Viral reservoir diversity in circulating PBMC and T cell subsets under suppressive ART

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Introduction

The primary infection of HIV-1 is usually caused by a single or few founder viruses. With the rapid establishment of a viral reservoir, HIV-1 can persist as integrated provirus for very long periods in resting memory T cells, even during fully suppressive therapy.

This HIV-1 reservoir remains very stable over long periods. So far, neither highly active ART nor 'shock and kill' strategies have been able to continuously reduce or eliminate the HIV reservoirs, making an eradication currently impossible. Even during extended periods of effective, suppressive immune control, a substantial viral dynamic and genetic adaptations are observed in HIV-1 positive individuals suggesting that the persisting reservoirs stay continuously active.

Methods

- Longitudinal analysis of proviral Env sequences was performed by next-generation sequencing (NGS) in HIV-infected individuals.
- HIV-1 proviral load and intracellular viral poly-A transcripts (pA), TTV (Torque-Teno-virus: TTV plasma levels serve as marker of immuno-suppression or immune reconstitution in HIV infection and in stem cell transplant recipients) load were quantified by qPCR.
- PBMCs, sorted and stimulated in cultured for 3 weeks for virus outgrowth, were monitored for viral reactivation by Tat-induced LTR-activation and HIV-1 protein expression using FACS.
- Single-genome sequencing (SGA) analysis of the 3' half of the HIV-genome was carried out to assess genetic integrity in free released SN virus.

Results

TN = Naïve T-cells; TCM = Central Memory T-cells; TTM = Transitional Memory T-cells; TEM Effector Memory T-cells

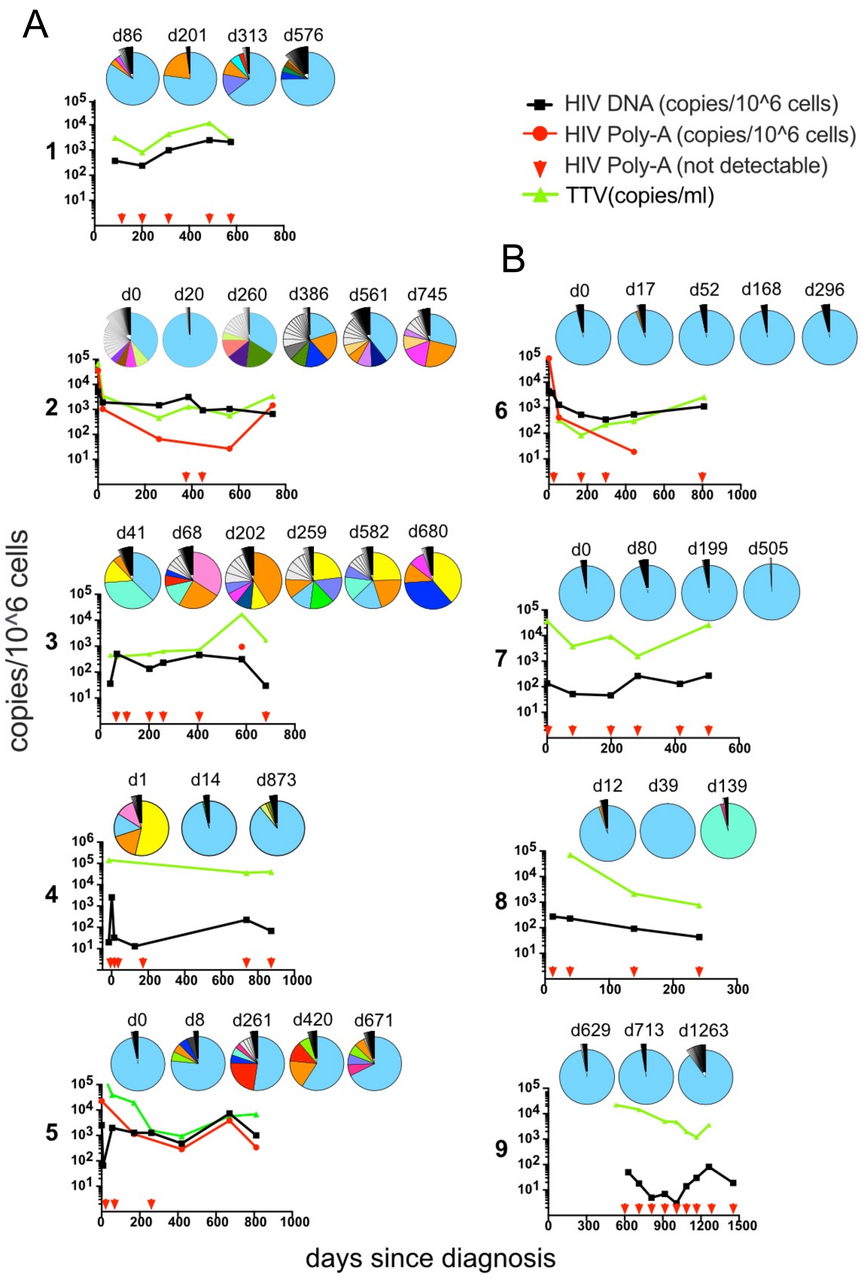

Figure.1 Dynamic changes of HIV reservoirs from time of Diagnosis. A: One group of individuals consistently presents with a high genetic diversity in the viral reservoir during all follow-up periods (1-5). B: A second group of individuals has only one dominating virus variant (6-9). NGS analysis of the V3 region of HIV proviral DNA is shown as pie charts. The 5 most frequent variants are depicted in color; variants with a proportion below 1% are pooled (black). Each color represents one virus variant.

❖ ***During acute infection, the early initiation of ART limits and reduces the genetic diversity of the viral reservoir.***

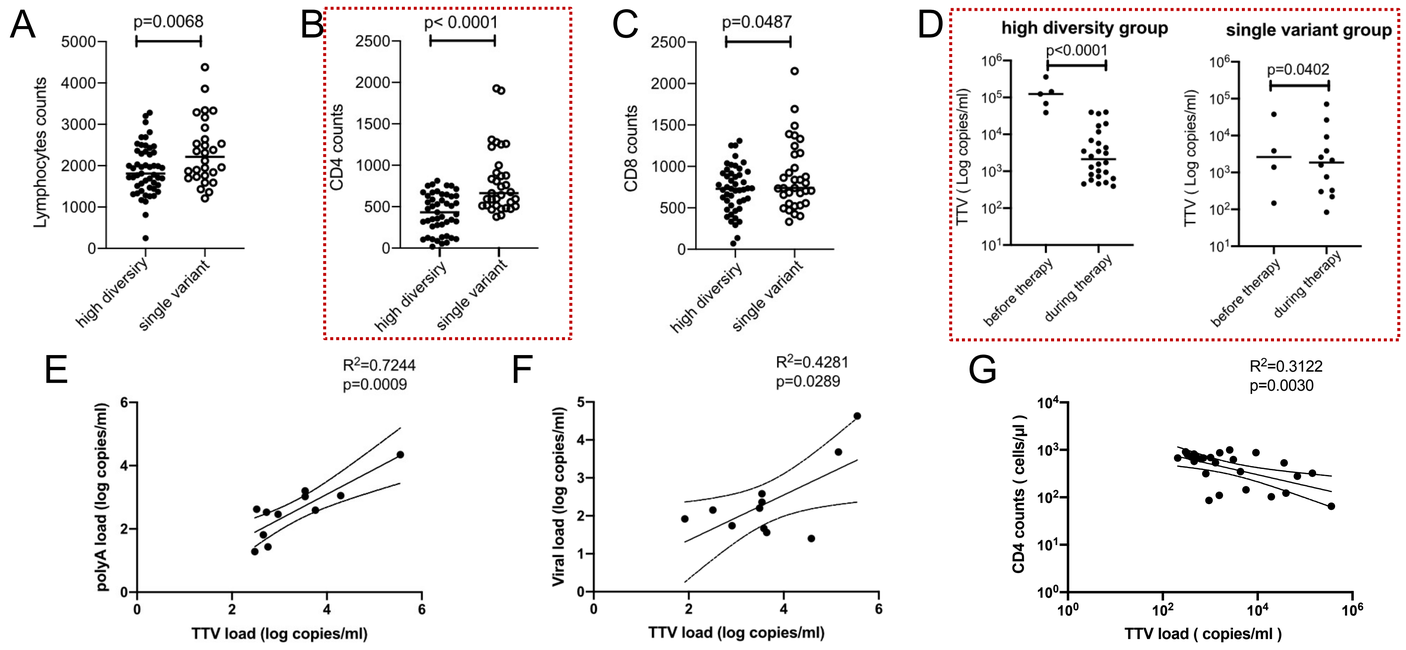

Figure.2 Immune profiling of individuals with highly diverse virus vs. the single variant group: A: lymphocyte count; B: CD4 count, C: CD8 count. D: Comparison of TTV load in both groups before and during suppressive therapy. E-G: Correlation between timepoints of detectable intracellular HIV polyA-RNA load (E), plasma viral load (F), CD4 counts (G) with the corresponding TTV load.

❖ ***The number of CD4 cells inversely correlates with HIV reservoir diversity.***

❖ ***TTV may be a suitable marker for the reservoir of transcriptionally active HIV positive cells.***

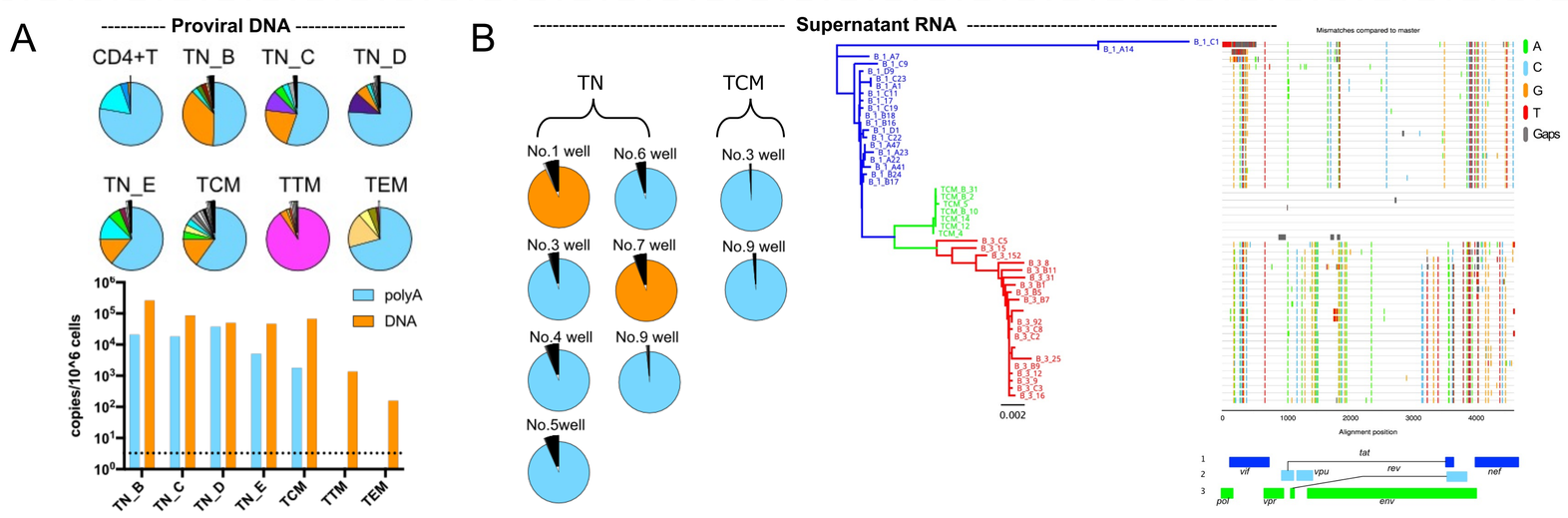

Figure.3 Dynamics of the HIV-1 reservoir in memory T cell subsets *(related to Figure1A)* **A:** Env V3 loop diversity, determined by NGS five days post stimulation, represented by pie charts for bulk PBMC and each T-cell subset. B: NGS V3 loop viral distribution in indicated memory compartments, phylogenetic tree and highlight plot of 3' half SGA sequence from individual outgrowth wells of TN and TCM ($TN_{well\ No.1}$: blue, $TN_{well\ No.3}$: red, $TCM_{well\ No.3}$: green). Each line represents one genomic sequence, mutations are color-coded according to nucleotide as depicted in the legend top right.

❖ ***Replication-competent HIV is typically found in TN and TCM cells, and viral reservoir diversity correlates with free viral genomes; in contrast, TTM and TEM cells present patterns of higher viral reservoir diversity, but do not actively contribute to the pool of infectious virus.***

Conclusions

- In acutely infected HIV individuals, early initiation of ART significantly **reduces and limits the genetic diversity** of the viral reservoir
- **Low lymphocyte counts** in a given individual correlate not only with high viral loads in blood but also with a **high genetic diversity**, even during continuously suppressive therapy.
- This study confirms the key reservoirs for **replication-competent HIV to be in TN and TCM**, which consistently contain a low genetic variability among intact viral variants.
- A distinct, significantly smaller population of archived HIV resides in **TTM and TEM** and is characterized by a high viral variability reflected by a diverse virus population. **These effector cell fractions might drive the evolution of new virus variants** even during suppressive therapy.

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