SINGLE-CELL MULTI-OMICS ANALYSIS OF HIV RESERVOIR CELLS BY NOVEL T-TRACE APPROACH

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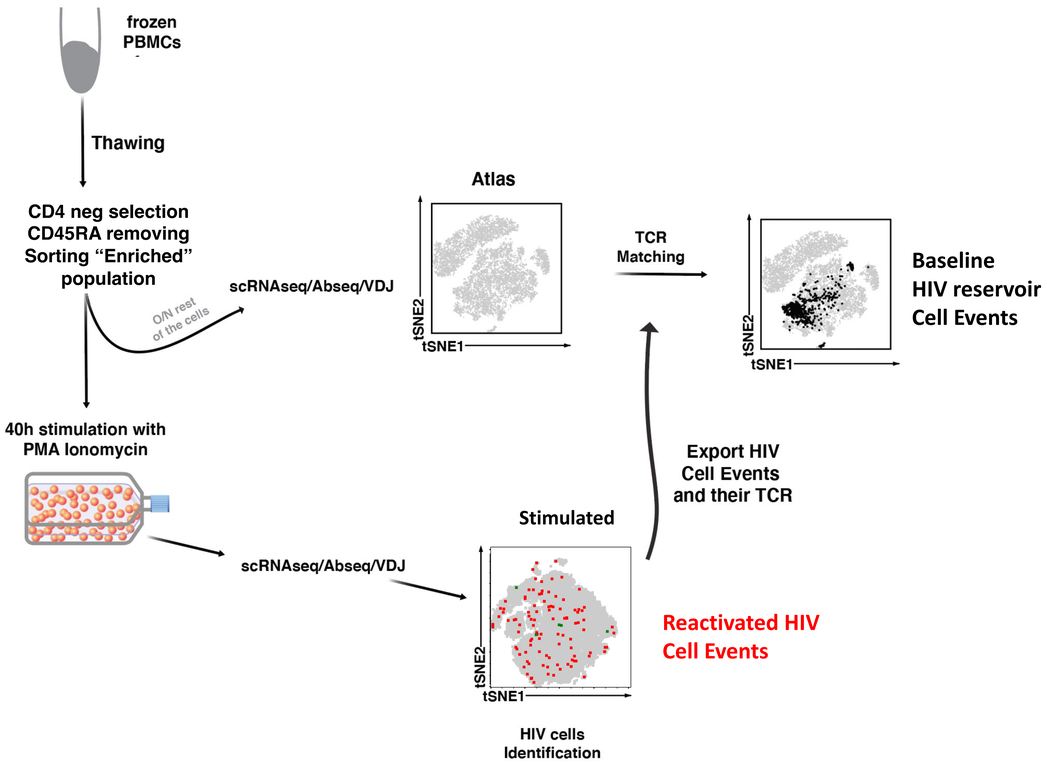
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BACKGROUND

We recently reported PP-SLIDE as a method taking advantage of high-dimensional phenotyping by CyTOF to phenotype and enrich for inducible reservoir cells from virally-suppressed people living with HIV *(Neidleman et al 2020)*. In the current study, we describe a new approach called T-TRACE (**T**CR **Tr**acing of Unperturbed **A**nalogous **Ce**ll), that takes advantage of multi-omics single-cell analysis (by single-cell RNAseq, Antibody-seq, and TCR sequencing) and the fact that the reservoir is largely clonally expanded, and which allowed us to conduct full-transcriptome analysis of reservoir cells capable of inducing HIV gene expression.

METHODS

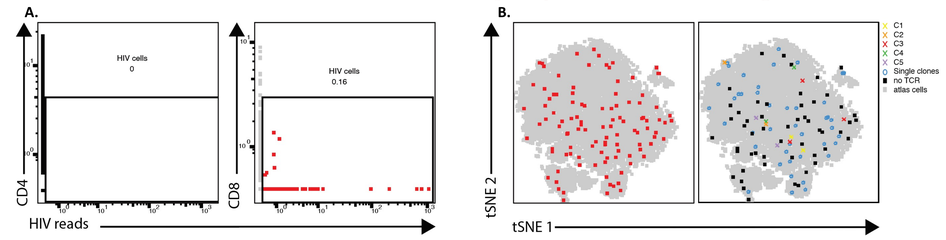


HIV reservoir cells identification by T-TRACE (TCR Tracing of Unperturbed Analogous Cell)

Cryopreserved PBMCs from an ART-suppressed donor were enriched for CD4 memory cells followed by cell sorting using a 7-parameter FACS panel designed based on prior PP-SLIDE analyses of this participant, in order to enrich for reservoir cells. A portion of the sorted cells were immediately subjected to simultaneous scRNA-seq, Antibody-Seq and VDJ-Seq to generate the "atlas" and define the baseline features of total T cells. The remaining cells were stimulated with PMA/ionomycin for 40 hours in the presence of ART and caspase inhibitor Z-VAD-FMK, and then analyzed using the same multi-omics platform. Induced reservoir cells from the stimulated sample were defined as those harboring HIV transcripts. TCR sequences from these cells were matched against all T cells in the atlas specimen for VDJ sequence matching in order to identify clonally expanded, inducible reservoir cells in their baseline (pre-stimulated) state. These identified cells from the atlas are referred as baseline HIV reservoir cells.

RESULTS

1. HIV inducible reservoir cells are clonally expanded and belong to 30 different clonotypes

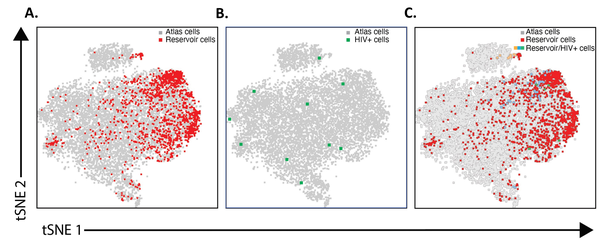


HIV-expressing cells identified among stimulated cells are clonally expanded and belong to different clonotypes.

A. Gating on HIV+ cells. Left panel shows gating of HIV RNA+ cells in an uninfected sample where no infected cells were detected. Right panel shows the gating HIV+ cells identified from the stimulated sample from ART-suppressed donor.

B. Left panel shows a tSNE plot depicting all stimulated cells in gray and HIV+ cells in red. Right panel shows HIV+ cells colored by clonotype . Thirty different clonotypes were identified among the HIV+ cells.

2. Multiple clonotypes of HIV reservoir cells identified by T-TRACE overlap with the transcriptionally active reservoir



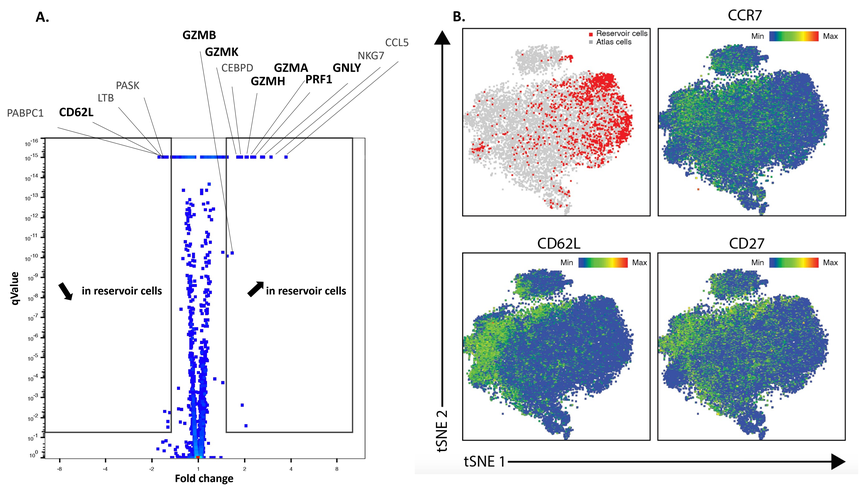
Baseline HIV reservoir cells can be identified by T-TRACE, and are made up of multiple clonotypes.

A. tSNE plot depicting all atlas cells in gray and all baseline reservoir cells identified by T-TRACE in red.

B. tSNE plot depicting all atlas cells in gray and transcriptionally active cells in green.

C. tSNE plot depicting all atlas cells in gray; select clones of baseline reservoir cells in orange, blue, and green; and the remaining baseline reservoir cells in red..

3. HIV reservoir cells exhibit a Tem phenotype with low CD62L expression and a cytolytic signature.



HIV reservoir cells exhibit a Tem phenotype and cytolytic features.

A. Volcano plot showing differentially expressed genes between baseline reservoir cells and the rest of the atlas cells. Cells within the two gates correspond to genes with at least 1.5-fold change expression and with $q < 0.05$, as determined by Mann-Whitney U Test.

Genes associated with Tem and cytolytic phenotypes are indicated in bold.

B. Upper left panel shows tSNE plot depicting all atlas cells in gray and baseline reservoir cells in red. Upper right and bottom panels show tSNE depicting atlas cells as colored heatmaps showing the relative expression levels of the Tcm markers CCR7, CD62L and CD27. Low expression of CCR7, CD62L, and CD27 are associated with a Tem phenotype.

CONCLUSIONS

T-TRACE allows full transcriptomic and surface phenotypic analysis of inducible, clonally-expanded reservoir cells in their baseline (non-stimulated) state. In-depth analysis of these reservoir cells revealed them to be primarily of a T effector memory phenotype ($CD62L^{lo}$) and to exhibit a cytolytic signature. Our data also suggest an overlap between the inducible and transcriptionally-active reservoir. Future application of T-TRACE on specimens from additional donors, including from longitudinal sampling, will provide insights into mechanisms of HIV persistence and potentially identify biomarkers of the inducible reservoir.