# MULTIOMICS PROFILING OF HIV-TRANSCRIBING CELLS IN PEOPLE WITH HIV ON SUPPRESSIVE ART

466

Julie Frouard[1,2], Xiaoyu Luo[1], Sushama Telwatte[3,4], Douglas Arneson[5], Jason Neidleman[1, 2], Kailin Yin[1,2], Antoine Chaillon[6], Rebecca Hoh[8], Steven G. Deeks[8], Sara Gianella[6], Davey Smith[6], Sulggi A. Lee[9], Phyllis Tien[3,7], Steven Yukl[3], and Nadia R. Roan[1, 2]

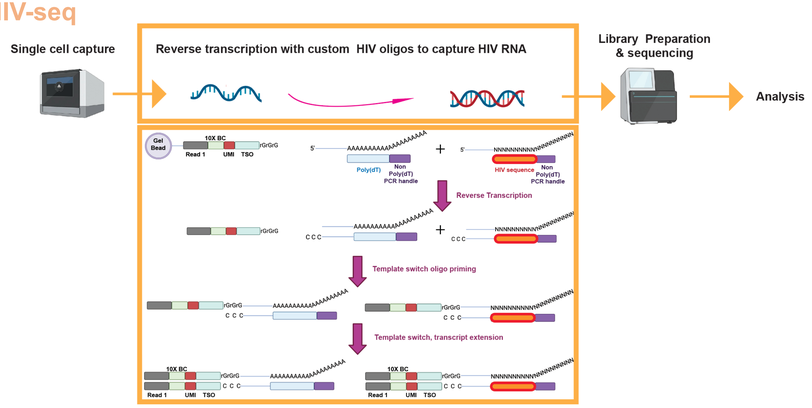
[1]Gladstone Institute of Virology and Immunology, University of California, San Francisco, CA 94158, USA, [2]Department of Urology, University of California, San Francisco, CA 94158, USA , [3]San Francisco Veterans Affairs (VA) Medical Center and University of California, San Francisco, CA, USA, [4]Department of Infectious Diseases, The University of Melbourne at the Peter Doherty Institute of Infection and Immunity, [5]Bakar Computational Health Sciences Institute, University of California, San Francisco, San Francisco, CA, USA., [6]Center for AIDS Research (CFAR), Division Infectious diseases, UCSD and VA San Diego Healthcare System, La Jolla, CA 92093-0679, USA, [7]Department of Medicine, University of California, San Francisco, CA 94158, USA, [8]Division of HIV, Infectious Diseases and Global Medicine, University of California, San Francisco, USA., [9]Zuckerberg San Francisco General Hospital and the University of California, San Francisco, CA 94110, USA.

## BACKGROUND

HIV reservoir cells actively transcribing HIV persist despite ART, but remain poorly characterized since standard single cell (sc)RNAseq approaches inefficiently identify these cells. In this study, we devised a comprehensive multiomics scRNAseq-based approach that increases identification of HIV- transcribing cells, enabling a deep characterization of these cells including from tissue compartments.

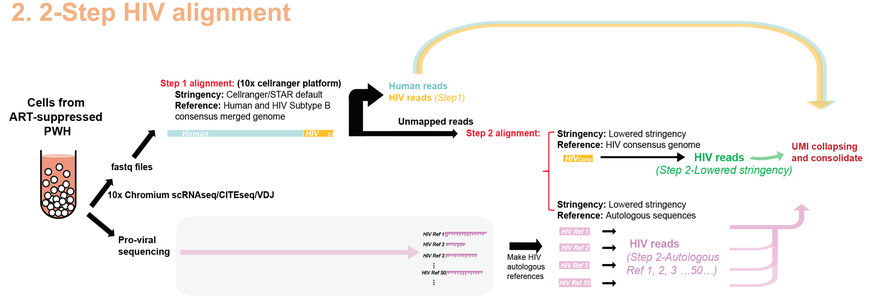
## METHODS

### 1. HIV-seq




After cell encapsulation using a 10X Chromium instrument, HIV-specific oligos targeting different regions of the HIV genome (gag, pol, env and tat ) 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.

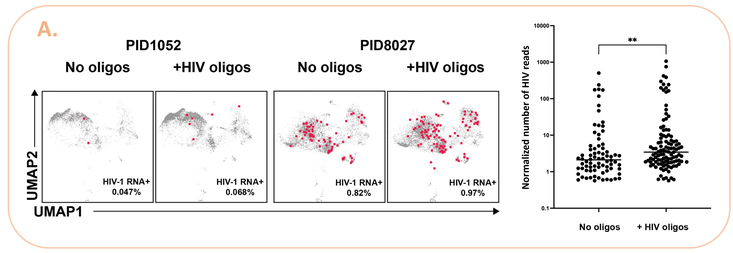
### 2. 2-Step HIV alignment

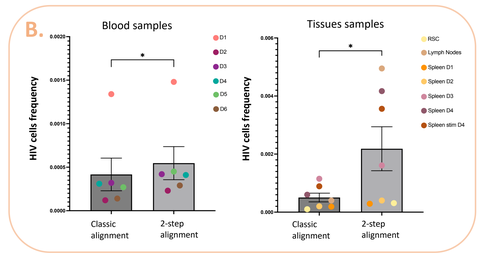



Cells isolated from ART suppressed PWH, obtained from blood and/or tissues, undergo the 10X pipeline to generate GEX, ADT, and VDJ libraries. Subsequently, sequencing is performed, with GEX libraries being subjected to the 2-step alignment method.
The first step entails performingconventional alignment using human genome appended with a consensus HIV genome. For this, we appended a subtype B consensus to the human GRCh38 genome. Following this initial alignment, unmapped reads are extracted and re-aligned to 1) the subtype B consensus sequence with reduced stringency, and 2) autologous viral sequences (from proviral sequencing) at a reduced stringency. The reduced stringency conditions were verified to not result in false positives (identification of HIV+ cells from people without HIV). Finally, after unique molecular index (UMI) collapsing, HIV reads from both steps are consolidated.

## RESULTS

### 1. HIV-seq and 2-step alignment methods increase detection of HIV RNA-expressing cells in blood and tissue samples




**Panel A. Left,** UMAP plots of two viremic donors showing HIV-1 RNA+ cells as red dots among total CD4+ T cells represented in gray, with vs. without the addition of HIV oligos. The percentage of HIV-1 RNA+ cells among CD4+ T cells is indicated in the lower right of each plot. **Right**, Normalized numbers of HIV reads in the absence vs. presence of HIV oligos, for each HIV-1 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 B.** Bar graphs of the mean and SEM of frequency of HIV RNA+ cells frequency identified from blood (left) or tissues (right) from ART-suppressed PWH, after running classic alignment vs. the 2-step alignment. Each dot represent a donor. *P ≤ 0.05 as determined using Wilcoxon test.

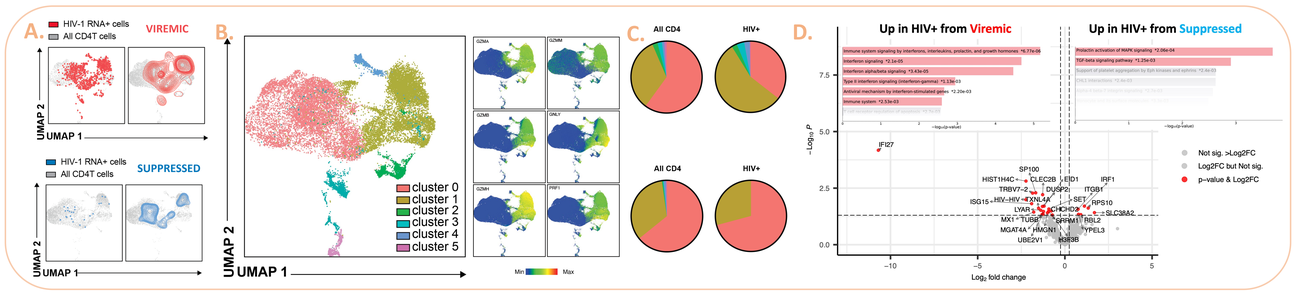
**Panel A.** UMAP plots depicting HIV-1 RNA+ cells as colored dots or contours, against a background of HIV-CD4+ T cells depicted in gray, from 4 donors. Samples are blood specimens from PWH before (viremic, in red) vs. after (suppressed, in blue) ART suppression.

**Panel B.** UMAP showing 6 clusters of CD4+ T cells defined by their transcriptome for all 4 participants combined. Smaller UMAPs are showing the expression levels of individual cytolytic markers.

**Panel C.** Pie graphs showing distribution among different cluster of all CD4+ T cells (left) or HIV-1 RNA+ CD4+ T cells (right) from viremic (top) and suppressed (bottom) samples.

**Panel D.** Volcano plot displaying up- and down-regulated genes in HIV-1 RNA+ CD4+ T cells from suppressed samples, compared to HIV-1 RNA+ CD4+ T cells from viremic samples, all donors combined. Pathways associated are indicated on top of each set of genes.

### 2. HIV RNA+ cells from viremic PWH are enriched for cytolytic cells, and suppressed in TGF-beta signaling relative to HIV RNA+ cells from suppressed PWH




### 3. HIV RNA+ cells are detected in diverse tissues from PWH under ART

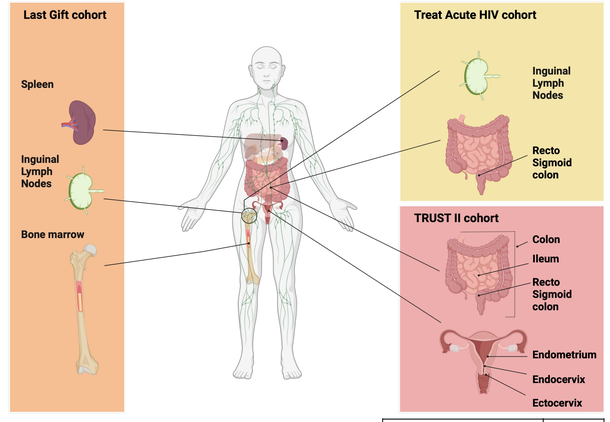
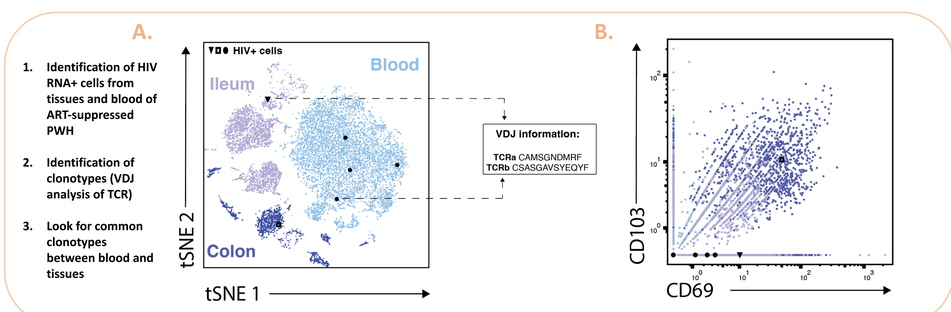



Table listing all tissues tested where HIV RNA+ cells were detected.

| Tissue type | HIV+ cells detected |
|---|---|
| Inguinal Lymph Nodes | ✓ |
| Spleen | ✓ |
| Colon | ✓ |
| Ileum | ✓ |
| Recto Sigmoid colon | ✓ |
| Endometrium | ✓ |
| Endocervix | ✓ |

### 4. Clones of HIV RNA+ cells between gut and blood of ART-suppressed PWH sharing the same VDJ sequence exhibit distinct phenotypes




**Panel A.** tSNE showing CD3+/CD8- blood cells, colonic cells, and ileum cells from an ART-suppressed PWH. HIV RNA+ cells are shown in black. The sequence of a shared TCR detected on HIV RNA+ cells from ileum and blood is indicated.

**Panel B.** 2D plot showing levels of expression of T resident memory markers CD69 and CD103.

## CONCLUSION

Our study presents new tools that improve detection of HIV RNA+ cells at the single-cell level, enabling us to establish an atlas of HIV-transcribing cells from PWH on suppressive ART. HIV-transcribing cells clonally expand and disseminate to multiple tissue sites where they adopt tissue site-specific features.

Acknowledgments: We are grateful to the generous participants who donated blood and/or tissue specimens to make this study possible.