Reduced HIV RNA Transcription During Long-Term ART is [Not] Associated with Increased Ki67⁺CD8⁺ T cells.

471

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BACKGROUND

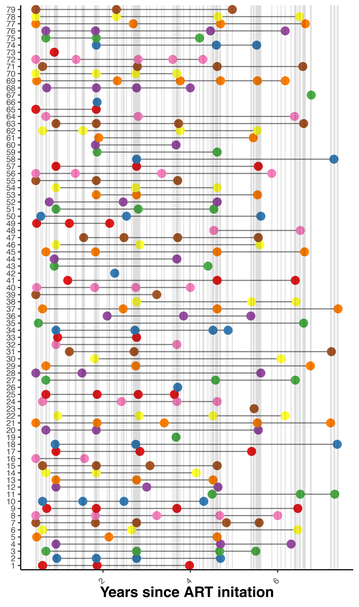
- Sex-based differences affect HIV persistence and immune responses.
- We investigate longitudinal T cell phenotypes and their association to measures of HIV persistence (size and activity) in 28 men and 51 women during long term suppressive ART.

STUDY COHORT

AIDS Clinical Trials Group Longitudinal Linked Randomized Trials (ALLRT):

- Longitudinal peripheral blood samples (N=242) between 1999 and 2013.
- 40-53 years of age at the time of ART initiation.
- Virally suppressed (<20 cp/mL) throughout the study period.
- Did not report taking any hormonal therapy.
- See **Figure 1** for timing of blood sampling, **Table 1** for cohort characteristics.

Figure 1 Timing of blood sampling



DATA GENERATED FOR EACH TIMEPOINT

- Markers of T Cell activation (HLA-DR⁺CD38⁺), cytotoxicity (CD107a⁺), cycling (Ki67⁺), exhaustion (TIGIT⁺PD-1 ⁺) on T cell memory subsets (central memory, effector memory, terminally differentiated), See **Figure 2** for gating strategy.
- Cellular HIV DNA and HIV RNA (by ddPCR).

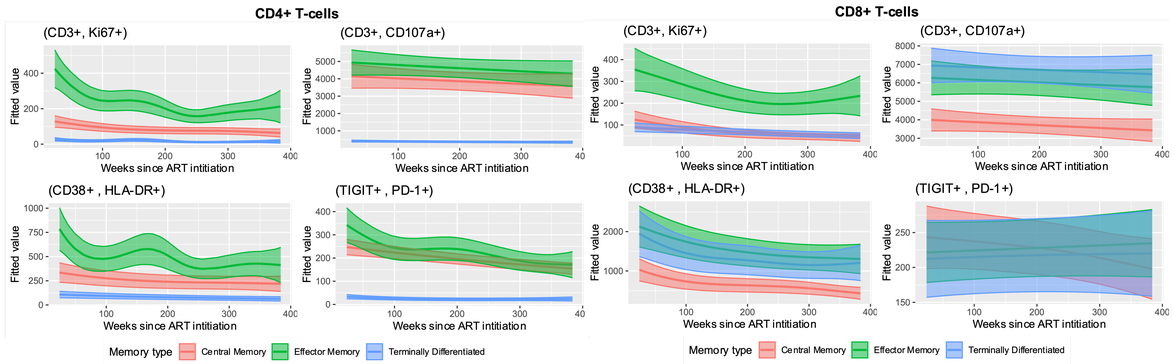
STATISTICAL ANALYSIS

- Immune cell trajectories were analyzed using a generalized additive model with negative binomial link function with random intercept.
- Association between immune cells and HIV reservoir measures were analyzed using a similar model with a gaussian link function.
- All p-values were adjusted for multiple comparisons using the Holm procedure in a closed testing framework.
- First a full model with covariates age, race, sex and time was compared to a null model; if significant, this test was followed by tests of individual effects.

Cytotoxic CD4⁺ and CD8⁺ T cells and exhausted CD8⁺ T cells remain elevated in PWH despite long term suppressive ART, suggesting ongoing antigen exposure.

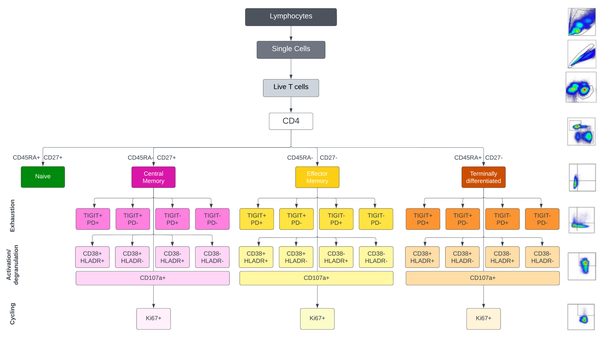
Deeper phenotypic analysis is likely needed to understand the interactions between sex, immune system and HIV persistence.

Figure 3. Markers of T Cell Cytotoxicity as well as CD8 T Cell exhaustion remain elevated despite long term suppressive ART



Legend: (A): CD4+ T cells, (B) CD8+ T cells. Colored line shows predicted value and shaded area indicates 95% confidence interval.

Figure 2 Flow cytometry gating



Legend: Cells were analyzed on a BD FACS Canto analyzer (BD Biosciences). Gating was performed using FlowJo (version 10)

RESULTS

Change in T cell phenotypes over time:

- Total CD4⁺ T cells increased, whereas CD8⁺ T cells decreased over time (p<0.001).
- Significant decrease in cellular markers of cycling (Ki67⁺) and activation (CD38⁺HLA-DR⁺) across all memory subtypes in CD4⁺ and CD8⁺ T cells (p<0.001). (**Figure 3**)
- Significant decrease in cellular markers of exhaustion (TIGIT⁺PD-1⁺) in all CD4⁺ T cells subtypes (p<0.01) but not in CD8⁺ T cells.
- Markers of T cells cytotoxicity (CD107a⁺) remained elevated over time.

Reservoir analysis: After correcting for multiple comparisons, we did not observe any significant associations between T cell phenotypes and measures of HIV reservoir (RNA and DNA) .

Table 1: Cohort Characteristics (N=79)

Characteristic	Women (n = 51)	Male(n = 28)	p-value
Race/Ethnicity			0.048
Black	25 (49%)	7 (25%)	
Hispanic	16 (31%)	9 (32%)	
White	10 (20%)	12 (43%)	
Age at ART initiation	45.0 (40.0, 53.0)	47.5 (40.0, 53.0)	0.142
IV drug use at entry			0.415
Never	46 (90%)	27 (96%)	
Previously	5 (9.8%)	1 (3.6%)	
Baseline CD4 (cells/μL)	219.5 (10.0, 659.0)	243.3 (11.0, 444.5)	0.498
Pre-ART HIV RNA (Log_{10} copy/mL)	4.6 (2.3, 6.0)	4.5 (3.6, 6.2)	0.51
Max follow-up time (years)	7.3 (1.6, 17.7)	5.1 (0.9, 13.8)	0.003
Number of visits	3.0 (1.0, 5.0)	3.0 (1.0, 6.0)	0.802

Legend: Median (range) shown for continuous variables and count (percentage shown for categorical variables. T-tests were used to compare continuous variables and Fisher's exact test or Chi-square test were used to compare categorical variables between men and women.

CONCLUSIONS

- Although markers of T cell activation and cycling keep decreasing during long term suppressive ART, cytotoxic CD4+ and CD8+ T cells and exhausted CD8+ T cells remain elevated in PWH, possibly because of ongoing antigen exposure.
- Although women showed a slower decline in HIV reservoir than men in this cohort (PMID: 34612493), we did not detect any sex-differences in T cell phenotypes, suggesting that deeper phenotypic analysis is needed.

LIMITATIONS

- Potential selection biases in the sampling; confounding factors such as race/ethnicity, difference in follow-up time between men and women.
- Importantly, women were not characterized for reproductive stages but were matched with men by age.

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