HIV-1-Specific T Cells From Post-treatment Controllers And Non-controllers

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Background

- A subset of individuals, **post-treatment controllers** (PTCs), experience sustained virologic control following **analytical treatment interruption** (ATI), whilst **non-controllers** (NCs) require re-initiation of antiretroviral therapy (ART) to suppress rebounding viremia.
- A deeper understanding of the **immunological mechanisms of PTC** is needed to uncover potential **biomarkers for predicting PTC** and inform the development of therapeutic strategies aimed at achieving a functional cure for HIV-1.

Methods

- We investigated the immune response in two PTCs (ID107 and 142) and two NCs (ID104 and 112) before ATI (**pre-ATI**) and at either **viral rebound** or several time points during **long-term ATI** (Figure 1).

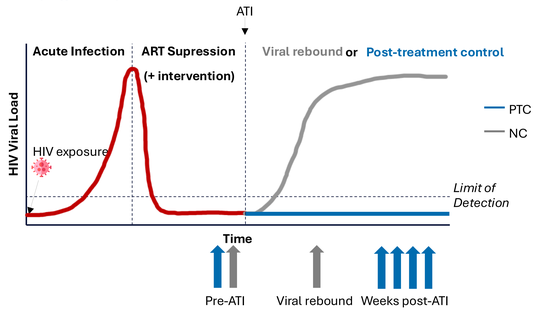


Figure 1: *Schematic overview of HIV Viral load in PTCs (blue) and NCs (grey) and sample timepoints for each group.*

- We used **spectral flow** cytometry-based **intracellular cytokine staining** and stimulated PBMCs with HIV-1 peptide mix (Pol, Gag, Nef, or Env) or negative control (DMSO).
- The panel consisted of markers of **lineage/differentiation** (CD3, CD4, CD8, CD16, CD19, CD45RA, CCR7, CD27, CD95), chemokine receptors (CCR4, CXCR5), proliferation (Ki67), **cytokines/degranulation** (IFNγ, TNFα, IL2, CD107a), transcription (FoxP3, Tbet, TCF1), effector proteins (Perforin, GzmB, GzmK, Granulysin) and immune checkpoints (TIGIT, CD39, PD-1).
- Cell were clustered using FlowSOM and visualised on a UMAP downsampled to 10,000 cells.
- **Polyfunctionality** was assessed by gating cells on cytokines and CD107a and quantifying the combinations of the four markers. **Pseudobulk background subtraction** was performed by calculating the percentage of marker-positive cells per cluster in the stimulated conditions and subtracting the corresponding percentage from the unstimulated control.

Analysis of two post-treatment controllers during long-term ATI revealed a **T cell memory phenotype** with better **capacity for viral control** by higher frequencies of HIV-1-specific **polyfunctional** memory T cells.

Results

- We clustered our data into **main lymphocyte populations** (Figure 2A) and filtered out NK cells, B cells, double positive T cells and double negative T cells (Figure 2B) for **subclustering** of CD4 T cells (cluster 9) and CD8 T cells (clusters 5 and 7) to **further refine T cell subsets** (Figure 2C, D). **PTC was associated with increased proportions** of CD8 Transitional memory, CD8 Pre-effectors, CD4 Central memory and EMRA, aTreg (only ID107) and **decreased proportions** of CD8 Naïve and Effector memory, CD4 Effector memory, and iTreg (Figure 2E, F).
- We excluded CD8 Naïve, CD4 Naïve, and Tregs to focus on the **CD8 and CD4 memory populations** (Figure 3A, B). Cells were then gated for cytokines (IFNγ, TNFα, IL2) and the degranulation marker CD107a (Figure 3C). When assessing polyfunctional T cells within the CD8 and CD4 memory compartments, PTCs exhibited higher proportions of polyfunctional CD8 and CD4 memory T cells (Figure 3D). The CD4 response was predominantly **IFNγ⁺TNFα⁺IL2⁺**, whereas the CD8 response was primarily **IFNγ⁺TNFα⁺CD107a⁺**. ID107 displayed a **dominant response to HIV-1 Gag** in both CD4 and CD8 memory subsets, while another PTC, ID142, exhibited responses to **HIV-1 Pol and Nef**, mainly within the CD8 memory compartment. Notably, the Nef response was largely monofunctional.

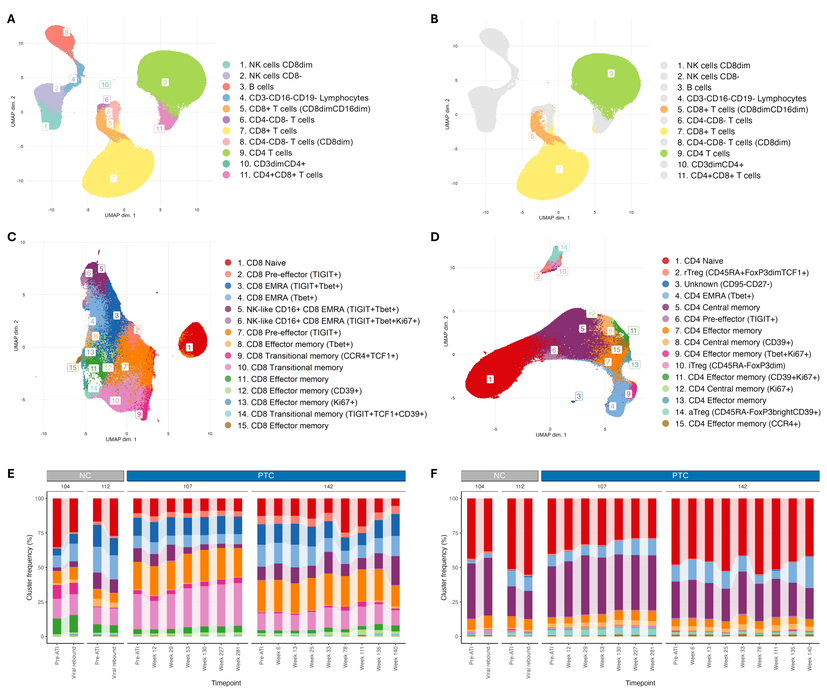


Figure 2: T cell composition. *(A, B) FlowSOM clustering of major lymphocyte populations visualized on a UMAP. Cells are coloured by cluster ID, with clusters selected for subclustering highlighted in (B). (C, D) UMAP representation of subclusters within the CD8 (C) and CD4 (D) T cell populations. (E, F) Stacked bar graphs depicting longitudinal changes in cluster frequencies of CD8 (E) and CD4 (F) T cells in NCs and PTCs. Colours represent cluster IDs, corresponding to the colour scheme in (C) and (D).*

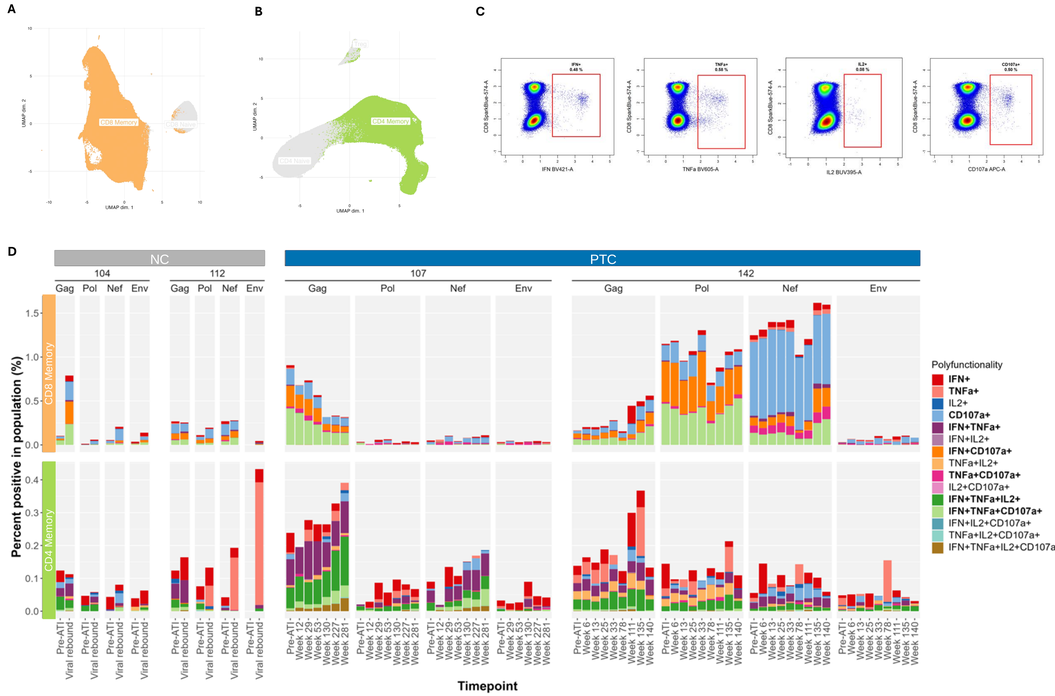


Figure 3: Memory T cell polyfunctionality. *(A, B) UMAP highlighting CD8 (A) and CD4 (B) memory T cell populations. (C) Representative gating example for cytokine positivity and the degranulation marker CD107a. (D) Stacked bar graph showing the longitudinal percentages of positive cells for each combination of markers (polyfunctionality). The top panel represents CD8 memory responses, while the bottom panel shows CD4 memory responses. Colours indicate marker combinations, with those exceeding 0.05% frequency highlighted in bold. The pre-ATI Env stimulation data for ID112 is missing due to insufficient cell numbers in the PBMC vial.*

Conclusions

- PTC was associated with higher proportions of CD8 TM, CD8 Pre-effectors, CD4 CM and EMRA, and lower proportions of CD8 Naïve and EM, CD4 EM, and iTreg.
- PTCs showed a CD8 and CD4 memory phenotype with better capacity for viral control by their higher frequency of HIV-1-specific polyfunctionality.

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