Transcriptional Profiles of HIV RNA+ Cells in PWH on ART Differ From Those During Active Viremia

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BACKGROUND

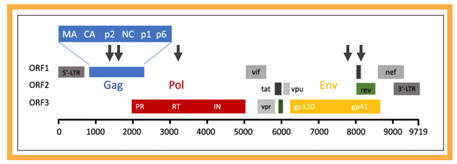
Active reservoir cells transcribing HIV can perpetuate chronic inflammation in virally suppressed people with HIV (PWH), and their relative abundance correlates with time to rebound after antiretroviral therapy (ART) interruption. Therefore, they represent an important target for new HIV therapies. Yet, little is known about their phenotypic and functional features. While scRNA-seq analysis has been performed on HIV RNA+ cells in the context of untreated infection, active reservoir cells remain largely uncharacterized due to the inefficiency of capturing HIV RNA+ cells from ART-suppressed PWH.

METHODS AND DESIGN

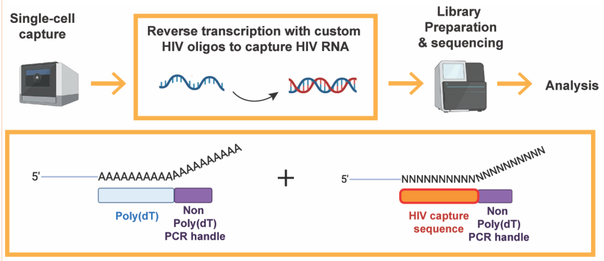
HIV-seq

A. probes

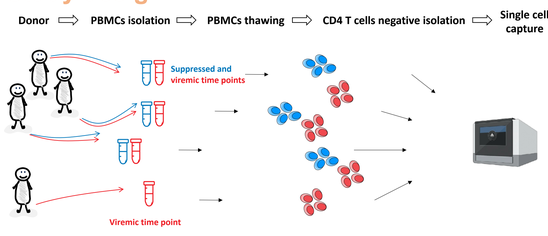
Capture Sequence target
PreGag (626-643)
Packaging (775-797)
Pol (2634-2662)
Rev Response Element (7832-7851)
Tat-Rev (8433-8459)



B. pipeline



C. Study design



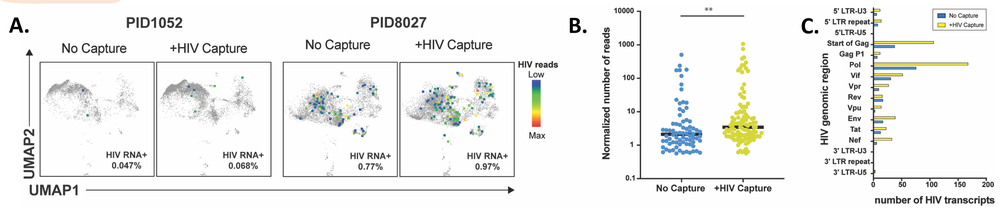
Panel A. Schematic illustrating the genomic location of HIV-specific capture sequences of HIV-seq. The name and nucleotide position (based off HXB2 annotation) of each of capture sequence are labeled in black.

Panel B. After cell encapsulation using a 10X Chromium instrument, HIV-specific oligos targeting different regions of the HIV genome and described in *Panel A* are added to the poly(dT) primers during the Reverse Transcription (RT) step of the 10X Genomics protocol. Libraries are then prepared according to the 10X Genomics protocol.

Panel C: Paired blood specimens from 3 PWH before vs. after ART suppression, along with an additional participant for whom only the viremic time point was available, were collected. Cryopreserved PBMCs were thawed, and CD4+ T cells were isolated by negative selection and used for single cell capture for HIVseq (and a classic 10X 5' scRNAseq pipeline (for Figure 1)).

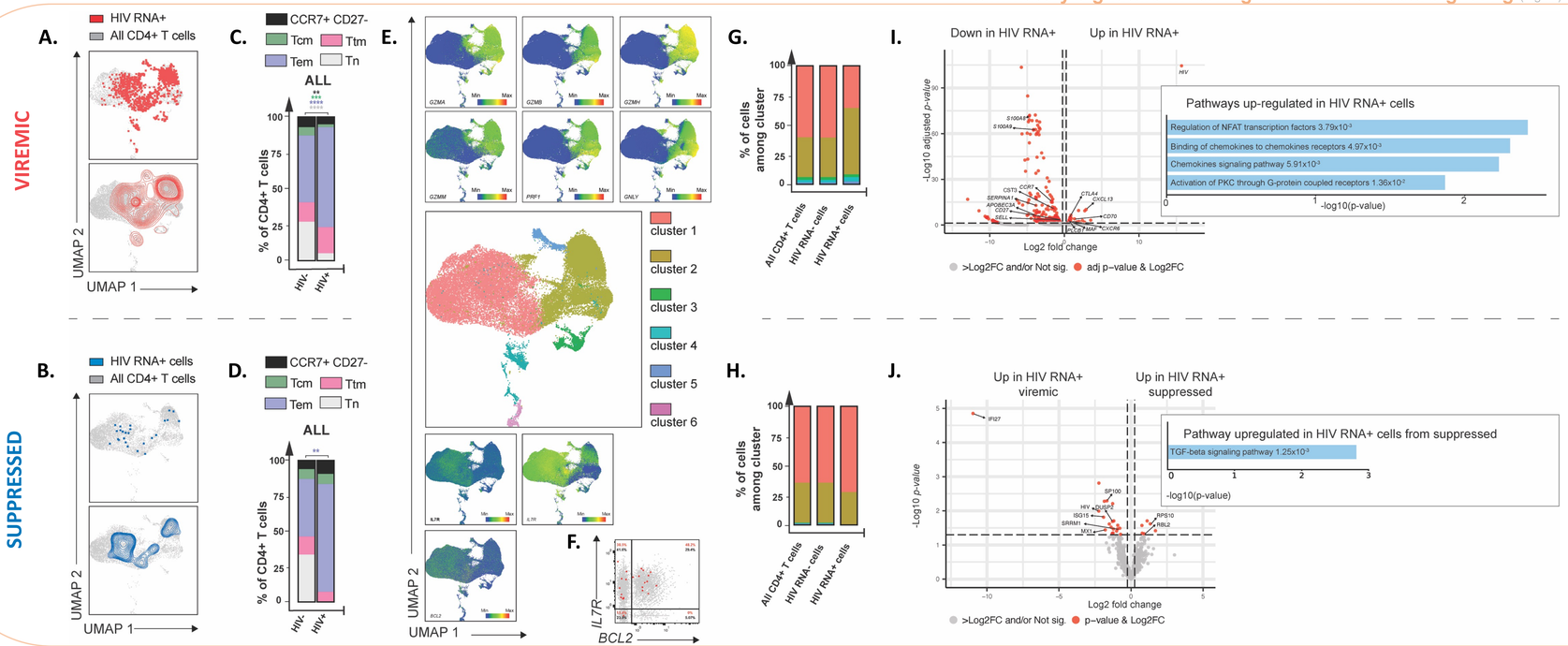
RESULTS

Figure 1 HIV-seq increases detection of HIV RNA-expressing cells throughout the entire genome



Panel A. UMAP plots of two viremic donors showing HIV RNA+ cells as colored dots among total CD4+ T cells represented in gray, with vs. without the addition of HIV oligos. The percentage of HIV RNA+ cells among CD4+ T cells is indicated in the lower right of each plot.
Panel B. Normalized numbers of HIV reads in the absence vs. presence of HIV oligos, for each HIV RNA+ cell identified from PID1052 and PID8027 in panel A. The lines for each condition correspond to median values. **P ≤ 0.01 as determined using a Mann-Whitney test.
Panel C. HIV reads from PID1052 and PID8027 were aligned to the HIV-1 subtype B consensus reference genome. The y-axis depicts individual HIV genes, and the x-axis shows the number of detected HIV transcripts, in the absence (blue) vs. presence (yellow) of the HIV capture sequences.

- **HIV RNA+ cells are enriched among Tem cells during both viremia and ART suppression** (Fig2.C & D)
- **HIV RNA+ cells from blood of viremic (but not ART-suppressed) PWH are enriched for cytolytic cells** (Fig2.E & G)
- **HIV RNA+ cells during viremia exhibit low expression of antiviral factors SERPINA and APOBEC3A** (Fig2.I)
- **HIV RNA+ cells from ART-suppressed PWH predominantly reside among a long-lived population of memory CD4+ T cells** (Fig2.E & F & H)
- **As compared to HIV RNA+ cells during viremia, those during ART exhibited anti-inflammatory signature involving elevated TGF-beta signaling** (Fig2.J)



Panels A&B. UMAP plots depicting HIV RNA+ cells as colored dots or contours, against a background of HIV- CD4+ T cells depicted in gray, from 4 and 3 donors respectively for viremic (A) and suppressed (B) samples.
Panels C&D. Bar graph of the distribution of HIV- and HIV+ cells among classic CD4+ T cell subsets for viremic (C) and suppressed (D) samples. **P ≤ 0.01, ***P≤ 0.001, ****P≤ 0.0001 as determined by generalized linear mixed model (GLMM).
Panel E. The center UMAP shows the 6 clusters of CD4+ T cells defined by their transcriptome (all participants combined). The smaller UMAPs show the expression levels of individual cytolytic (top) and long lived (bottom) markers.
Panel F: 2D plot with HIV RNA+ CD4+ T cells depicted in red and HIV RNA- CD4+ T cells depicted in gray. Percentages of HIV RNA+ cells and HIV RNA- CD4+ T cells in each quadrant are indicated in red and black, respectively, for each population.
Panels G&H. Stack graphs showing cluster distribution among total CD4+ T cells, or among HIV RNA- and RNA+ CD4+ T cells, from viremic (G) and suppressed (H) PWH.
Panel I. Volcano plot displaying up- and down-regulated genes in HIV RNA+ CD4+ T cells vs. HIV RNA- CD4+ T cells from viremic samples, with select transcripts annotated. Red dots correspond to transcripts with 0.25log2 fold-change expression and with an adjusted p value < 0.05, as determined by genewise quasi F-tests. Associated pathways upregulated in HIV RNA+ cells are indicated on the top right of the plot. The p-value representing the statistical significance of enrichment of the gene set withing the pathway is indicated next to each pathway.
Panel J. Volcano plot displaying up- and down-regulated genes in HIV RNA+ cells during viremia vs. ART suppression, with select transcripts annotated. Red dots correspond to transcripts with 0.25log2 fold-change expression and with a non-adjusted p value < 0.05, as determined by gene-wise quasi F-tests. Associated pathways associated with the volcano plots are indicated on the top right of the plot. The p-values representing the statistical significance of enrichment of the gene set within the pathways are depicted to the right of each pathway name.

Figure 2

CONCLUSION

HIV-seq enables better capture of HIV transcripts for scRNA-seq analysis, revealing HIV RNA+ cells from ART-suppressed PWH to differ from HIV RNA+ cells during untreated infection. HIV RNA+ cells from blood of ART-suppressed PWH do not preferentially exhibit cytolytic features, but instead preferentially exhibit immunosuppressive TGF-β signaling pathways and express markers of cell survival and stemness.

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