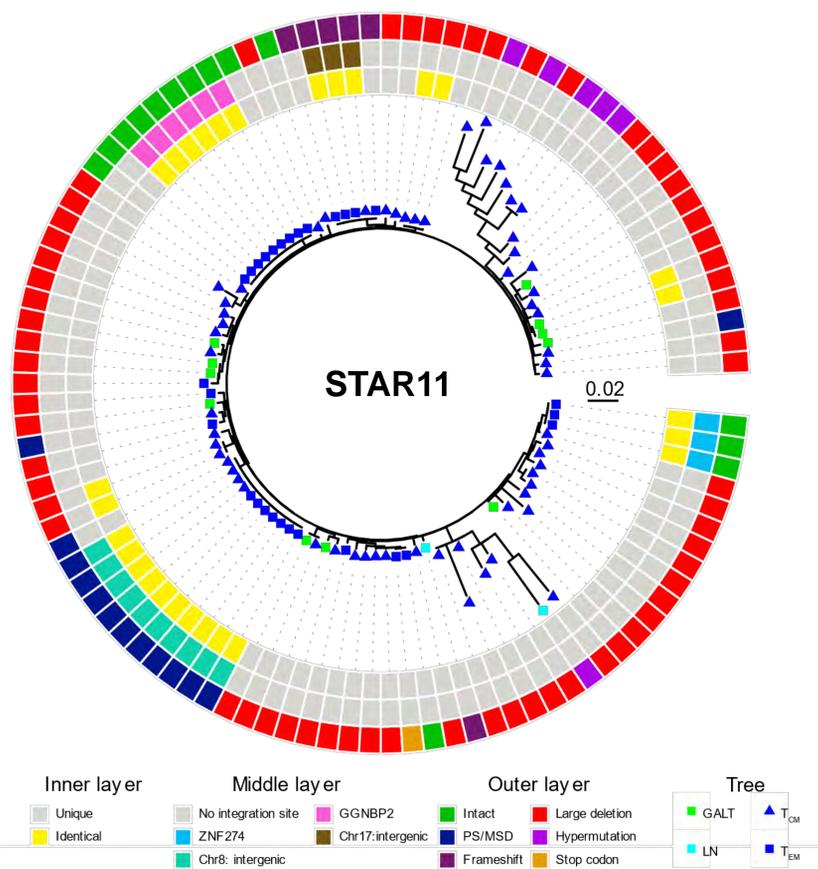


Figure 3: Maximum likelihood tree of all proviral STAR 11 FLIPS sequences. Inner circle layer showing identical sequences based on FLIPS alignment. Middle circle layer displaying matching V1V3 *env* between trimmed FLIPS proviruses and MIP-Seq SGS V1V3 sequences, suggesting link between IS and FLIPS provirus. Outer circle layer showing genetic characterization based on FLIPS sequences.

Participant STAR 10: Defective proviruses match the *env* region of rebounding plasma virus

- 149 IS and 132 proviral genomes were sequenced while only 3 unique intact proviruses (2%) were identified.
- A cluster of 17 identical defective proviruses were linked to an IS (9% of all IS) in **STAT5B** located in T_{NA}, T_{TM} and T_{CM}.
- Comparing trimmed FLIPS to SGS V1V3 *env* sequences, two 100% matches were found between a defective provirus (large internal deletion or ψ deletion) and a plasma HIV RNA sequence during rebound.

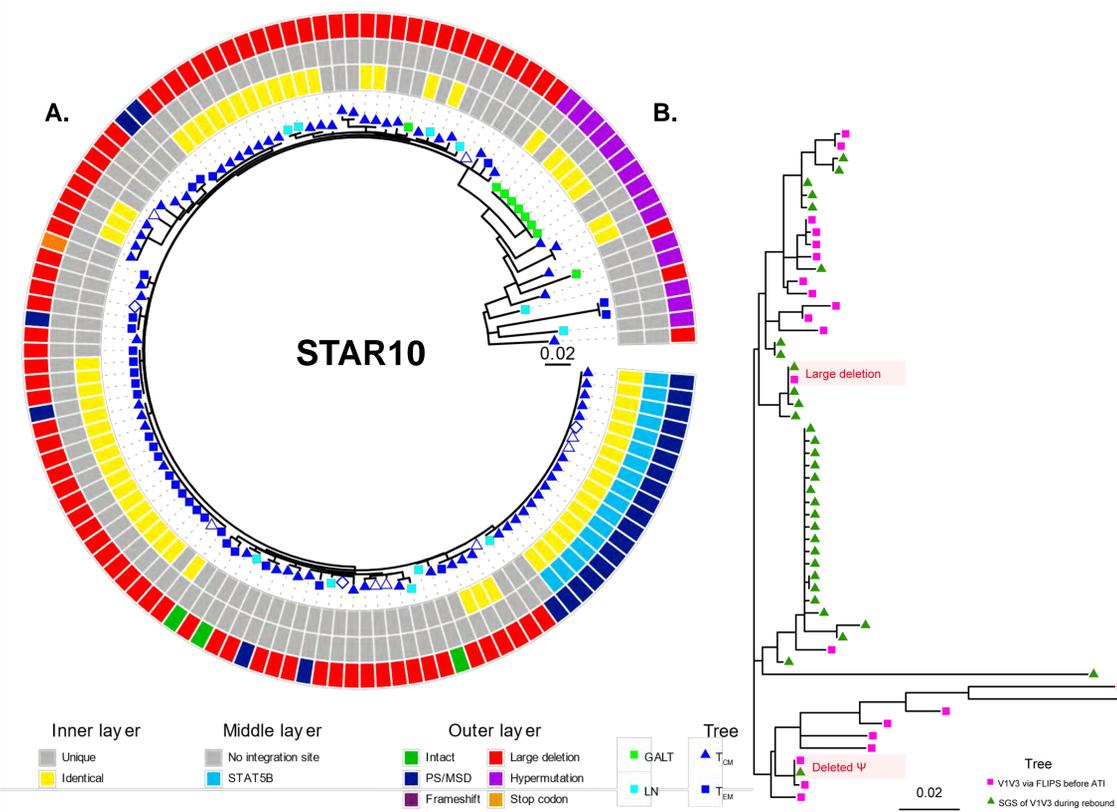


Figure 4: Maximum likelihood tree of all proviral STAR10 FLIPS sequences A) Inner circle layer showing identical sequences based on FLIPS alignment. Middle circle layer displaying matching V1V3 *env* between trimmed FLIPS proviruses and MIP-Seq SGS V1V3 sequences, suggesting link between IS and FLIPS provirus. Outer circle layer showing genetic characterization based on FLIPS sequences. B) Tree showing unique V1V3 sequences from trimmed FLIPS and V1V3 SGS on plasma from rebound.

CONCLUSIONS

- The proviral landscape across different participants is highly variable as illustrated in these three HIV-1 infected individuals.
- Determining the source of viral rebound based on the analysis of subgenomic regions should be interpreted with caution as illustrated in participant STAR10.

ADDITIONAL KEY INFORMATION

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