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Pre-ATI CD4⁺ T-cell Immune Activation Capacity Identifies Postintervention Controllers

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BACKGROUND

Broadly neutralizing antibodies (bNAbs) increase the likelihood of sustained viral suppression after ART interruption. While CD8⁺ T cells and autologous antibodies have been implicated in postintervention control, the role of CD4⁺ T cells remains less defined.

Which CD4⁺ T-cell features characterize postintervention controllers?

Defining these features may help predict postintervention control and inform curative HIV-1 interventions.

Enrichment of early memory states, pre-activation, and robust antiviral responses to HIV-1 stimulation characterize CD4⁺ T cells in postintervention controllers

CONCLUSIONS

Across complementary modalities, unstimulated memory CD4⁺ T cells from PICs exhibit a less differentiated phenotype and a pre-activated transcriptional state. Upon HIV-1 stimulation, CD4⁺ T cells from PICs display enhanced polyfunctionality and antiviral gene expression. Importantly, these features are present prior to treatment interruption. Identifying or therapeutically inducing this immune state may increase the likelihood of postintervention control.

METHODS

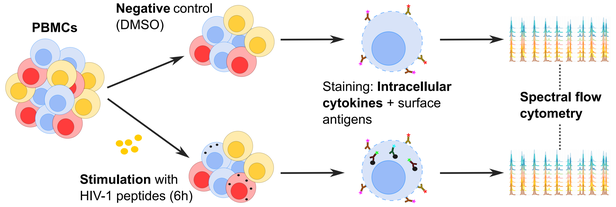
1. 5 Postintervention Controllers From 4 bNAb Studies

Postintervention Controllers (PICs) | Non-Controllers (NCs)

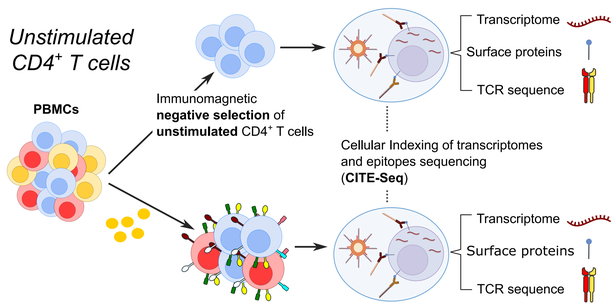
	Years off ART	Intervention
Mendoza *et al.*, Nature, 2018	8.5	3BNC117 + 10-1074
Gaebler *et al.*, Nature, 2022	6.5	3BNC117 + 10-1074
Gunst *et al.*, Nature Medicine, 2022	7.5	3BNC117 + Romidepsin
Gunst *et al.*, Nature Medicine 2023	4	3BNC117 + 10-1074
Gunst *et al.*, Nature Medicine 2023	2.5 (rebound)	3BNC117 + 10-1074

Time points: pre-ART, pre-ATI, ATI, Late ATI (multiple)

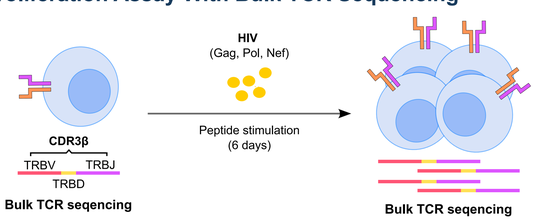
2. Spectral Flow With Intracellular Cytokine Staining



3. CITE-Seq With Paired αβ TCR Sequencing

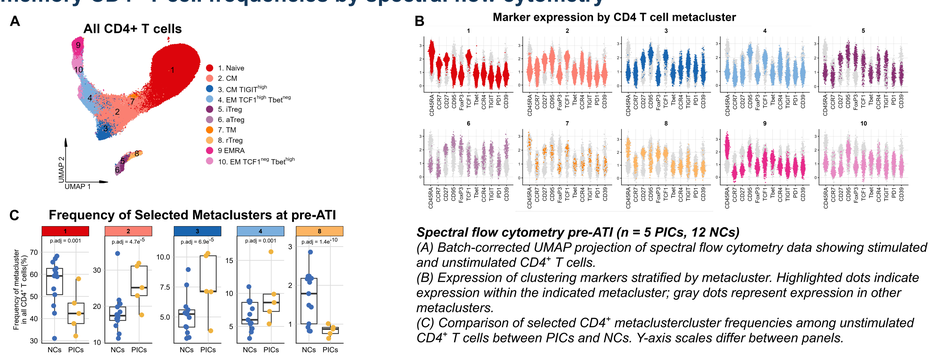


4. Proliferation Assay With Bulk TCR Sequencing



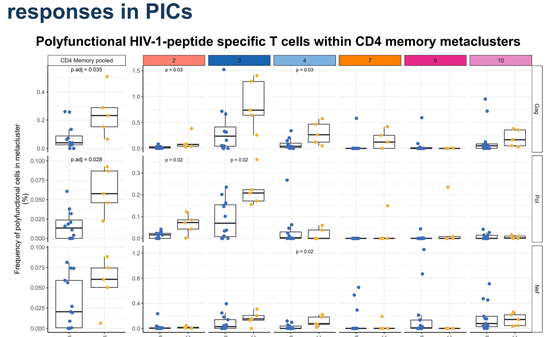
RESULTS

1. In the unstimulated pre-ATI state, PICs show higher central memory and TIGIT^{high} central memory CD4⁺ T-cell frequencies by spectral flow cytometry



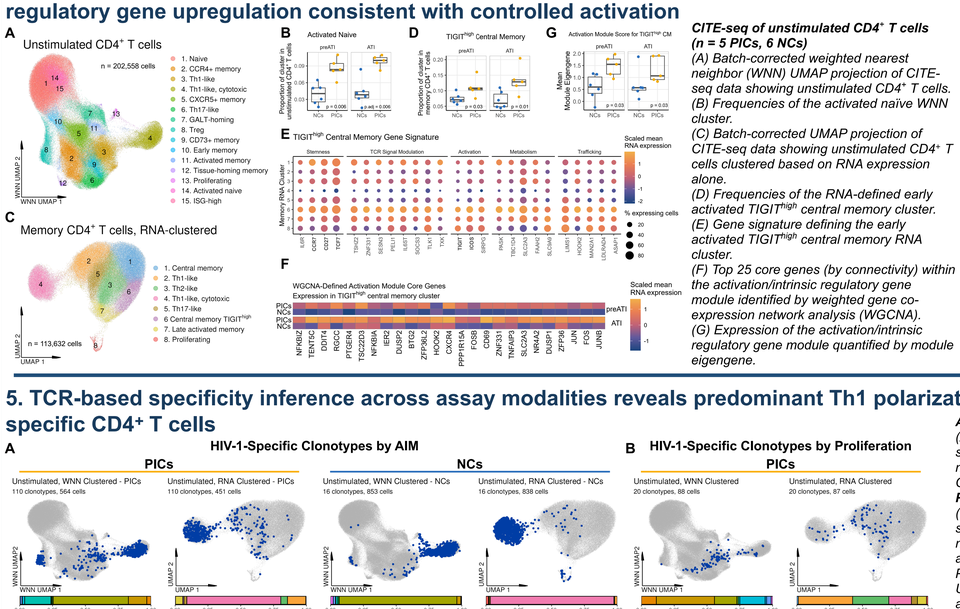
Spectral flow cytometry pre-ATI (n = 5 PICs, 12 NCs)
(A) Batch-corrected UMAP projection of spectral flow cytometry data showing stimulated and unstimulated CD4⁺ T cells.
(B) Expression of clustering markers stratified by metacluster. Highlighted dots indicate expression within the indicated metacluster; gray dots represent expression in other metaclusters.
(C) Comparison of selected CD4⁺ metaclustercluster frequencies among unstimulated CD4⁺ T cells between PICs and NCs. Y-axis scales differ between panels.

2. Pre-ATI HIV-1 peptide stimulation (6h) reveals enhanced polyfunctional CD4⁺ T-cell responses in PICs



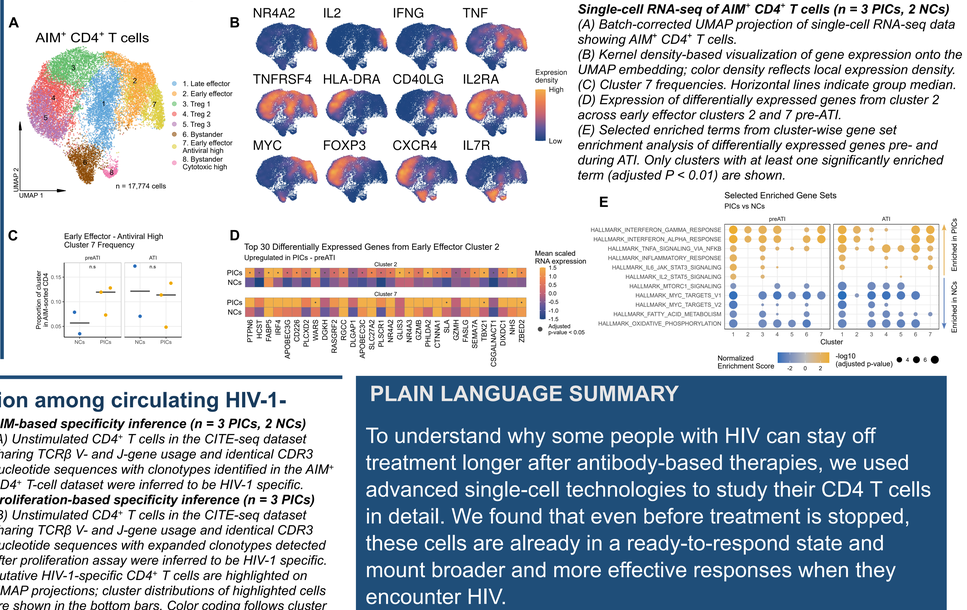
Spectral flow cytometry with intracellular cytokine staining pre-ATI (n = 5 PICs, 12 NCs)
Frequency of polyfunctional CD4⁺ T cells (expressing ≥2 markers) stratified by epitope within the total memory CD4⁺ compartment and by metacluster.
Markers assessed: IFN-γ, TNF-α, IL-2, and CD107a.
Y-axis scales differ between panels; values represent the prcentage of polyfunctional cells within the indicated metacluster.

3. In the unstimulated state, PICs show increased frequencies of activated naïve and early activated TIGIT^{high} central memory CD4⁺ T cells with coordinated activation/intrinsic regulatory gene upregulation consistent with controlled activation



CITE-seq of unstimulated CD4⁺ T cells (n = 5 PICs, 6 NCs)
(A) Batch-corrected weighted nearest neighbor (WNN) UMAP projection of CITE-seq data showing unstimulated CD4⁺ T cells.
(B) Frequencies of the activated naïve WNN cluster.
(C) Batch-corrected UMAP projection of CITE-seq data showing unstimulated CD4⁺ T cells clustered based on RNA expression alone.
(D) Frequencies of the RNA-defined early activated TIGIT^{high} central memory cluster.
(E) Gene signature defining the early activated TIGIT^{high} central memory RNA cluster.
(F) Top 25 core genes (by connectivity) within the activation/intrinsic regulatory gene module identified by weighted gene co-expression network analysis (WGCNA).
(G) Expression of the activation/intrinsic regulatory gene module quantified by module eigengene.

4. Pre-ATI HIV-1 peptide stimulation (20h) reveals enhanced antiviral gene expression and interferon-γ/α response enrichment in CD4⁺ T cells from PICs



Single-cell RNA-seq of AIM⁺ CD4⁺ T cells (n = 3 PICs, 2 NCs)
(A) Batch-corrected UMAP projection of single-cell RNA-seq data showing AIM⁺ CD4⁺ T cells.
(B) Kernel density-based visualization of gene expression onto the UMAP embedding; color density reflects local expression density.
(C) Cluster 7 frequencies. Horizontal lines indicate group median.
(D) Expression of differentially expressed genes from cluster 2 across early effector clusters 2 and 7 pre-ATI.
(E) Selected enriched terms from cluster-wise gene set enrichment analysis of differentially expressed genes pre- and during ATI. Only clusters with at least one significantly enriched term (adjusted P < 0.01) are shown.

5. TCR-based specificity inference across assay modalities reveals predominant Th1 polarization among circulating HIV-1-specific CD4⁺ T cells

AIM-based specificity inference (n = 3 PICs, 2 NCs)
(A) Unstimulated CD4⁺ T cells in the CITE-seq dataset sharing TCRβ V- and J-gene usage and identical CDR3 nucleotide sequences with clonotypes identified in the AIM⁺ CD4⁺ T-cell dataset were inferred to be HIV-1 specific.
Proliferation-based specificity inference (n = 3 PICs)
(B) Unstimulated CD4⁺ T cells in the CITE-seq dataset sharing TCRβ V- and J-gene usage and identical CDR3 nucleotide sequences with expanded clonotypes detected after proliferation assay were inferred to be HIV-1 specific. Putative HIV-1-specific CD4⁺ T cells are highlighted on UMAP projections; cluster distributions of highlighted cells are shown in the bottom bars. Color coding follows cluster annotations in previous panels.

PLAIN LANGUAGE SUMMARY

To understand why some people with HIV can stay off treatment longer after antibody-based therapies, we used advanced single-cell technologies to study their CD4 T cells in detail. We found that even before treatment is stopped, these cells are already in a ready-to-respond state and mount broader and more effective responses when they encounter HIV.