STABILITY AND INSTABILITY OF THE CELLULAR HIV RESERVOIR AFTER REBOUND DURING ATI

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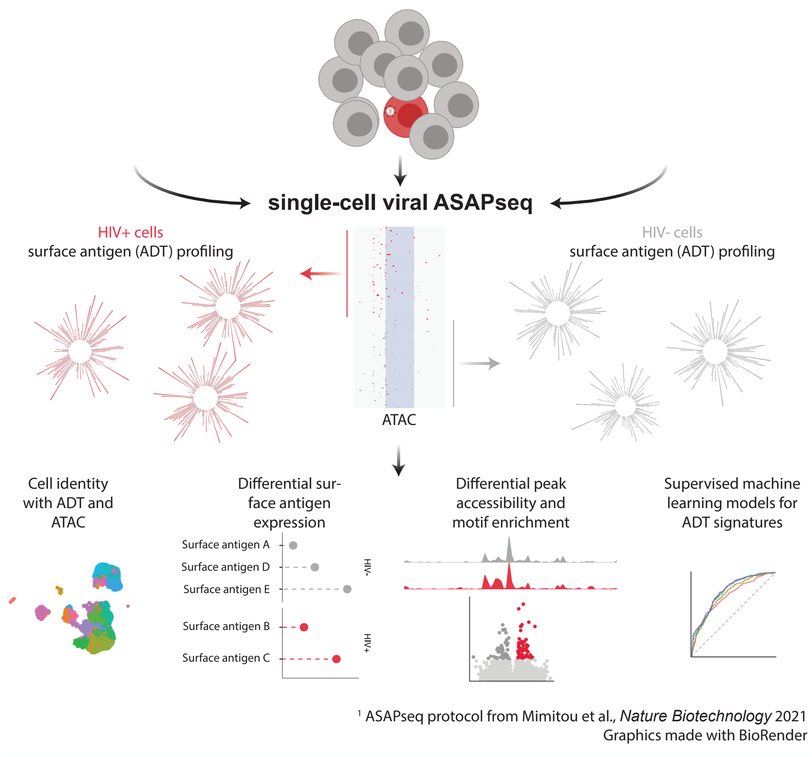
Background

The complex pool of infected cells that comprise the HIV reservoir can be distributed amongst CD4+ T cell subsets with varied functional and compartmental characteristics. Recent studies during treatment interruption (ATI) in passive immunotherapy trials have demonstrated that reservoir reseeding can coincide with viral rebound. However, whether reseeding is associated with compositionally distinct cellular populations is unknown.

Aims

Identify and characterize the HIV reservoir with single-cell resolution and without any *ex vivo* manipulation to assess the stability of the reservoir after ATI.

Methods



[1] ASAPseq protocol from Mimitou et al., *Nature Biotechnology* 2021
Graphics made with BioRender

Sample and Donor information

Individual	Sex	Time on ART before trial start (years)	Total cells	HIV+ cells (% of total cells)	Total HIV DNA copies per 1e6 CD4+ T cells (%)*
A01	M	3.6	14021 (pre-ATI) 27065 (post-ATI)	9 (0.06%; pre-ATI) 6 (0.02%; post-ATI)	185 (0.019% pre-ATI) 293.8 (0.029% post-ATI)
A08	M	4.2	18427 (pre-ATI)	46 (0.25%; pre-ATI) 36 (0.22%; post-ATI)	1791.2 (0.18% pre-ATI) 1564.5 (0.16% post-ATI)
A09	M	5.6	44331 (pre-ATI) 32998 (post-ATI)	67 (0.15%; pre-ATI) 36 (0.11%; post-ATI)	1297.3 (0.13% pre-ATI) 1221.8 (0.12% post-ATI)

Age was not reported at the participant level for the original study (Bar et al., *NEJM* 2016). However, the age range was reported as between 34 and 52 years.
*from Salantes et al., *JCI* 2018

Direct ex vivo atlas of infected cells from PBMC of ART-treated PLWH

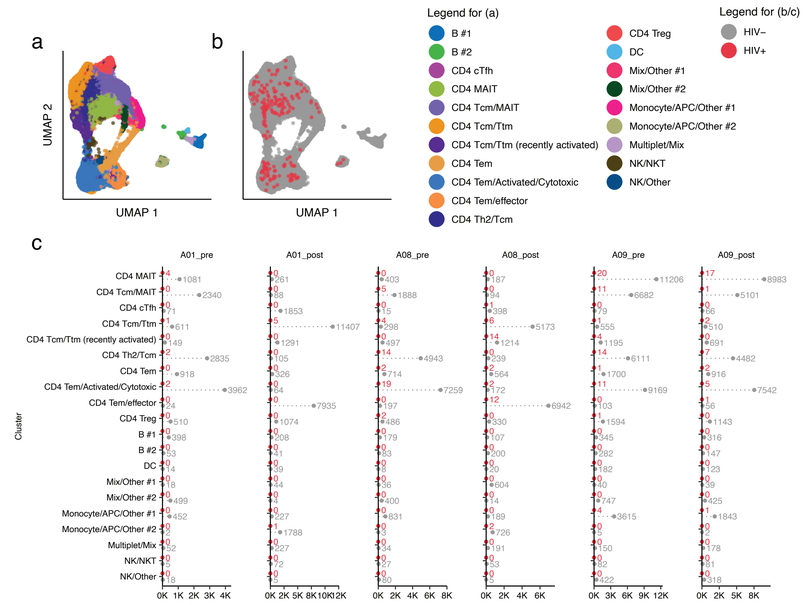


Figure 1: (a) UMAP representation of ATAC component colored by manually annotated cell phenotypes. (b) UMAP representation of ATAC component colored by detection of HIV reads. (c) Absolute count of cell numbers based on annotated clusters.

(In)stability of HIV+ cellular phenotype after ATI

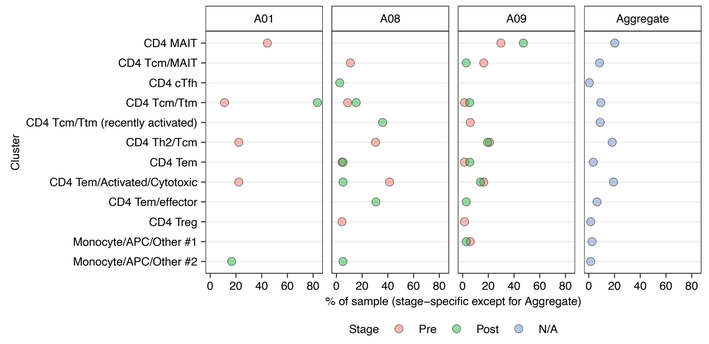


Figure 2: Perecentage of HIV+ cells found by cluster separated by donor and collection stage (pre-ATI vs post-ATI); x axis represents the percent of HIV+ cells in each specific sample that were found in each annotated cluster. The right panel indicates the aggregate values across the entire ART- treated dataset.

Differential surface protein expression between memory grouped HIV+ cells after ATI

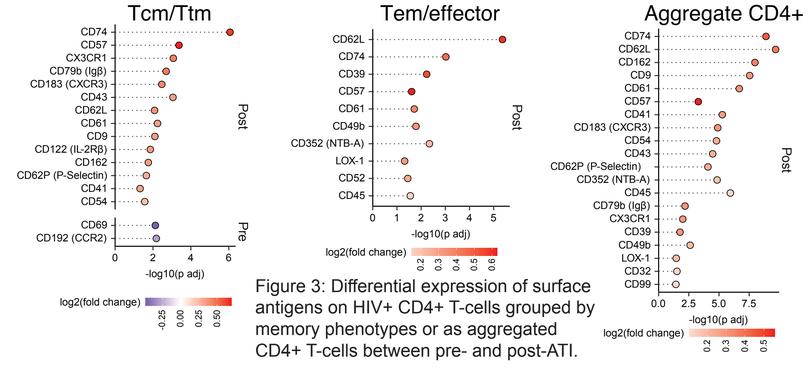
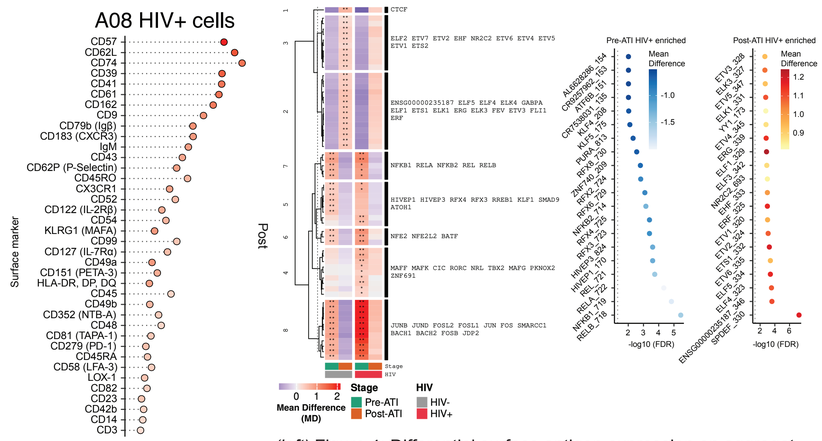


Figure 3: Differential expression of surface antigens on HIV+ CD4+ T-cells grouped by memory phenotypes or as aggregated CD4+ T-cells between pre- and post-ATI.

Differential expression and regulation after ATI



(left) Figure 4: Differential surface antigen expression on aggregated HIV+ CD4+ T-cells by individuals A08 or A09 between pre- and post-ATI. *(middle)* Figure 5: Motif comparison in accessible chromatin regions of cells grouped by timepoint (pre vs post-ATI) and infection. Showing motifs with FDR < 0.05 and MD > 0.5, indicating significant enrichment for cells in the cluster as compared with all other cells (*P < 0.05; **P < 0.01). *(right)* Figure 6: Motif comparison between HIV+ pre-ATI and post-ATI cells. The top 20 most significant motifs are shown. The dotted line indicates a –log10 transformed FDR value of 0.05. Color = MD in chromVAR deviations.

Conclusions

- Used our single-cell strategy to phenotypically (surface antigen + epigenetics) profile HIV+ cells at basal state pre- and post-ATI + VRC01 immunotherapy.
- Extent of viral rebound AUC may be associated with greater changes in reservoir phenotype
  - Reservoir modulation was specifically associated with the emergence of recently activated Tcm/Ttm cells at the post-ATI timepoint
  - Profound intra- and inter-personal heterogeneity of cellular phenotypes of HIV+ cells

Future Directions

- Validation of various markers as a biomarker of ATI on HIV+ cells.
- Determine if these findings are specific to ATI + VRC01 or if ATI alone is sufficient for the observed differences.
- Add resolution with simultaneous RNA capture (TEAseq/DOGMAseq) for comparison of pre vs post-ATI as well as HIV- vs HIV+.

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