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CITE-Seq and Single Cell αβ TCRseq Unravels HIV-1-Specific GZMB Subset in Post-intervention Controllers

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BACKGROUND

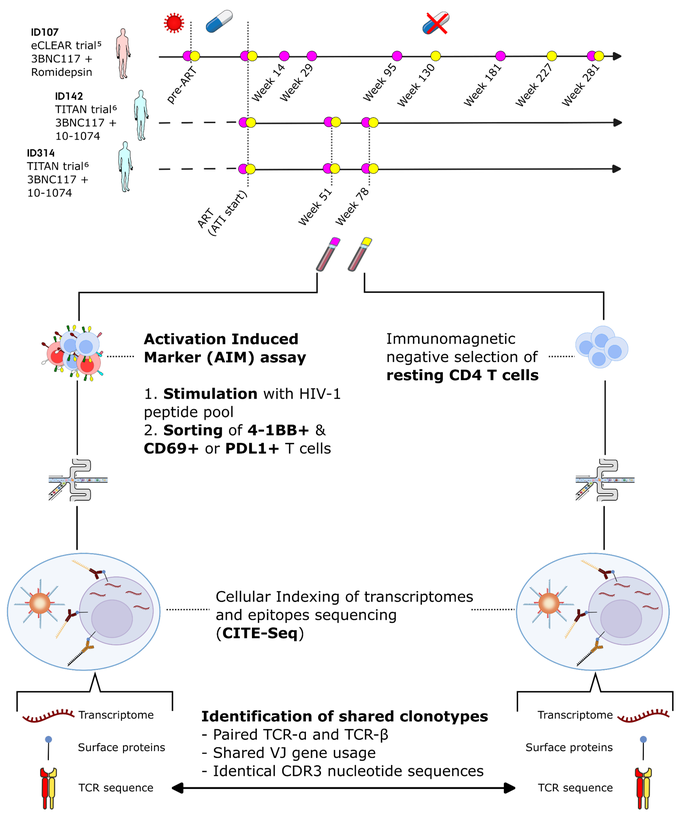
Cytotoxic CD4 T cells

- expand in chronic viral infections[1]
- mediate direct MHC class II-restricted cytolysis [2]
- can target HIV-1-infected cells and inhibit HIV-1 replication[3,4]

Do cytotoxic CD4 T cells evolve after antiretroviral therapy interruption? Do cytotoxic CD4 T cells contribute to ART-free virological control?

To answer these questions, we investigated **antigen specificity and temporal development** of cytotoxic CD4 T cells in 3 post-intervention controllers.

METHODS



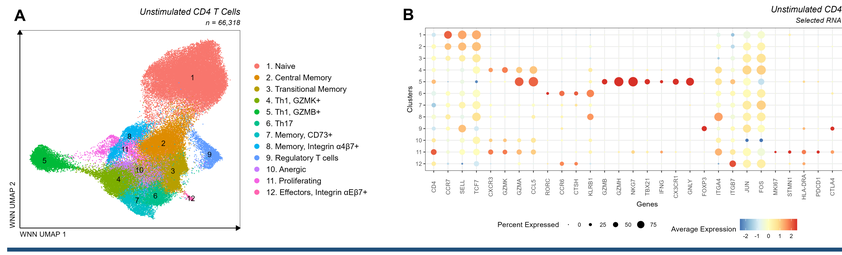
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Expanded clones of terminally differentiated, cytotoxic GZMB+ CD4 T cells – predominantly HIV-1-specific – remained stable over years in post-intervention controllers

RESULTS

1. CITE-Seq reveals GZMB+ CD4 T cells with a cytotoxic transcriptional and surface protein profile

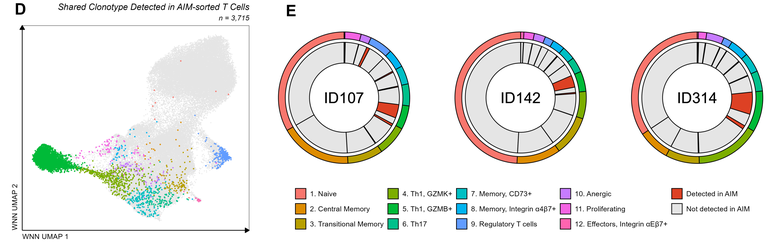


(A) Batch-corrected Weighted Nearest Neighbor (WNN) UMAP projection of CITE-Seq data, showing CD4 T cells from eleven samples*. Clustering and visualization are based on a weighted combination of gene and surface protein expression data.*

(B) Dot plot showing key marker gene expression per cluster*.*

(C) Heatmap showing surface protein expression per cluster. *Values represent the average DSB-corrected expression, measured as the number of standard deviations from the expected noise. Markers with average expression below 4 were considered non-expressed. Only proteins expressed in at least one cluster are shown.*

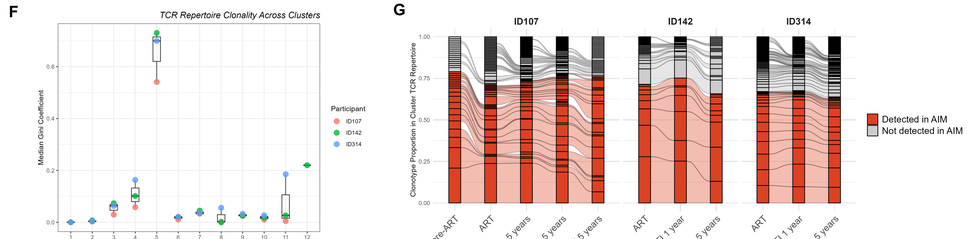
2. Shared TCR sequences identify AIM-responsive clones. Most of them are GZMB+ CD4 T cells



(D) Unstimulated CD4 T cells sharing TCR sequences with AIM-sorted T cells. *WNN UMAP coordinates match those in Figure A. AIM-responsive clones are colored according to their cluster, non-detected clones are gray.*

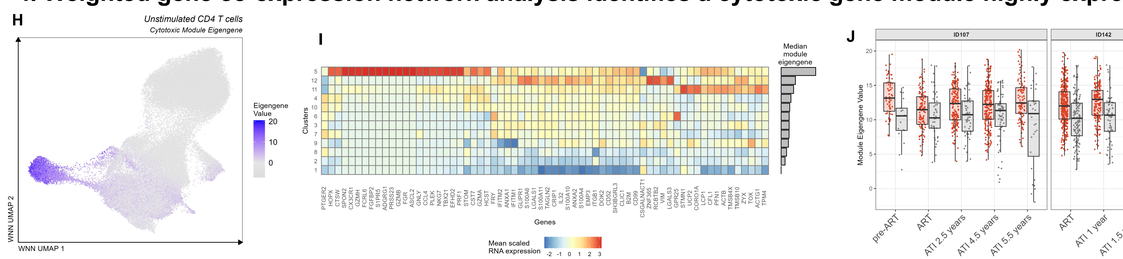
(E) Cluster distribution of unstimulated CD4 T cells sharing TCR sequences with AIM-sorted T cells. *The outer ring represents cluster abundance, while the inner ring indicates the frequency of AIM-detected clones per cluster.*

3. GZMB+ CD4 T cells exhibit a high degree of clonality. Clonal families remain stable over years.



(F) Median Gini coefficient across all visits for each cluster in each participant **with cluster coefficient distributions represented by boxplots.**
(G) Alluvial plot showing the frequency of each participant's GZMB+ clones over time. *AIM-detected clones at any time point are highlighted in red. Time points on the x-axis are categorical and not proportionally scaled.*

4. Weighted gene co-expression network analysis identifies a cytotoxic gene module highly expressed by AIM-responsive clones



(H) Single-cell eigengene values for the identified gene module. *WNN UMAP coordinates match those in Figure A. Cells are colored according to module eigengene values.*

(I) Heatmap of scaled average gene expression in the identified module. *Columns (genes) are hierarchically clustered based on expression, and rows (clusters) are ordered by median module eigengene.*

(J) Single-cell module eigengene values in the GZMB+ cluster at each time point*, faceted by participant and stratified by AIM-detection status*

CONCLUSIONS AND FUTURE DIRECTIONS

GZMB+ CD4 T cells in these post-intervention controllers exhibit **high clonality** and **coordinated cytotoxic gene expression** patterns.

Most belong to **AIM-responsive clones**, suggesting HIV-1 specificity.

These putative HIV-1-specific clones **persist over time** with **sustained cytotoxic module expression**.

The next steps for **validation** may include:

- **Luciferase reporter assays** to confirm TCR specificity
- **Killing assays** to assess cytotoxic activity.