MULTI-COMPARTMENT FRESH SINGLE CELL STUDIES IN AN INTERNATIONAL HIV RESEARCH SETTING

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BACKGROUND

- Single cell methods enhance the resolution at which cells in blood and tissues can be studied in people with HIV (PWH) and guide defining the population of infected cells across the body.
- Capturing the native multi-omics state of cells obtained from multiple tissue compartments and from rare and vulnerable cells in cerebrospinal fluid (CSF) while avoiding artifacts that may arise from cryopreservation remains a challenge for single cell studies, more so in resource limited settings.

METHODS

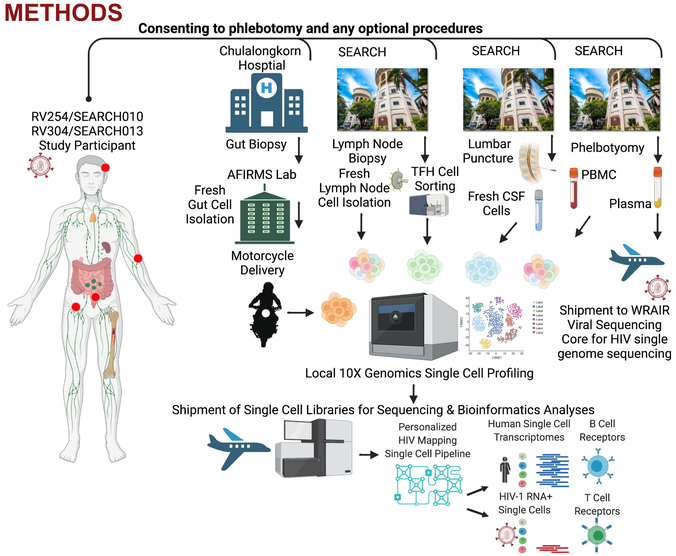
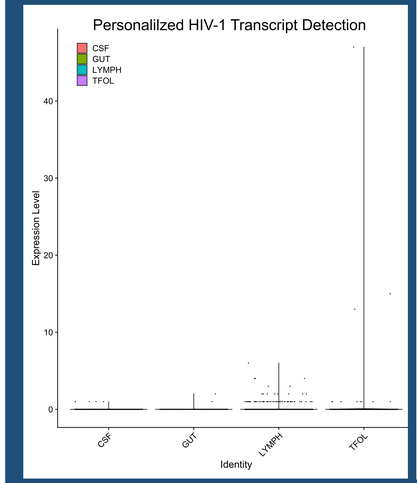


Figure 1. Fresh cells were isolated from gut and LN, CSF, and blood over 2 days from an ART naïve PWH with chronic HIV (CHI) (**Participant 1**, Figure 1A), and from gut and blood from a PWH on suppressive ART initiated during acute HIV infection (AHI) (**Participant 2, Data not shown**). We also leveraged flow cytometry cell sorting capabilities of fresh T follicular helper cells from LN for multi-omics single cell profiling of 5,712 cells.

- To demonstrate single cell studies of fresh cells from PWH are logistically feasible in a resource limited setting, we built upon the RV254/SEARCH010 and RV304/SEARCH013 studies enrolling people with acute and chronic HIV (AHI & CHI) in Bangkok, Thailand where uptake of optional procedures including leukapheresis, lumbar puncture (LP), gut biopsy, and lymph node (LN) biopsy is high.
- The 10X genomics platform was used locally to generate single cell transcriptome and T-cell/B-cell receptor data from fresh specimens within hours of sampling. The 10X Genomics CellRanger pipeline 6.1.1, Seurat 4.0 suite, and scRepertoire toolkit v1.0.2 (powerTCR approach to clone size distribution and Startrac clonotype metrics) was used for analysis.

Mapping to a personalized HIV-1 viral reference sequence in ART naïve chronic HIV infection enhanced detection of single cells containing HIV-1 transcripts in fresh CSF, gut, lymph node, and sorted CXCR5+ T follicular helper cells.

Figure 2. Log normalized expression levels of HIV-1 transcript in single cells across compartments. Reads were mapped to a concatenated human (GRCh38) and personalized HIV viral reference sequence obtained by single-genome amplification and sequencing of HIV-1 viruses from plasma.



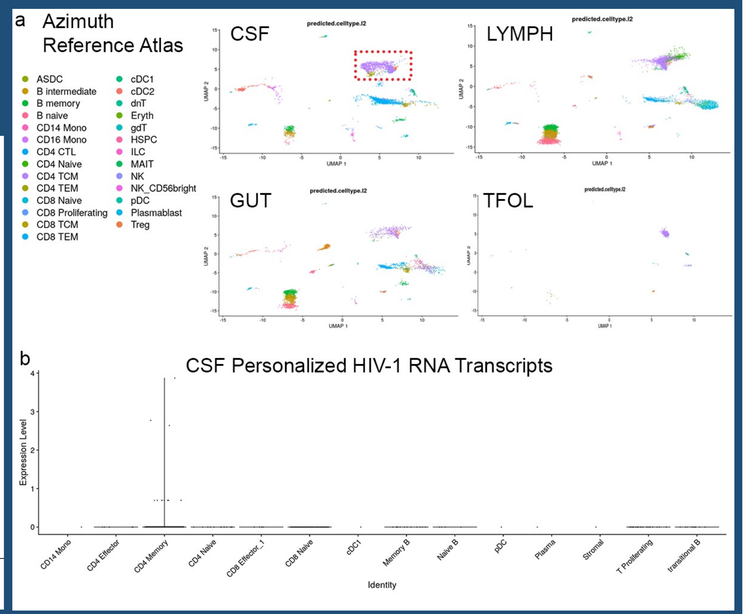


Figure 3. **A.** Reference-based mapping of CSF, LN, Gut, and T follicular helper cells using Azimuth Reference Atlas. Predicted immune cell types displayed by compartment in UMAP. **B.** Deeper sequencing of CSF single cell library reveals all detectable HIV transcripts were identified in CD4 memory cells in the CSF compartment.

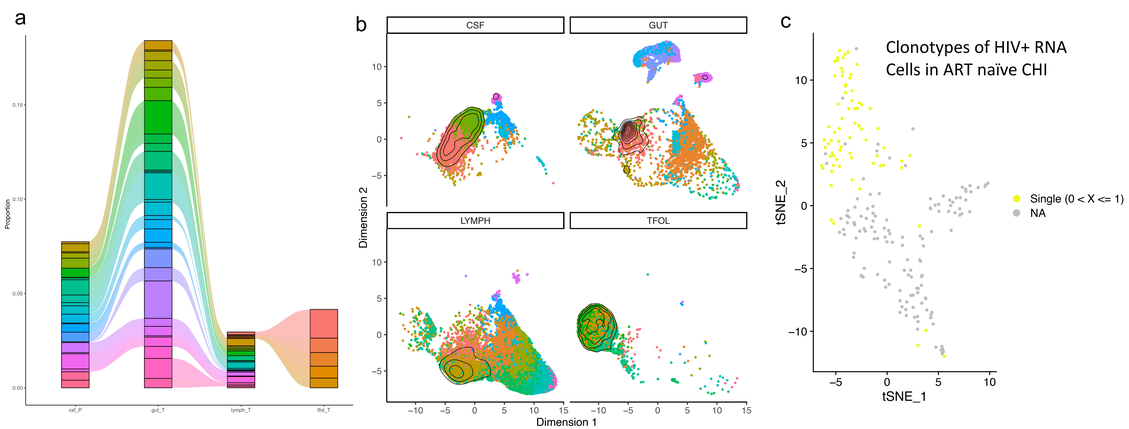


Figure 4. **A.** Comparison of single cell clonotypes between CSF, lymph node and sorted CXCR5+ T follicular helper cells use the amino acid sequence of the CDR3 region. **B.** Visualized distribution of clonal frequency overlaid onto dimensional reduction plots. **C.** Distribution of clonotypes in HIV+ RNA cells with paired TCR sequence.

RESULTS

- Multi-omics single cell data was obtained for 28,400 freshly isolated lymph node cells, 5,968 gut cells, and 5,614 CSF cells from Participant 1. We also leveraged flow cytometry cell sorting capabilities of fresh T follicular helper cells from LN for multi-omics single cell profiling of 5,712 cells from Participant 1.
- To enhance detection of HIV viral transcripts in the CHI ART naïve participant, we generated an individualized near full-length patched viral sequence from concurrent plasma from the same donor to align sequencing reads and detected HIV transcript containing cells in all compartments with heterogenous single cells either producing high or low HIV transcripts (Figure 2).
- All HIV transcript containing cells in the CSF were identified in inferred CD4 memory T cells (Figure 3B).
- Utilizing the paired T cell receptor single cell data from the CHI participant, we tracked overlapping clonotypes in the CSF, gut, and lymph node. Clonotype tracking of sorted CXCR5+ T follicular helper cells revealed exclusive shared clonotypes with lymph node only (Figure 4).
- Based on 71 single transcriptionally active HIV+ cells with a paired TCR captured across compartments, all T cell clones are unique in cells containing HIV transcripts from CSF, gut, lymph node, and CXCR5+ T follicular helper cells in ART naïve CHI (Figure 4).

CONCLUSIONS

- We demonstrate the logistical feasibility of generating single cell multi-omics data from fresh cells from blood, CSF, LN, and gut in PWH in Bangkok.
- A personalized HIV mapping approach can be used to pinpoint infected single cells in multiple tissue compartments including the CNS.
- Future research should investigate the dynamics of active and latent HIV under clonal expansion that may vary in tissues and the central nervous system compared to findings in peripheral blood.
- Multidisciplinary teams and investments in international HIV research settings will enable cross-compartmental multi-omics studies to further interrogate the active and latent HIV reservoir in PWH.

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