Antiretroviral therapy substantially repairs CD4 T cell dysregulation in people living with HIV

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BACKGROUND

- Immune homeostasis is a broad term that can include maintenance of immune cell number, phenotype, function and, for adaptive immunity, specificity.
- Under normal signaling, homeostatic cytokines, IL-2, IL-7 and IL-15 bind cognate receptors on immune cells to drive reconstitution of lymphocytes, generation and maintenance of T cell memory.
- In untreated HIV infection, immune homeostasis is progressively dysregulated, resulting in an inverted CD4:CD8 T cell ratio and loss of immune memory evidenced by significantly diminished response to vaccination.
- Recent studies show that on average 60% of the HIV reservoir sequences shared greatest genetic identity with circulating plasma HIV from the 12 months prior to ART initiation.
- These studies suggest that the post-ART window is critical to the restoration of immune homeostasis and health in people with HIV and also entry of HIV infected cells into the stable HIV reservoir

MASS CYTOMETRY PANEL

Antibody	Function
CD45	leukocytes
CD19	B cell
CD3	T cell
CD4	T cell
CD8a	T cell
CD14	monocyte
CD56	NK
CD16	NK
TCRgd	γδ

Antibody	Function
CD45RA	memory
CCR10	T memory
CD28	T memory
CD27	T memory
CD95	T memory
CCR7	T memory
CD99	T memory

Antibody	Function
CD127	T memory, Treg
CD31	RTE
CD38	activation
CD25	activation
HLA-DR	activation
CD57	activation
Bcl-2	survival
Ki-67	cell cycling

Antibody	Function
FoxP3	Treg
PD-1	exhaustion
CCR5	homing
CCR4	homing
CCR6	homing
CXCR5	homing
CXCR3	homing

Figure 1. 31 marker mass cytometry panel designed to phenotype immune cells as people initiate ART (Sponaugle et al., Cytometry A 2022)

ANTIRETROVIRAL THERAPY DRIVES CHANGES ACROSS MULTIPLE LEUKOCYTE LINEAGES

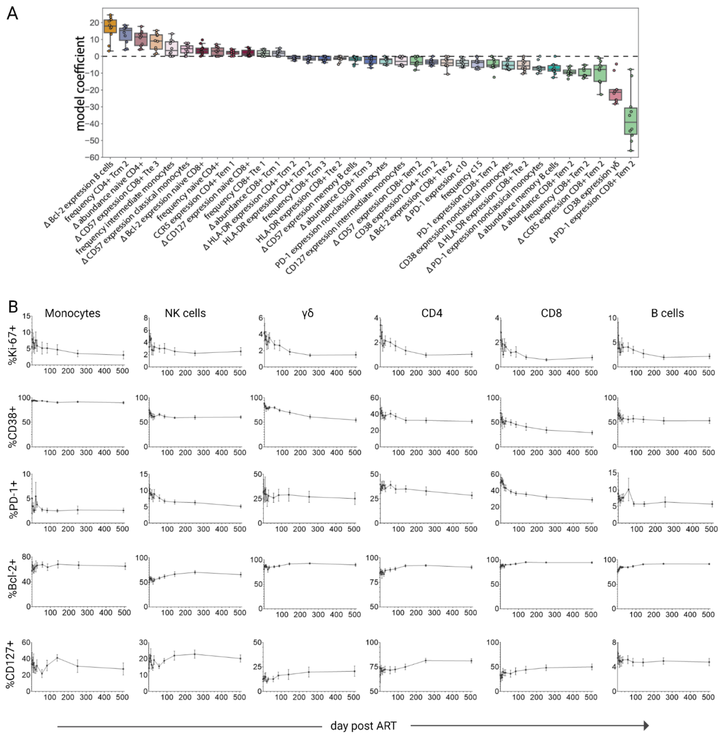

Figure 2. (A) Elastic net regression analysis showing mass cytometry immune features from unsupervised metaclusters that are predicted to be associated with time following ART initiation (B) Manual gating analysis of CD14+ monocytes, total NK cells, γδ T cells, CD4+ T cells, CD8+ T cells and CD19+ B cells showing average %frequency of indicated markers from day 0 to day 504 post-ART initiation, n=10 mean +/- SEM.

ART INITIATION INDUCED DYNAMIC CHANGES IN CD4+ T CELL POPULATIONS

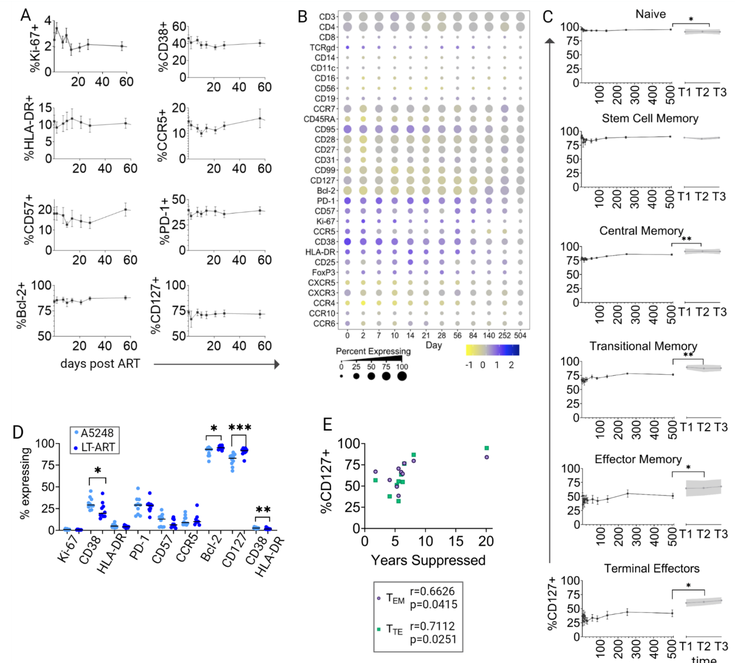

Figure 3. (A) Mean frequency of **CD4+ T cell populations** in the 60 days post-ART in A5248 (n=10). (B) Mean log2fold change in CD4+ T cell marker expression (yellow to blue) over time in A5248 (n=10) relative to day 504 post-ART. (C) Mean frequency of CD127+ cells in naïve and memory CD4+ T cells post-ART in A5248 vs. LT-ART (n=10, gray) spanning 17-26 months. (D) % frequency of CD4+ T cell subpopulations in A5248 participants at day 504 post-ART (n=10) vs. with durably, suppressed (average 6.7 years) participants in LT-ART cohort (n=10, average of 3 time points collected over 17-26 months). (E) Correlation between years virally suppressed and %CD127+ on effector memory (T_{EM}) and terminal effector (T_{TE}) CD4+ T cells in the LT-ART cohort.

Table 1. Quasibinomial fixed-effects model quantifiying changes in leukocyte populations over time

Population	A5248 (%pts/ 30 days)	LT-ART (%pts/ 30 days)
B cells	+0.20	--
Bcl-2 on B cells	+1.00	--
Memory B cells	-0.15	--
Plasmablasts	-0.08	--
Cytotoxic NK cells	+0.48	--
Pro-inflammatory NK cells	--	--
CD56- NK cells	-0.52	--
TCRγδ	-0.05	--
CD38 on TCRγδ	-1.57	--
CD8 T cells	-0.59	-0.22
CD4 T cells	+0.68	+0.17
CD4+ T cell populations: T_N	+0.33	--
T_{SCM}	--	--
RTE	--	--
T_{CM}	--	--
T_{TM}	-0.13	--
T_{EM}	-0.27	--
T_{TE}	-0.03	--
$CD99^{HI}$	--	--

CD4+ T cell Populations	A5248 (%pts/ 30 days)	Lt-ART (%pts/ 30 days)
T_{H1}	--	--
T_{H2}	+0.12	--
T_{H17}	--	--
$T_{H17.1}$	-0.07	--
T_{H9}	-0.10	--
T_{H22}	+0.01	--
T_{reg}	-0.08	--
T_{FH}	+0.32	--
T_N CD127+	--	--
T_{SCM} CD127+	+0.27	--
T_{CM} CD127+	+0.63	--
T_{TM} CD127+	+0.65	--
T_{EM} CD127+	--	--
T_{TE} CD127+	--	--
$CD99^{HI}$ CD127+	+0.57	
Ki-67+	-0.11	--
CD57+	-0.28	--
CD38+ HLA-DR+	-0.34	--
PD-1+	-0.64	--
CD25+	--	--
CCR5+	-0.21	--

CD8+ T CELLS EXHIBIT MORE RAPID MEMORY RESTORATION THAN CD4+ T CELLS AND NK CELLS

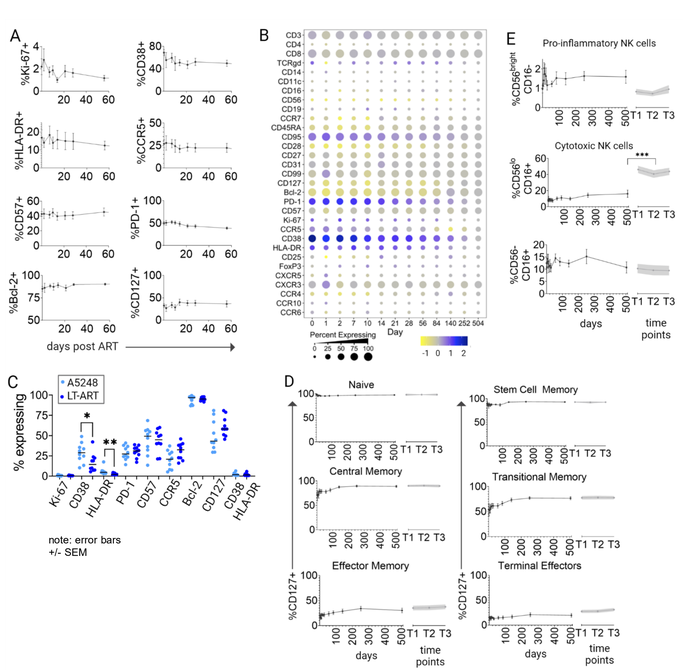

Figure 4. (A) Mean % frequency of **CD8+ T cell populations** in the 60 days post-ART in A5248 (n=10). (B) Mean log2fold change in CD8+ T cell marker expression in A5248 relative to day 504 post-ART. (C) % Frequency of CD8+ T cell populations in A5248 at day 504 post-ART and LT-ART cohort. (D) Mean frequency of CD127+ cells T cells in A5248 vs. LT-ART (n=10, gray) over time points, T1-T3 spanning 17-26 months. (E) Mean frequency of NK cell subsets post-ART in A5248 vs. LT-ART

HIV-SPECIFIC CD4+ T CELL PROLIFERATION WAS NOT DETECTABLE IN THE FIRST YEAR POST-ART

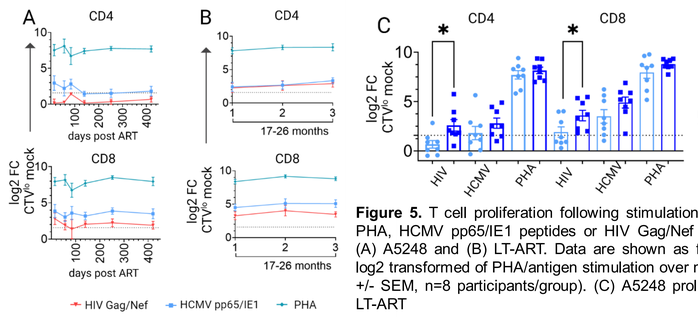

Figure 5. T cell proliferation following stimulation with either PHA, HCMV pp65/IE1 peptides or HIV Gag/Nef peptides in (A) A5248 and (B) LT-ART. Data are shown as fold change log2 transformed of PHA/antigen stimulation over mock (mean +/- SEM, n=8 participants/group). (C) A5248 proliferation vs. LT-ART

CONCLUSIONS

- ART induced immune restoration *with different kinetics* across multiple leukocyte lineages, impacting expression levels of multiple survival and activation markers.
- Leukocytes lineages, including CD4 T cell populations, were highly stable in durably suppressed individuals
- CD8 T cell memory phenotypes restored more rapidly than CD4 T cells post-ART initiation similar to patterns observed following myeloablation and HSCT transplant.

FUTURE DIRECTIONS

- Use IPDA to investigate how ART and early changes in CD4+ T cell subsets associate with HIV reservoir kinetics
- Complement above with mathematical modeling of memory restoration dynamics (Ruy Ribeiro, LANL)