The interplay between epigenetics and IFN-signaling modulate HIV reservoir seeding and persistence

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BACKGROUND

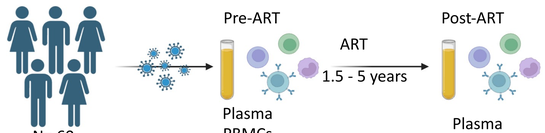
The persistence of the HIV reservoir remains a major barrier for cure. Even under long-term antiretroviral therapy (ART), the reservoir endures and drives viral rebound during analytical treatment interruption (ATI). Evidence shows that smaller reservoir sizes on ART are associated with delayed rebound after ATI. Therefore, uncovering the mechanisms that naturally limit reservoir size in people on long-term ART could inform strategies aimed at reservoir elimination, ultimately enabling sustained remission without rebound and advancing efforts toward a functional HIV cure.

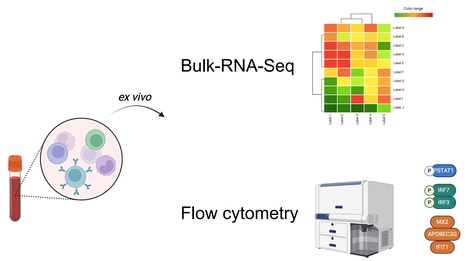
Pre-ART viremia levels is the dominant driver of HIV reservoir seeding, linking CD4 activation/TNF-α inflammation and transcription-permissive chromatin remodeling to higher total HIV-DNA levels

On ART, smaller reservoir size is associate with IFN antiviral programs and IFN-γ$^+$ T cells; while higher reservoir sizes are associated with increase with T cell activation/differentiation

METHODS

Plasma and peripheral blood mononuclear cells (PBMCs) from 60 PWH sparing 3-logs on HIV reservoir size on-ART (<10 to >1000 copies/million cells) were collected (pre- and >3 years post-ART). Plasma viral load (pVL) and HIV-DNA levels in CD4+T cells were quantified.





Innate and adaptive immune profiles were analyzed by flow cytometry. *Ex vivo* antiviral responses and histone acetylation/methylation marks were assessed and validated in an *in vitro* in a HIV infection model. Bulk RNAseq analysis was performed in PBMC.

RESULTS

Fig. 1. Plasma viral load (pVL) was associated with HIV-DNA levels prior to ART, which in turn predicted reservoir size post-ART

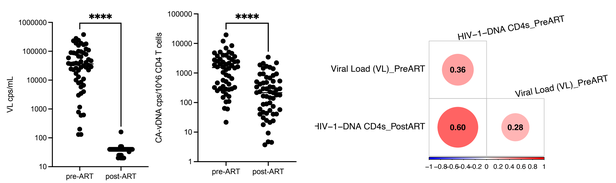
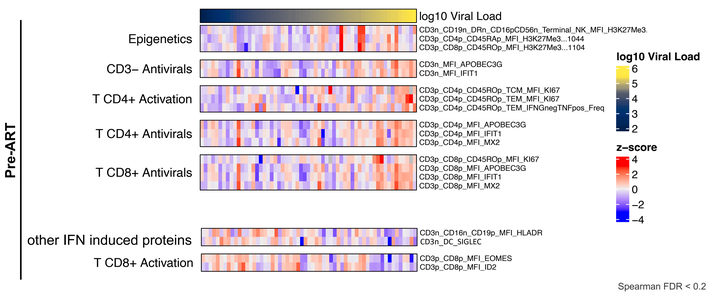


Fig. 2. Pre-ART lower viremia is associated with antigen presentation, CD8 effector programs, and TGF-β–linked metabolic pathways

Pre-ART expression of HLA-DR and Siglec-1 (B and DC cells); and CD8 T cell effector function (EOMES, ID2) are inversely correlated with VL
Pre-ART CD4 activation (ki67) and CD4/CD8 antiviral machineries are associated with higher VL (pre-ART)



Lower viremia is associated with higher TGF-β signaling pathway, protein secretion, and heme metabolism
Higher viremia is associated with cellular activation and higher IFNs signatures

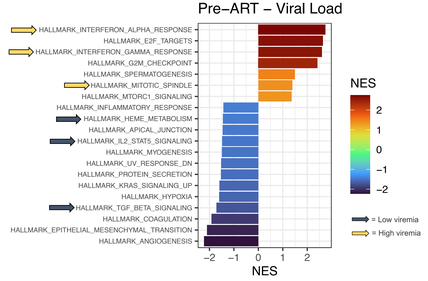
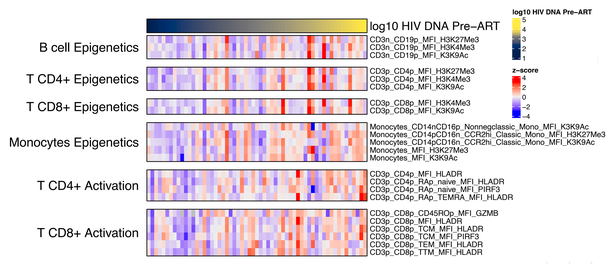
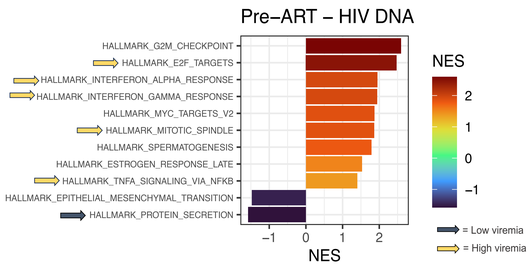


Fig. 3 Pre-ART reservoir seeding is driven by T cell activation and chromatin remodeling, and restrained by innate sensing and cytokine programs

Epigenetics modifications in all subsets are associated to higher viral seeding



Lower HIV seeding at pre-ART is supported by protein secretion (cytokines)
Higher HIV seeding at pre-ART is fueled by T cell activation and TNF-α signaling



cDCs, and expression of pIRF3 and SIGLEC-1 on innate cells are associated with less vDNA seeding

feature pre-ART	rho vs vDNA pre-ART
CD3n_CD19n_CD56nCD19n_CD14nDRp_CD11cpCD123n_cDCs_Freq	-0.31
CD3n_not_CD16n_CD19p_CD16pDRp_nonnegclassic_mono_MFI_PIRF3	-0.3
cDCs_MFI_SIGLEC-1	-0.28

CONCLUSIONS

Overall, higher pre-ART viremia is associated with higher HIV-DNA seeding, supported by CD4 activation/TNF-α inflammation with chromatin remodeling that enriches productive infection in transcriptionally active (histone-marked) cells. Viral control and reduced seeding aligned with better antigen presentation, CD8 effector programs, TGF-β–linked metabolism, innate sensing, and cytokine secretion. On ART, smaller reservoirs is associated with IFN antiviral programs and IFN-γ$^+$ T cells, while higher reservoir sizes are associated with persistent T cell activation/differentiation.

PLAIN LANGUAGE SUMMARY

· Before treatment, immune cells are highly susceptible to viral infection and replication because of their activated state, resulting in increased plasma viremia and greater HIV reservoir seeding.

· During treatment, antiviral molecules contribute to reducing the size of the viral reservoir; however, if immune activation persists, virus-harboring cells may proliferate, leading to an expansion of the reservoir.

Fig. 4. Productive HIV infection is higher on cells expressing histones related to active transcription

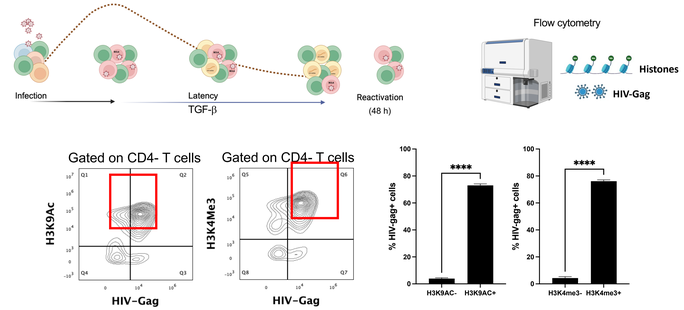
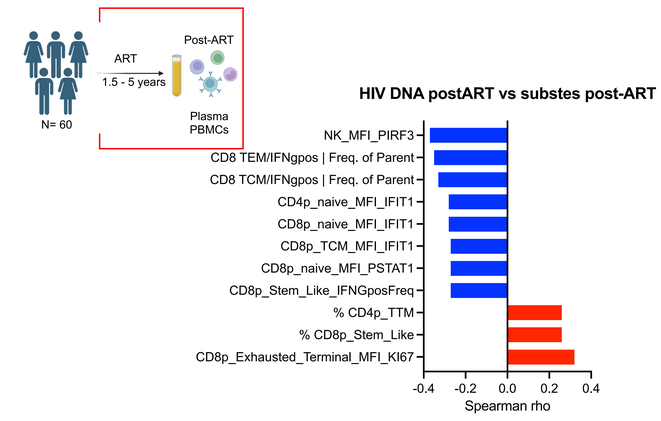
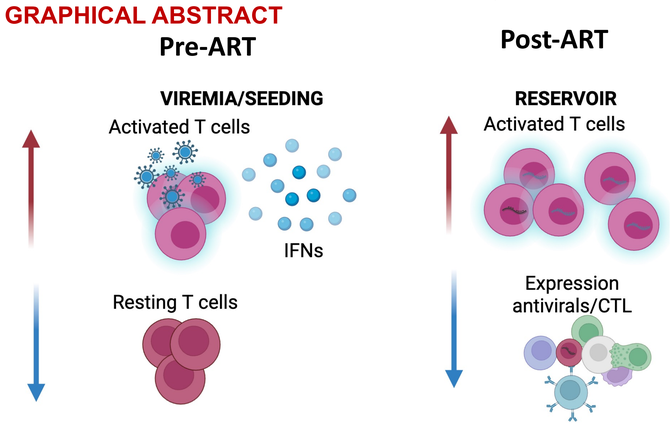


Fig. 5. Lower reservoir size on ART is associated with IFN-antiviral programs and IFN-γ$^+$ T cells; while higher reservoir sizes are supported by increased T cell activation and differentiation



GRAPHICAL ABSTRACT



ACKNOWLEDGEMENTS

RIDHIV MDC for HIV Cure Research
NIAID/NHLBI/NIDDK/NINDS/NIDA UM1AI164562
Schematic figures created in https://BioRender.com

R37-AI141258
ORIP/OD P51 OD011132 (ENPRC)
CFAR – AI050409

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