Distinct HIV Transcriptional Signatures Across Reservoir-Defined Tissue Clusters

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BACKGROUND

- Despite ART, HIV reservoirs persist and vary across tissues, reflecting differences in tissue-specific HIV transcriptional regulation.[1,2]
- The extent to which **HIV transcriptional cascade** - from HIV DNA to early transcripts (e.g., unspliced [us] HIV Gag RNA) and late transcripts (e.g., multiply spliced [ms] HIV Tat/Rev RNA) - varies across tissues remains unclear (**Fig.1**).[1,2]

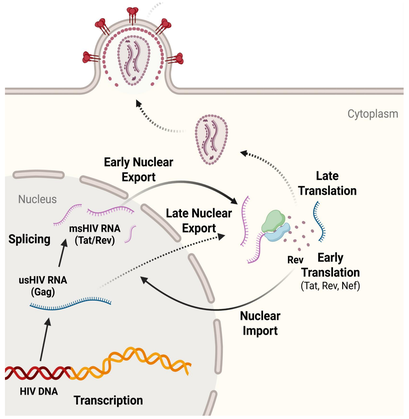


Figure.1 HIV transcriptional cascade
HIV DNA generates viral transcripts that remain unspliced (usGag, reflecting early transcriptional activity) or undergo splicing to produce multiply spliced transcripts (msTat/Rev, reflecting completion of transcription). Figure made with Biorender.

STUDY AIM

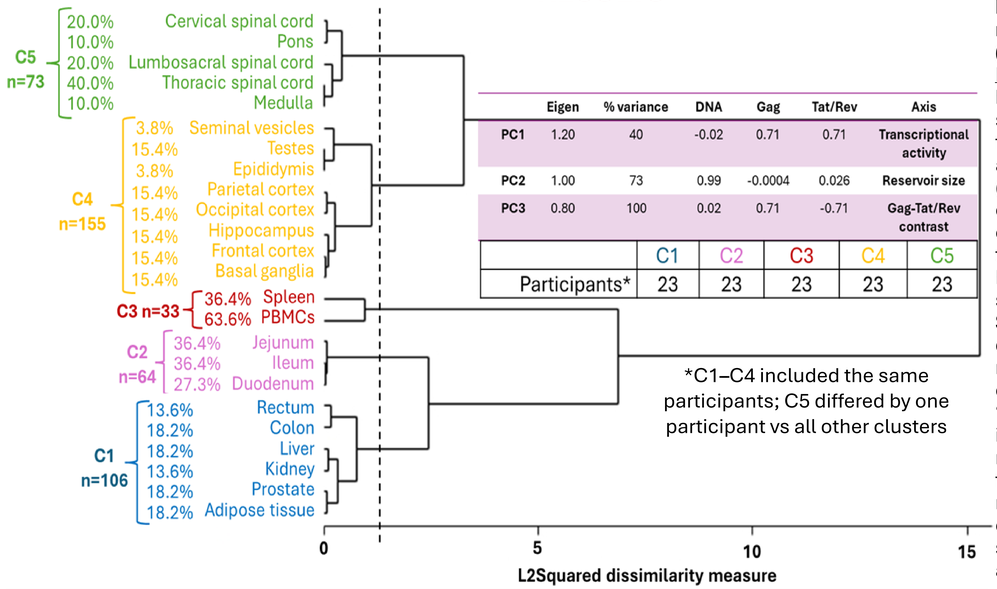
To assess how tissue-specific environments are associated with HIV transcription under suppressive ART.

METHODS

- Collected 24 distinct tissue types at rapid autopsy from 24 Last Gift participants on long-term suppressive ART.
- Quantified HIV DNA, usGag RNA, and msTat/Rev RNA by ddPCR (in bulk tissues).
- Performed full-length env single-genome sequencing to assess viral diversity and identify intact and hypermutated clones.
- Defined tissue clusters using Principal Component Analysis (PCA) of HIV metrics followed by hierarchical clustering (**Fig.2**).
- Assessed differences in transcription activity across clusters using mixed-effects models with interaction terms and structural equation modeling, adjusting for demographics, last/nadir CD4, duration of HIV and ART, last ART regimen, and number of clones.

Tissue clusters defined by HIV reservoir profiles showed distinct transcriptional behaviors. These differences indicate that residual viral activity under suppressive ART varies by tissue context and is not explained by HIV burden alone.

RESULTS



	Eigen	% variance	DNA	Gag	Tat/Rev	Axis
PC1	1.20	40	-0.02	0.71	0.71	Transcriptional activity
PC2	1.00	73	0.99	-0.0004	0.026	Reservoir size
PC3	0.80	100	0.02	0.71	-0.71	Gag-Tat/Rev contrast

	C1	C2	C3	C4	C5
Participants*	23	23	23	23	23

*C1–C4 included the same participants; C5 differed by one participant vs all other clusters

Fig.2 Tissue Clusters based on HIV reservoir profiles (size and activity)
Dendrogram of the hierarchical clustering solution showing the five tissue clusters (C) and the contribution (%) of sample types to each cluster. Principal components (PC) with the relative weight of HIV metrics are also shown. Bottom table: Summary of the differences in HIV reservoir across clusters. Labels (e.g., "highest", "moderate") indicate statistical rank groups derived from FDR-adjusted mixed-effects pairwise comparisons: clusters sharing the same label are not significantly different, while different labels indicate significant differences. Example, for HIV DNA levels, C2 ~ C3 > C1 > C5 > C4 (no differences within tied groups).

	C1	C2	C3	C4	C5
HIV DNA	Higher	Highest		Lowest	Lower
HIV Gag RNA	Lowest	Highest		Moderate	
HIV Tat/Rev RNA	Highest	Moderate		Highest	Lowest
Env diversity	Highest			Lowest	Moderate
% clones with hypermutated env	No statistical differences				
% clones with intact env	Lowest		Moderate	Highest	

Table 1. Study population, n=24

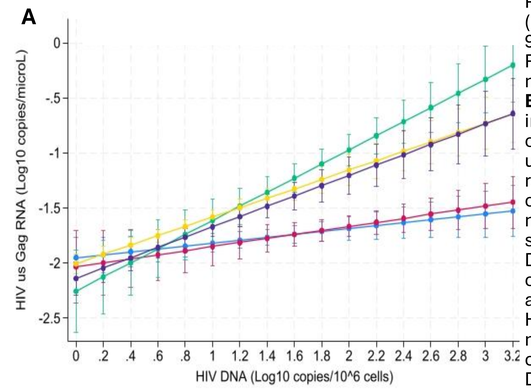
Male, %	83.3%
Age, y°	65±13
White, %	83.3%
CD4⁺ count, cells/μL°	263±193
CD4⁺ nadir, cells/μL°	153±150
ART duration, y°	14±9
Last ART duration, y°	2±1
HIV duration, y°	24±10
Cause of death, %	
Cancer	58.3%
Organ failure	29.2%
ART regimen, %	
2NRTI-INI	58.3%
2NRTI-NNRTI/PI	8.3%
Dual	20.8%
Others	12.5%

°mean ± standard deviation

- 24 participants (**Tab.1**) contributed to 438 samples. Across clusters (Cs), HIV transcription cascade differed in:
  - **Roadblocks:** C3-C5 showed a progression from DNA→us→ms (all path coefficients $p<0.05$), whereas us→ms path was weak in C1 ($p=0.15$), and DNA→us was weak in C2 ($p=0.12$).
  - **Efficiency:** At comparable HIV DNA levels, early transcription was the most efficient in C3 (steepest increase in usGag levels, aβ +0.62, $p<0.001$ vs other clusters; **Fig.3A**). At comparable HIV usGag RNA levels, late transcription was the most efficient in C2 (aβ +0.51, $p<0.001$ vs other clusters; **Fig.3B**).

RESULTS

Fig.3 HIV Transcriptional Efficiency differs across tissues: predicted marginal relationships among HIV measures, stratified by tissue clusters



Predicted levels (margins and 95%CI) of usGag RNA in **A** and msTat/Rev RNA in **B** increased with increasing levels of HIV DNA and usGag RNA respectively, in a cluster-specific manner: the steepest HIV DNA–usGag RNA coupling in C3 (**A**) and the steepest HIV usGag RNA-msTat/Rev RNA coupling in C2 (**B**). Differences across clusters reflect variation in the relationships between HIV metrics, not in their absolute levels (which are shown in Fig.2).

- **Role of intact clones:** higher abundance of intact env clones increased early transcriptional efficiency in C1 (aβ +0.44, $p=0.028$), while reduced it in C5 (aβ –0.46, $p=0.051$; n samples sequenced = 283).
- Env diversity was significantly higher in C1-C3 but did not correlate with transcription characteristics.

CONCLUSIONS

HIV persistence under ART is shaped by tissue-specific regulation of HIV transcription, highlighting the need for cure strategies tailored to transcriptional control rather than reservoir size alone.

ADDITIONAL KEY INFORMATION

References: [1]Telwatte S, PlosPathogens 2018; [2]Yukl S, Sci Transl Med 2018.

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