Heterogeneous Antiretroviral (ARV) Class Effects on Tissue HIV Reservoirs

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BACKGROUND

- Tissue Environments (TEs) and ARV pharmacokinetics/pharmacodynamics may differentially shape HIV reservoirs in the human body.
- In PBMCs, PI-based regimens are associated with higher cell-associated HIV DNA and HIV RNA than NNRTI-based therapy.[1]
- Blood studies show ARV class–specific reservoir signatures (e.g., 2LTR DNA, RNA transcripts),[2,3] but data from tissues are lacking.

STUDY AIM

To determine the association between ARV classes (PI, NNRTI, INSTI) and HIV reservoir size and transcriptional activity across TEs during long-term viral suppression

METHODS

- Rapid autopsies (≤6 hours) were performed in Last Gift participants on long-term suppressive ART.
- 32 tissue types were grouped into 10 TEs based on pharmacokinetics and reservoir features (**Fig.1**)
- Bulk tissue HIV DNA, unspliced (us) Gag RNA, and multiply spliced (ms) Tat/Rev RNA were quantified by ddPCR.
- Mixed-effects models tested interactions between ARV class (last regimen) and TE on reservoir metrics, adjusting for demographic, clinical, and ART factors.
- Observed interaction margins (real regimen mix) and counterfactual margins (covariates set to isolate each ARV class to assess pure class effects) were compared using pairwise contrasts with false discovery rate (FDR) correction.

Tab.1 Study population, n=28

Male, %	86%	**HIV duration, y°**	25±10
Age, y°	65±13	**Dual/mixed ART, %**	32.0%
White, %	82.1%	**On NRTI, %**	93.0%
Undetectable viremia, %	100%	**On NNRTI, %**	36.0%
CD4+ count, cells/μL°	260±187	**On INSTI, %**	36.0%
CD4+ Nadir, cells/μL°	154±155	**On PI, %**	29.0%
ART duration, y°	15±9	**Cancer, %**	60.7%
Last ART duration, y°	2±1	**Organ failure, %**	28.6%

Legend: °Mean ± standard deviation.

Antiretroviral classes were associated with distinct tissue-specific HIV reservoir patterns. Differences were most evident in early HIV transcription, suggesting that residual viral activity vary across tissues and drug classes, with potential implications for persistent tissue injury and cure

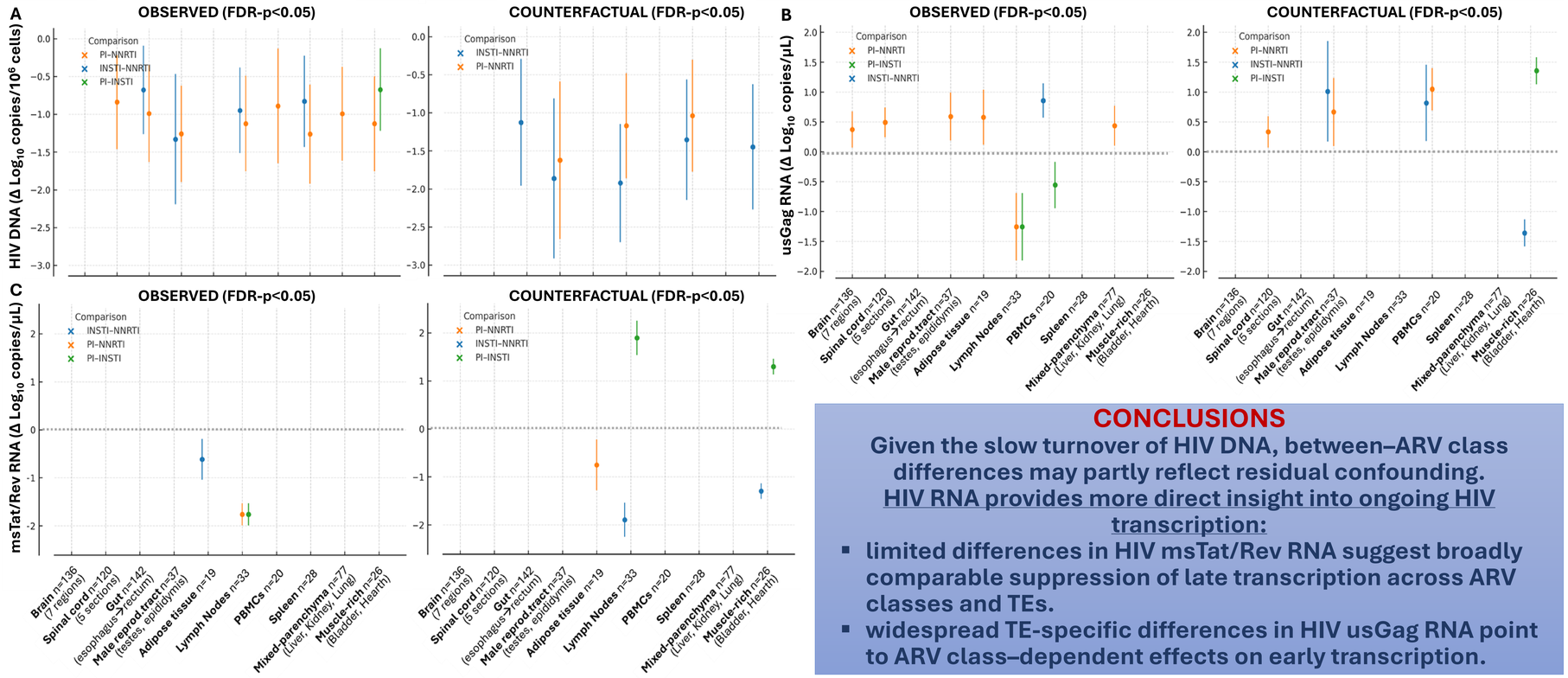
RESULTS

- Twenty-eight participants contributed 638 samples (**Tab.1**).
- **HIV DNA** levels showed a consistent ranking across TEs, with NNRTI > INSTI ≈ PI (**Fig.1A**):
  - HIV DNA levels were lower with PI- than NNRTI-based regimens in 7 TEs, lower with INSTI- than NNRTI-based regimens in 4 TEs, and lower with PI- than INSTI-based regimens in 1 TE (all FDR-adjusted p < 0.05), with no class effect in brain or adipose tissue.
  - The largest differences (0.5–1 $\log_{10}$ copies/10^6 cells) were observed in gut, lymph nodes, testes/epididymis, and spleen, with concordant counterfactual estimates.

RESULTS

- **HIV usGag RNA** levels showed stronger TE dependence and larger ARV class differences (0.2–1.2 $\log_{10}$ copies/μL; **Fig.1B**):
  - Levels were lower with PI than INSTI/NNRTI in lymph nodes, higher with PI than NNRTI in five other TEs, and higher with INSTI than PI/NNRTI in PBMCs (all FDR-adjusted p<0.05).
  - Counterfactual estimates were concordant in spinal cord and testes/epididymis, shifted in PBMCs (lower on NNRTI than PI/INSTI), and revealed additional differences in muscle-rich TE.
- Across TEs, between–ARV class differences in **HIV msTat/Rev RNA** levels were limited (**Fig.1C**):
  - Levels were lower with PI- than NNRTI-based regimens in adipose TE, and lower with INSTI- than PI/NNRTI-based regimens in lymph node and muscle-rich TEs.

Fig.1 Associations between ARV Classes and HIV Reservoir metrics across Tissues (FDR-significant differences only)
Point estimates with ±1 standard error represent pairwise differences in predicted margins from mixed-effects models. Blue = INSTI–NNRTI; Orange = PI–NNRTI; Green = PI–INSTI: it reads as the result of estimate in the first class minus estimate in the second class; positive/negative values mean higher/lower HIV metric in the first-class vs the second one.



CONCLUSIONS

Given the slow turnover of HIV DNA, between–ARV class differences may partly reflect residual confounding.
HIV RNA provides more direct insight into ongoing HIV transcription:

- **limited differences in HIV msTat/Rev RNA suggest broadly comparable suppression of late transcription across ARV classes and TEs.**
- **widespread TE-specific differences in HIV usGag RNA point to ARV class–dependent effects on early transcription.**

ADDITIONAL KEY INFORMATION

References: [1]Pasternak, eLife 2021; [2]Kiselinova et al, JAC 2015; [3]Suzuki et al, J Virus Erad 2025

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