Transcriptomics of Proviral Latency Reversal in Reservoirs of Youth With Perinatal HIV-1

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BACKGROUND

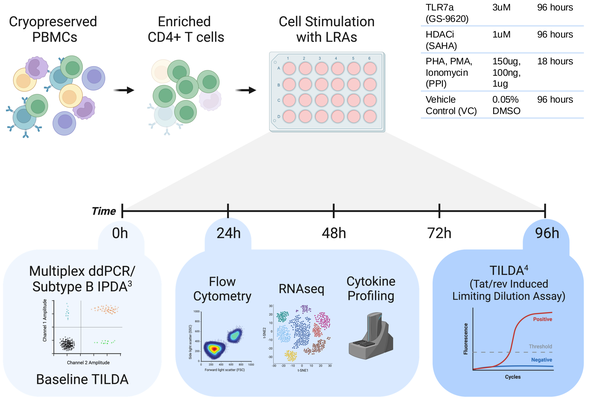
Latency reversing agents (LRAs) such as histone deacetylase (HDAC) inhibitors and TLR agonists reactivate HIV-1 from latency in adults[1,2,5,]. We examined the latency reversal effects of the TLR7 agonist GS-9620, the HDAC inhibitor SAHA, and their combination in *ex vivo* studies of enriched CD4+ T cells from adolescents with perinatal HIV-1 (AdLWPH), aiming to inform latency reversal strategies to purge reservoirs in this population. **We hypothesize that a combination of an innate immune enhancer and epigenetic modifier will reverse latency in perinatal HIV-1 infections.**

Perinatal HIV-1 reservoirs under long-term virologic suppression can be **reactivated** with **innate immune enhancers** and **epigenetic modifiers**, highlighting their therapeutic potential to **purge HIV-1 reservoirs** in this population.

CONCLUSIONS

- Proviruses persisting in perinatal HIV-1 infections are susceptible to reactivation with LRAs.
- Total and intact HIV-1 DNA is associated with greater proviral reactivation.
- LRAs drive CD4+ T cell activation and inflammatory responses through CD69, HLA-DR, TNF-α, and IL-6 upregulation.
- Proviral reactivation is associated with upregulation of proinflammatory pathways, including IFNγ, TGF-β, TNFα and IL-2.

METHODS



LRA	Conc.	Stim. Duration
TLR7a (GS-9620)	3uM	96 hours
HDACi (SAHA)	1uM	96 hours
PHA, PMA, Ionomycin (PPI)	150ug, 100ng, 1ug	18 hours
Vehicle Control (VC)	0.05% DMSO	96 hours

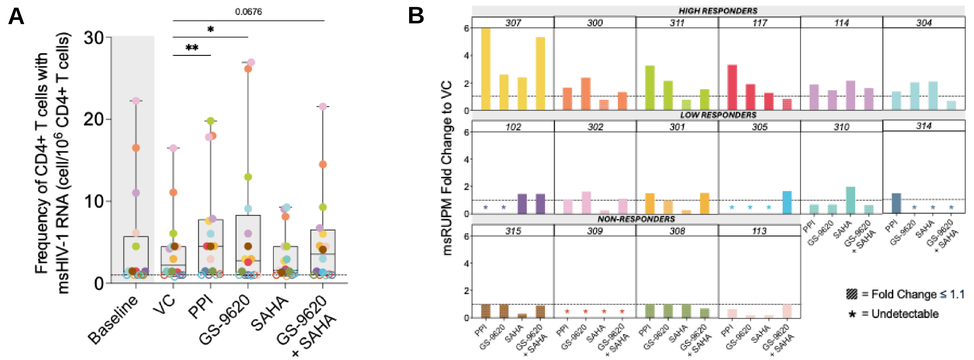
STUDY POPULATION

N	Race	Sex	HIV-1 Subtype	
16	Black/AA (81.25%) White or Mixed (12.5%) Asian (6.25%)	Female (81.25%)	A1 (6.25%) B (50%) C (12.5%)	A/E (6.25%) A/G (12.5%) G (12.5%)

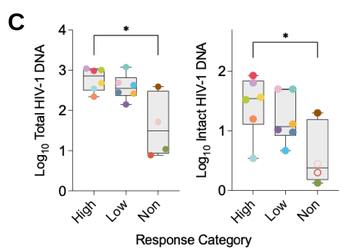
Age (yr)	Duration of Suppression (yr)	Total HIV-1 DNA (cpm) CD4s	*Inferred Intact HIV-1 DNA (cpm) CD4s (N=14)	% Intact HIV-1 DNA (cpm) CD4s (N=14)
17.8 [13.8-20.1]	10.4 [6.0-16.2]	366.7 [179.5-724.1]	14.4 [4.1-43.2]	5.5 [3.4-7.6]

Median [IQR]
** = Double positive (Gag + Env) droplets*

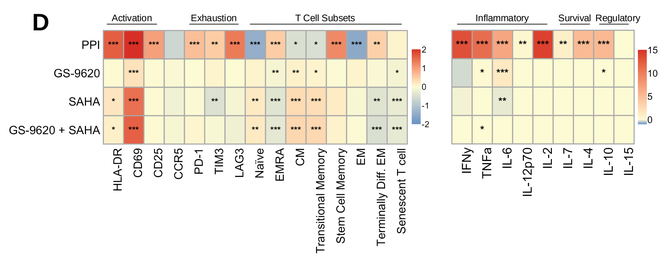
LRA Responses are Heterogenic in Perinatal HIV-1 Infections



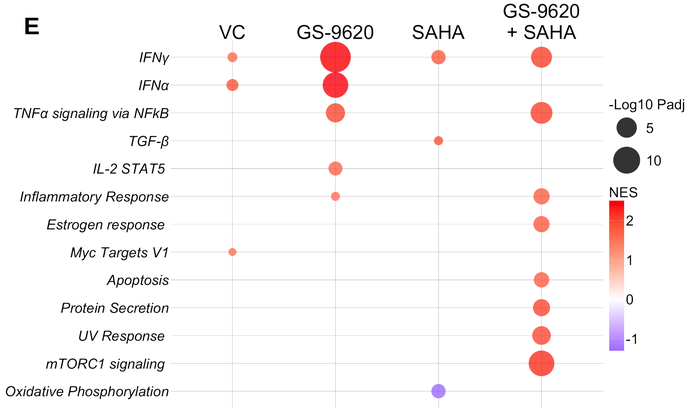
Proviral Reactivation is Associated with Total and Intact HIV-1 DNA

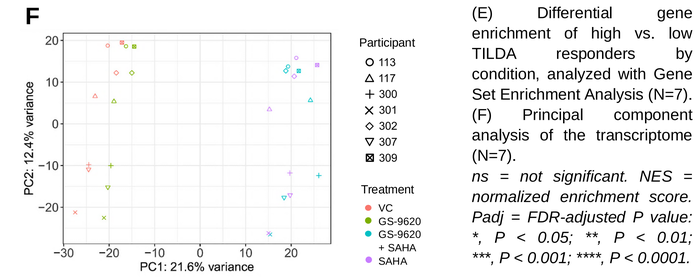


LRAs Drive T cell Activation and Inflammatory Responses



Proviral Reactivation is Associated with Upregulation of Distinct Proinflammatory Pathways





(A) Frequency of CD4+ T cells with msHIV-1 RNA (msRUPM) by treatment condition, measured by TILDA (N=16). **(B)** msRUPM fold change (FC) relative to VC. Categorization of participants as High (FC > 1.1 for ≥3 LRAs), Low (FC > 1.1 for ≥1 LRA), or Non-Responders (no FC >1.1). Dotted line = 1.1. Two-sided signed Wilcoxon test against VC; P-value < 0.05. **(C)** Total and intact HIV-1 DNA measured by IPDA, by response category. Mann-Whitney test; P-value < 0.05. **(D)** Immunophenotypes and cytokine profiles after 24-hour LRA stimulation. Color scale = log2FC relative to VC. Two-sided signed Wilcoxon test against VC*, *P < 0.05; **, P < 0.01; ***, P < 0.001*

(E) Differential gene enrichment of high vs. low TILDA responders by condition, analyzed with Gene Set Enrichment Analysis (N=7). (F) Principal component analysis of the transcriptome (N=7).
*ns = not significant. NES = normalized enrichment score. Padj = FDR-adjusted P value: *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001.*

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