Residual HIV Viremia Associates With Reservoir Size, Not With Immune Activation or Inflammation

B2-355

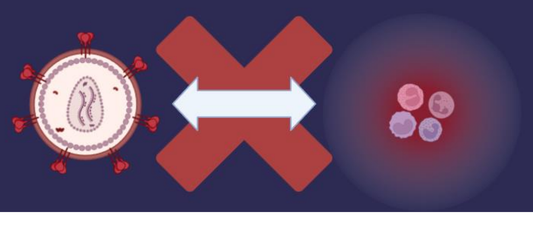
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Background

- Upon initiation of antiretroviral therapy (ART), people living with HIV (PLHIV) generally raipdly reach a viral load (VL) that is unquantifiably low for commercial PCR tests during routine VL monitoring
- However, while unquantifiable, the PCR tests can discriminate between unquantifiable low, but detectable VL (<40 c/ml, residual viremia, RV) and undetectable VL (Target Not Detected, TND).
- About one-third of PLHIV in the 2000HIV cohort had RV, and these individual were >2x as likely to develop CVD[CROI 2024 K2-783]
- Whether RV drives or is driven by systemic immune activation remains debated.
- In two cohorts of PLHIV, we examined associations between RV, systemic Immunologic effects, and viral reservoir composition and size.

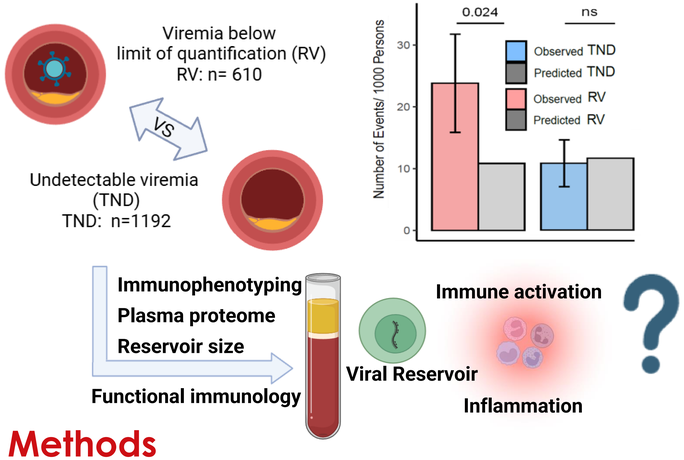
Residual Viremia < 40 copies/ml does **not** drive or is **not** driven by systemic inflammation or immune activation



2 cohorts of virally supressed PLHIV
Discovery Validation

2x

<40 ≠ 0

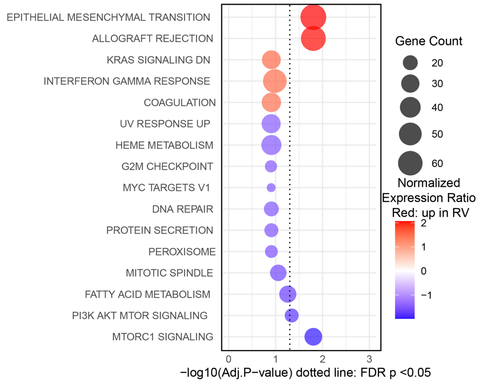


Methods

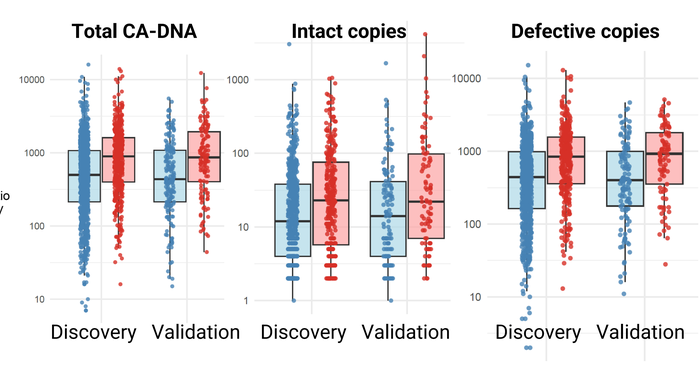
Participants: From the 2000HIV cohort encompassing 1895 virally supressed PLHIV, compared were those with negative PCR at baseline to those with unquantifiable yet detectable VL (<40c/ml, RV)

Instruments: Ex-vivo PBMC cytokine production: PBMCs were incubated with different stimuli for 24 hours and 7 days and cytokine levels were measured in supernatants- ~3000 targeted plasma proteins were measured with Olink® Explore panel. High dimension immunophenotyping of PBMCs to identify 403 populations. Proviral Cell Associated-HIVDNA (CA-DNA) in circulating CD4 T cells were analyzed, along with intactness ratio using the Rainbow assay.

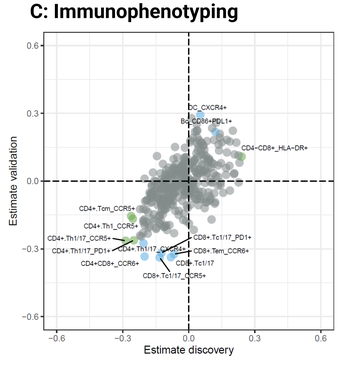
A: GSEA plasma proteins



B: Viral Reservoir / 10^6 CD4 T-cells TND RV

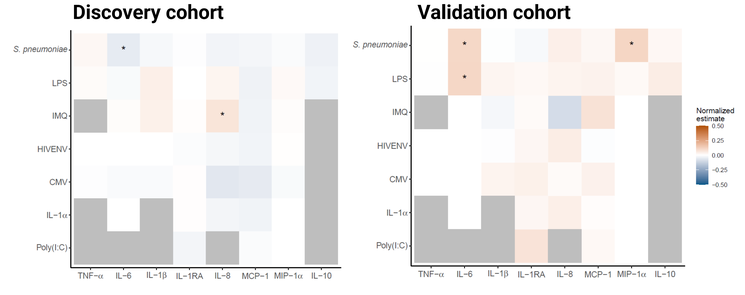


C: Immunophenotyping



Significant in discovery Significant in validation

D: cytokine production 24 hour ex vivo stimulation



Methods

Analysis: P values were FDR corrected in discovery cohort and nominal in validation cohort. Ex-vivo stimulation: rank based regression for linear models, normalized estimates are shown in heatmap. Plasma proteome: Differential expression analysis: NPX values were compared between the groups in a linear model, followed by gene set enrichment analysis (GSEA) using Hallmark library onproteins expressed in the same directionin both cohorts. Immunophenotyping: Linear regression was performed on inverse rank transformed data. Viral reservoir: Uncorrected untransformed data is shown, statistical testing was performed using Mann-Whitney U test. Defective was defined as total copies of proviral DNA per million CD4 T-cell minus the number of intact as determined by the Rainbow assay. Total intact was defined as by the Rainbow assay.

Results

No significant differences between RV and TND in:

- **plasma protein** abundance (GSEA revealed 4 enriched pathways with FDR p<0.05, including epithelial mesenchymal transition which links to impaired mucosal barrier function and HPV malignancies) fig. A.
- **immune cell** populations, their activation markers, fig. C.
- **cytokine production** capacity , fig. D.

Viral reservoir:

Higher total CA-DNA copies per million CD4-T cells median [IQR]:
-discovery RV 875 [387-1600] vs. TND 481 [191-1047], p<0.00 and
-validation RV 873 [331-1908] vs. TND 442 [200-1092], p<0.00.
-intactness ratio was similar, both intact and defective copies were higher in RV. fig. B.

Conclusions

Residual HIV viremia below limit of quantification < 40c/ml, while known to be associated with cardiovascular disease, **does not drive nor is driven by systemic inflammation or immune activation**, rather it was associated with viral reservoir size.

***Future**: Besides recognizing RVs relevance, interventions should target the transcriptionally active (be it intact or defective) viral reservoir, viral products itself or its interactions within the body directly.*

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2000HIV study: Vos et al. Front Immunol 2022; 13:982746
Sponsored by ViiV Healthcare, **www.2000HIV.com**