# Basal Proviral Transcription and Immune Correlates in Well-Suppressed Youth with Perinatal HIV-1




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PAVE Pediatric Adolescent Virus Elimination
A Martin Delaney Collaboratory

## BACKGROUND

In adults with HIV-1, persistent proviral transcription persists in 73-77%[1,2] despite years (6 to 10) of sustained virologic suppression (VS), reflective of the persistence of a pool of transcriptionally competent proviruses that may be targets for ART-free remission and cure therapies[3].

The extent to which transcriptionally active proviruses persist and influence the immune milieu in adolescents and young adults living with longstanding perinatal HIV-1 (AYAPHIV) is unknown and the basis for this study.

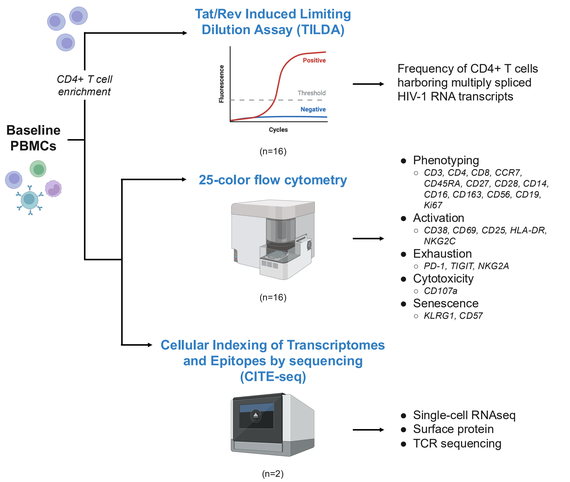
**Youth with perinatal HIV-1 harbor transcriptionally active proviruses despite long-term ART. This high basal proviral transcription is associated with higher effector memory CD4+ and CD8+ T cells with lower immune activation profiles.**

## CONCLUSIONS

- **63%** of adolescents and young adults living with perinatal HIV-1 on long term ART harbor proviruses that are basally expressing multiply spliced HIV-1 RNA transcripts of uncertain etiology.
- High basal proviral transcription was associated with **higher central and effector memory CD4+ and CD8+ T cell** populations.
- Youth with high basal proviral transcription had **lower levels of HLA-DR+ and CD38+** effector memory CD4+ and CD8+ T cells, and lower $CD56_{dim}$ HLA-DR+ NK cells.
- However, high basal proviral transcription was associated with **higher CD25 expression** in CD4+ T cells.
- CITE-Seq reveals differences in **inflammatory, stress, regulatory, and metabolic pathways** in association with basal proviral transcription.

**These findings have implications for targeting the proviral reservoir in youth with perinatal HIV-1.**

## METHODS



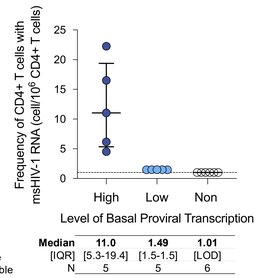

| Participant Demographics | Median [*IQR*] | | |
|---|---|---|---|
| N | 16 | | |
| Race | Black/AA (81.25%)<br>White or Mixed (12.5%) | Asian (6.25%) | |
| Sex | Female (81.25%) | | |
| HIV-1 Subtype | A1 (6.25%)<br>B (50%) | C (12.5%)<br>A/E (6.25%) | A/G (12.5%)<br>G (12.5%) |
| Age (yr) | 17.8 *[13.8-20.1]* | | |
| Duration of Suppression (yr) | 10.4 *[6.0-16.2]* | | |
| Total HIV-1 DNA (cpm) CD4s | 366.7 *[179.5-724.1]* | | |
| *Inferred Intact HIV-1 DNA (cpm) CD4s | 14.4 *[4.1-43.2]* | | |
| % Intact HIV-1 DNA (cpm) CD4s | 5.5 *[3.4-7.6]* | | |

* = Double positive (Gag + Env) droplets
cpm = copies per million

## RESULTS

### Multiply spliced Tat/Rev HIV-1 RNA transcripts are detected at baseline in 63% of AYAPHIV on long-term ART

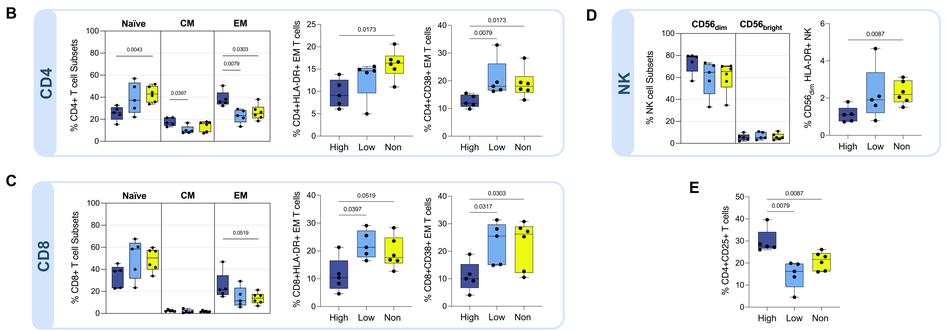
**(A)** TILDA was used to measure the frequency of transcriptionally competent proviruses at baseline. Enriched CD4+ T cells from virologically suppressed AYAPHIV (median 10.4 years) with perinatal HIV-1 for frequency of CD4+ T cells with multiply spliced (ms) HIV-1 RNA. Median and IQRs are listed. Three patterns of expression were detected: High, Low and Non-expressed.




| | High | Low | Non |
|---|---|---|---|
| Median [IQR] | 11.0 [5.3-19.4] | 1.49 [1.5-1.5] | 1.01 [LOD] |
| N | 5 | 5 | 6 |

Basal levels of ms HIV-1 RNA transcripts were not correlated with total HIV-1 DNA, intact HIV-1 proviruses, duration of virologic suppression or presumed age at virologic suppression, although the sample size is small (n=16).

### High basal proviral transcription was associated with lower HLA-DR+ and CD38+ effector memory CD4+ and CD8+ T cells, and lower $CD56_{dim}$ HLA-DR+ NKs




Baseline PBMC immunophenotyping from virologically suppressed AYAPHIV (n=16). **(B)** CD4+ **(C)** CD8+ T and **(D)** NK cell subsets and activation proportions, stratified by the three patterns of basal proviral expression: High, Low and Non-expressed. **(E)** Proportion of total CD4+ T cells expressing CD25. Mann-Whitney U test; FDR-adjusted P value = *<0.05*.

No differences in immune exhaustion markers (PD-1, TIGIT) were observed in CD4+ and CD8+ T cells.

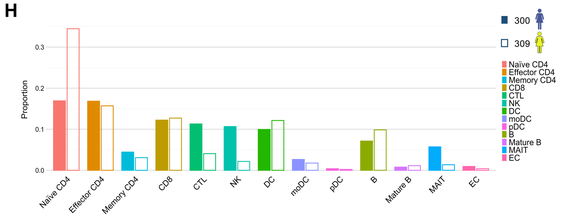
### CITE-Seq reveals differences between a high and non-expressor

**F**

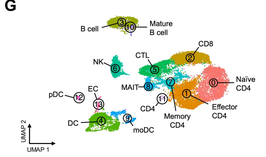
| | 300 | 309 |
|---|---|---|
| Sex | F | F |
| Age (yr) | 18 | 18 |
| HIV-1 Subtype | B | B |
| Duration of Suppression (yr) | 16 | 17 |
| Total HIV-1 DNA/$10^6$ CD4 | 218.8 | 7.7 |
| Intact HIV-1 DNA/$10^6$ CD4 | 15.8 | <2.0* |
| Baseline msRUPM | 16.51 | 1.01* |

* = *undetectable*

**H**



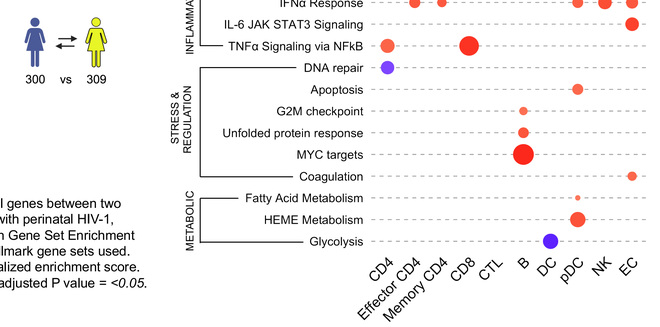

**G**




**(F)** Two participants with perinatal HIV-1, matched by age, sex, and duration of virologic suppression but varied by reservoir size and basal proviral transcription were analyzed by CITE-seq. **(G)** UMAP plot displaying the distribution of PBMCs **(H)** and the proportions of each cluster cell type between participants (n=2). MAIT = mucosal-associated invariant T cells; EC = endothelial cells.

### Basal proviral transcription is associated with enriched inflammatory, stress, regulatory, and metabolic pathways

**I**




**(I)** Differential genes between two participants with perinatal HIV-1, analyzed with Gene Set Enrichment Analysis. Hallmark gene sets used. NES = normalized enrichment score. Padj = FDR-adjusted P value = *<0.05.*


## ACKNOWLEDGEMENTS

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## REFERENCES


1. Procopio FA, et al. A Novel Assay to Measure the Magnitude of the Inducible Viral Reservoir in HIV infected Individuals. EBioMedicine. 2015. doi:10.1016/j.ebiom.2015.06.019
2. Dubé M, et al. Spontaneous HIV expression during suppressive ART is associated with the magnitude and function of HIV-specific CD4+ and CD8+ T cells. Cell Host Microbe. 2023. PMID: 37708853
3. Dhummakupt A, et al. Differences in inducibility of the latent HIV reservoir in perinatal and adult infection. JCI Insight. 2020. PMID: 31999647


## PLAIN LANGUAGE SUMMARY

In youth with perinatally acquired HIV, even when well controlled with ART, many still have active HIV in their immune cells. This ongoing activity is linked to changes in the immune system, such as lower immune cell activation, informing the design of cure strategies for youth with perinatal HIV.