CD4⁺ T cells harboring inducible HIV genomes seem to display a senescent-like phenotype

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Introduction

The major obstacle to HIV cure is the persistence, despite antiretroviral therapy (ART), of a pool of latently infected cells.

Several mechanisms could explain this survival: maintenance of latency, immune escape, resistance to apoptosis, proliferation …

We hypothesized that cells of the inducible, translation competent reservoir (p24⁺ cells) display a different phenotype from uninfected cells, which confers them the ability to persist through time on ART.

Materials & Method

Longitudinal cohort:

- 12 participants (11PWH + 1 PWOH), all males 35-60 years old
- At least 4 years post ART

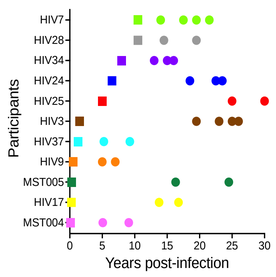


Figure 1: Timepoints sampled
Squares (■) represent the beginning of ART, circles (●) represent the times of sampling. Samples from the same PWOH were used for all experiments as a negative control.

Strategy: **HIV-Flow**

2 antibodies targetting p24

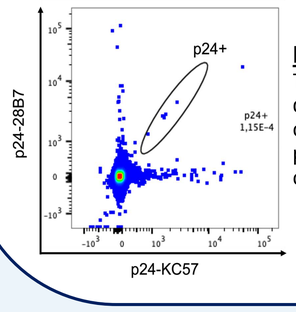


Figure 2: Example of p24 staining
Two antibodies are used, targetting two different epitopes of the p24 protein (viral capsid). For added specificity, only the double positive cells (inside the diagonal gate) are considered p24⁺.

Protocol

1) PBMCs are thawed and enriched in CD4⁺ T cells.

2) CD4⁺ T cells are stimulated 24h
(12,5µg/mL anti-CD3 and 5µg/mL anti-CD28)

3) Cells are harvested and stained according to the HIV-Flow strategy
Common: CD3, CD4, CD8, CD45RA, CCR7, Live/Dead, p24
Panel 1: TIGIT, PD-1, CTLA-4, CD127
Panel 2 : IL-2, IL-17, TNFα, IFNγ
Panel 3 : Granzymes A, B and K, CD57, KLRG1
Panel 4 : Tbet, GATA3, FoxP3, Bcl-6

Gating strategy

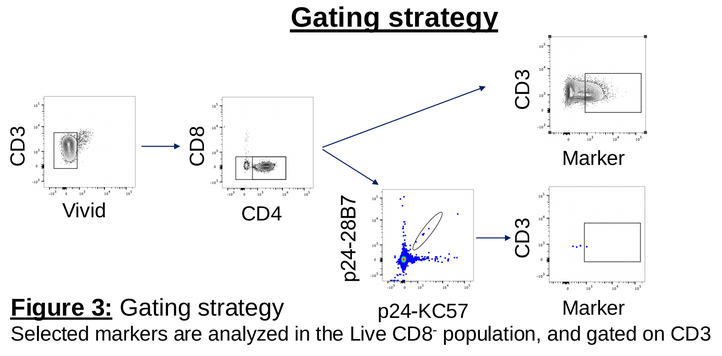
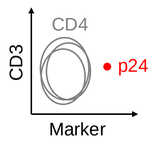


Figure 3: Gating strategy
Selected markers are analyzed in the Live CD8⁻ population, and gated on CD3

Legends

The dot plots show an example of gating. Grey lines and dots are Total CD4 and the red dots are the p24+ cells.



For each marker the MFI is compared between the Total CD4 population and the p24+ cells.

Each ●/● represents a participant.

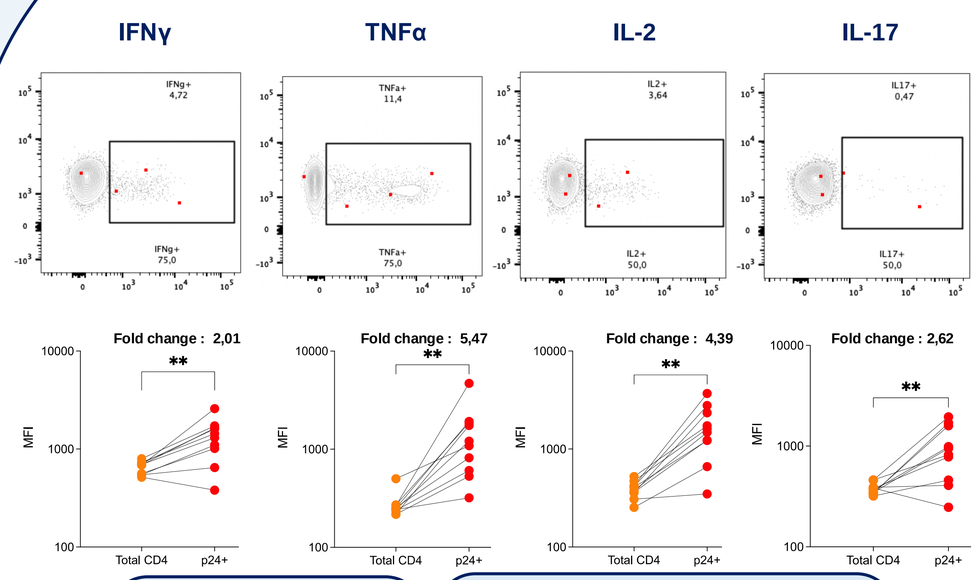
Statistical differences where determined using Wilcoxon test (Paired, non parametric).

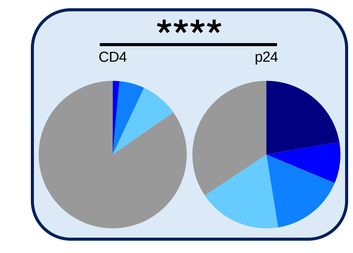
The pie charts show the co-expression of the markers in the Total CD4 population compared with the p24+ cells
Statistical differences where determined using Fisher's exact test)



Results

Cytokine production

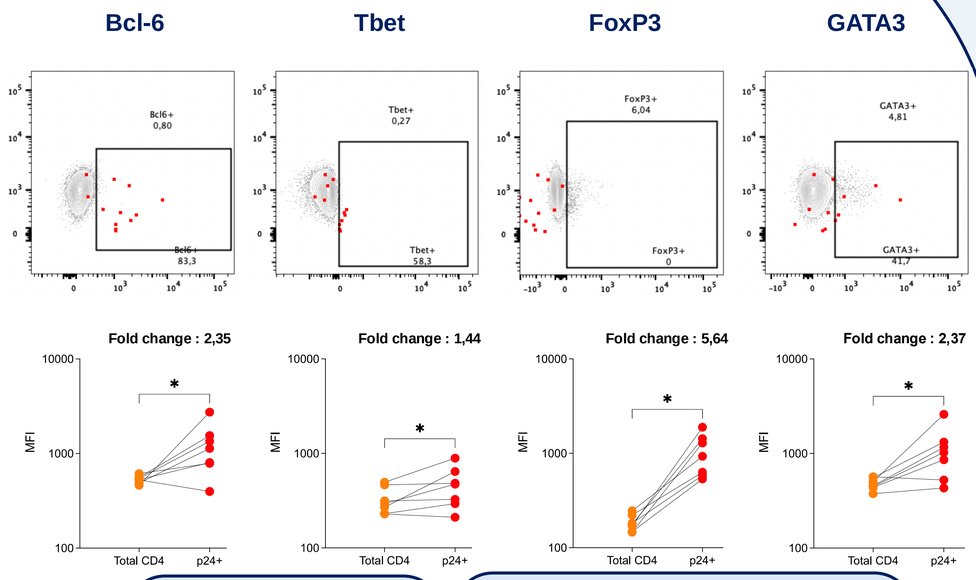


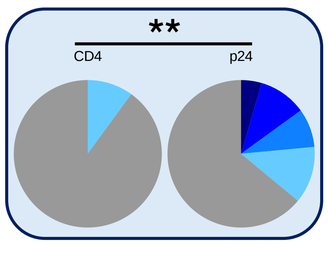


p24+ are enriched in IFNγ, TNFα, IL-2 and IL-17.

Furthermore, they co-express these cytokines more than the general CD4⁺ T population

Transcription factors

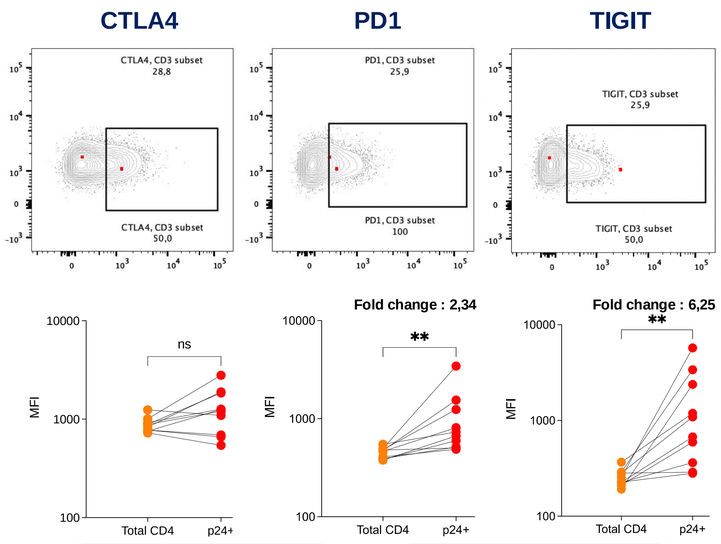


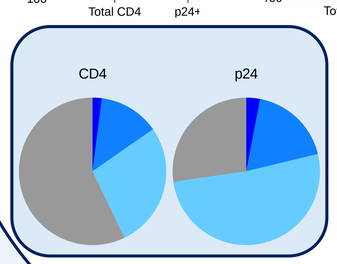


p24+ are enriched in Bcl-6, Tbet, FoxP3 and GATA3.

Surprisingly, where none of the CD4+ T cells co-express these markers, 25% of the p24+ cells do.

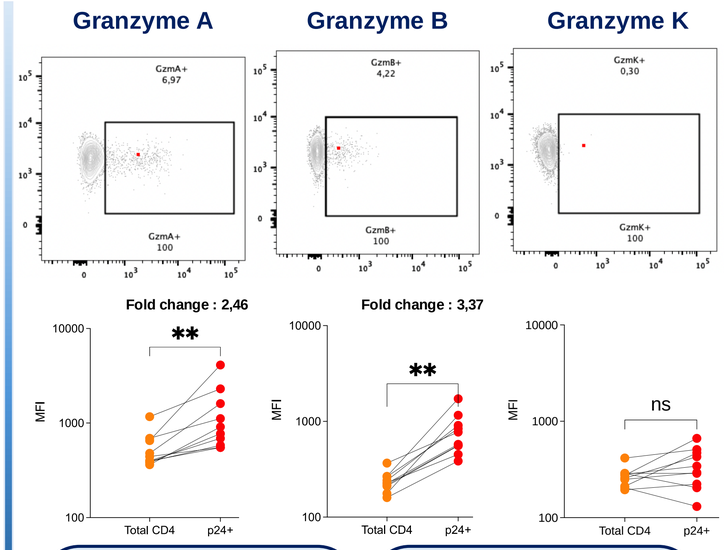
Immune checkpoint molecules

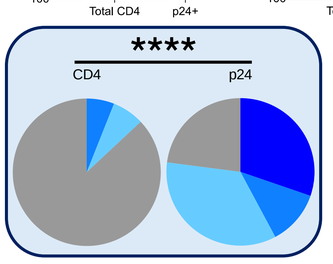




p24+ cells are enriched in PD1 and TIGIT.

Granzymes

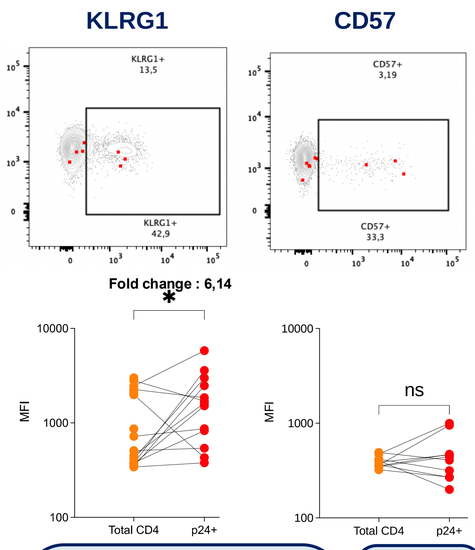


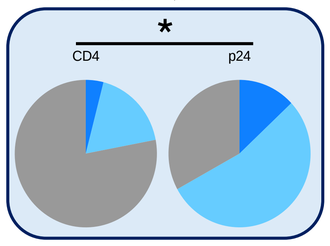


p24+ are enriched in Granzymes A and B.

Furthermore, they co-express these granzymes more than the general CD4⁺ T population

Senescence markers





p24+ cells are enriched in KLRG1

Conclusion

Our data suggest that cells of the inducible, translation competent reservoir (p24+) display phenotypic markers that could help explain their survival.

Interestingly, several of these markers could also be linked to a senescent-like phenotype (increased cytotoxicity, increased cytokine function, expression of senescence markers like KLRG1, expression of PD1 and TIGIT). The development of a panel of more specific senescence markers (downregulation of CD28 and CD27, increased SA-β-Gal…) could help further investigate this hypothesis.

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Conflict of Interest Disclosure: "I have no conflicts of interest"