

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

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## ABSTRACT MESSAGE

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

- Replication-competent infectious virus is mainly found in the TN and TCM cell subsets which is in agreement with the diversity of free viral genomes, while TTM and TEM present patterns of new emerged viral reservoir that lack the presence of infectious virus.
- A number of lymphocytes and CD4 cells in peripheral blood typically correlates inverse with the viral reservoir diversity in the cells.
- Expression of cell-associated HIV-RNA (mostly mRNA) does not imply the production and release of intact, infectious virus particles.

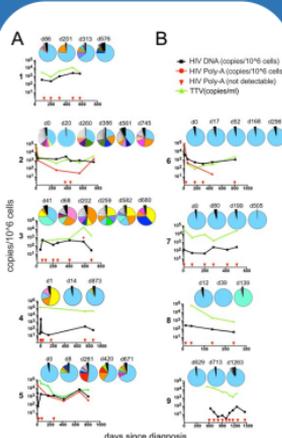
## BACKGROUND

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

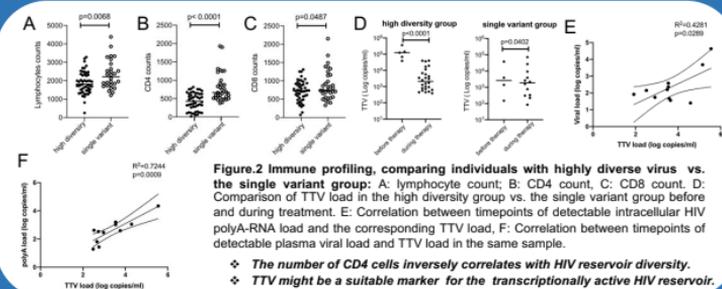
- Longitudinal analysis of proviral Env sequences was performed by next-generation sequencing (Nextera Illumina) in HIV-infected individuals.
- HIV-1 proviral load and intracellular viral poly-A transcripts (pA), TTV load (Torque-Teno-virus) were quantified by qPCR. TTV plasma levels serve as marker of immunosuppression or immune reconstitution in HIV infection and in stem cell transplant recipients.
- PBMCs, sorted and 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 viral in free released SN virus re-activation.

## RESULTS



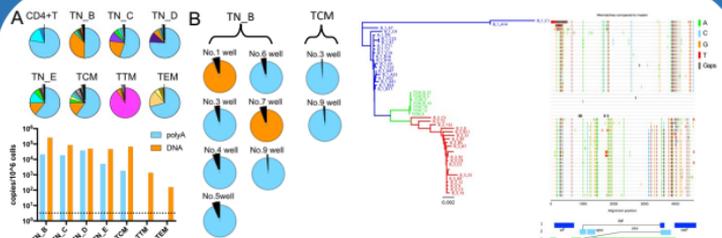
**Figure.1 Dynamic changes of HIV reservoirs from time of diagnosis.** A: One group of individuals consistently presents with a high level of genetic diversity in the viral reservoir during all follow-up periods (1-5). B: In contrast: Individuals with only one dominating virus variant (6-9). NGS analysis of the V3 region of HIV proviral DNA is illustrated by pie charts. Only the top 5 variants are depicted in color; variants with a proportion below 1% are colored (black). Each color represents one virus variant.

❖ **Individual HIV reservoir diversity is not directly correlated with proviral DNA load or intracellular viral poly-A load.**



**Figure.2 Immune profiling, comparing 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 the high diversity group vs. the single variant group before and during treatment; E: Correlation between timepoints of detectable intracellular HIV poly-A-RNA load and the corresponding TTV load; F: Correlation between timepoints of detectable plasma viral load and TTV load in the same sample.

- ❖ **The number of CD4 cells inversely correlates with HIV reservoir diversity.**
- ❖ **TTV might be a suitable marker for the transcriptionally active HIV reservoir.**



**Figure.3 Dynamics of the HIV-1 reservoir inside memory T cell subsets.** A: Viral V3 loop diversity, determined by NGS five days post stimulation, represented by pie charts for bulk PMBC and each T-cell subset. B: NGS V3 loop distribution inside respective memory compartment. Phylogenetic tree and highlight plot of 3' half SGA sequences 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 3' half genome sequence, and mutations are color-coded according to nucleotide, see the legend top right.

❖ **Replication-competent HIV typically stems from TN and TCM cells, and their viral reservoir diversity is consistent with free viral genomes; in contrast, TTM and TEM cells present patterns of higher and distinct viral reservoir diversity, but does not contribute to the pool of infectious virus.**

## CONCLUSIONS

- The presence of low lymphocyte counts in a given individual correlates not only with high virus levels in blood but also with a high genetic diversity, even during continuous suppressive therapy.
- We confirm the key reservoirs for replication-competent HIV to be in TN and TCM, which consistently contain a small number of intact viral variants.
- A distinct, significantly smaller contribution of archived HIV resides in TTM and TEM and is characterized by a high viral variability represented by a mixed virus population. These cell fractions may drive the evolution of new virus variants even during suppressive treatment.