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MEASURING HIV-1 RESERVOIR DYNAMICS DURING LONG-TERM ANTIRETROVIRAL THERAPY

HIV Dynamics and Replication Program
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BACKGROUND

- Quantifying the HIV-1 reservoir is essential for development and evaluation of HIV-1 cure strategies. The intact proviral DNA assay (IPDA), which distinguishes intact from defective proviruses by targeting the HIV packaging signal and *env* regions, indicates that defective proviruses decay more rapidly than intact proviruses in HIV-infected individuals on ART (Bruner et al.,2018, Peluso et al.,2020). We have shown that simultaneous quantification of conserved regions of HIV-1, LTR and *gag*, reveals the dynamics of HIV proviral landscape (Anderson *et al*.,2020). To investigate the dynamics of total, intact, and defective proviruses during ART, we performed IPDA and a multiplexed assay that simultaneously quantifies HIV-1 LTR and *gag.*

METHODS

- Peripheral blood mononuclear cells (PBMC) were obtained from participants at pretherapy (n=16), after short-(2-8 years on ART; n=12), intermediate-(8-16y; n=13), and long-(16-21y; n=12) term ART.
- Genomic DNA was extracted from 1e6 PBMC using an in-house guanidine-based extraction method to minimize DNA shearing. For IPDA, gDNA was digested with restriction enzyme to improve droplet formation and increase the input DNA/reaction while preserving intact HIV sequences (Levy et *al.,*2021).
- Levels of HIV-1 DNA LTR and *gag* (Anderson et *al*.,2020) and intact, 3' deleted/hypermutation, and 5'-deleted proviruses (IPDA) (Bruner et *al*.,2018) were measured by droplet digital PCR systems.

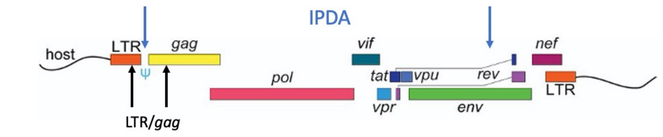


Figure 1: Primers used in LTR/gag assay (black) and IPDA (blue)

- The lower level of quantification (LLQ) of the two techniques was 5 copies/1e6 PBMC. Samples with detectable HIV-1 DNA under LLQ were assigned to 5 copies/1e6 PBMC. Samples with undetectable HIV-DNA were excluded from correlation analyses.
- HIV-1 DNA levels measured by the two assays were analyzed by Pearson correlation and fold-changes of HIV-1 DNA levels from pretherapy to multiple timepoints on ART were evaluated by Spearman correlation.

RESULTS

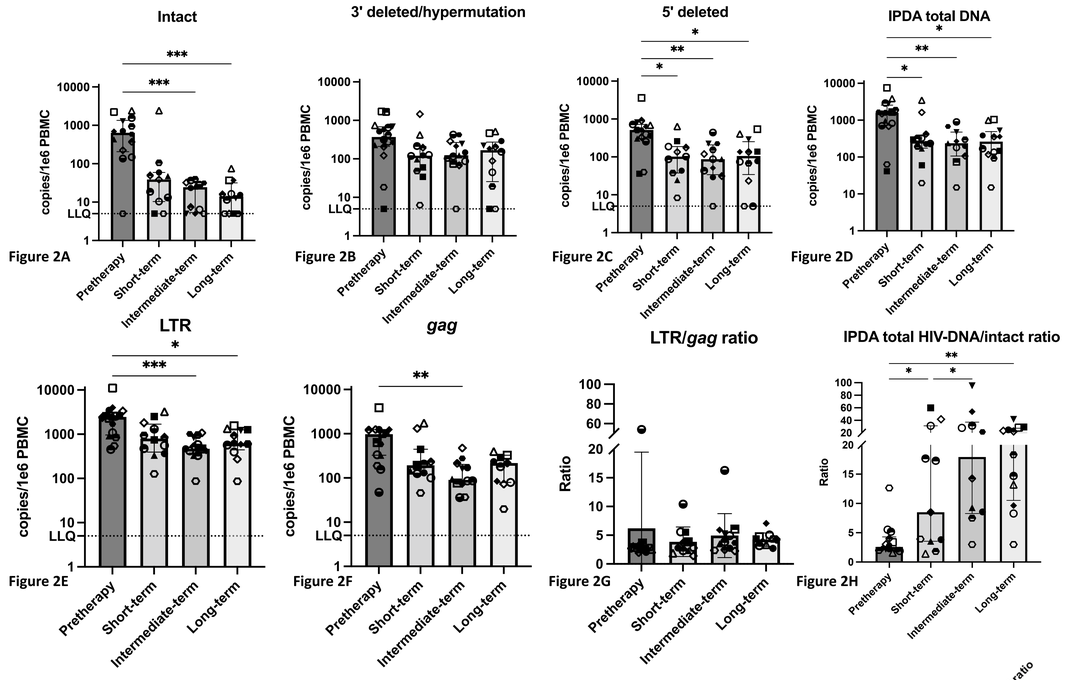
1) Patient characteristics

Characteristic	Median (IQR)
Age (n=16)	65 (58-69)
Sex, male	14
Log10 (Pretherapy viral load, copies/mL)	4.80 (4.45-5.07)
Nadir CD4 (cells/mm3)	170.5 (146.8-305.5)
Pretherapy CD4 count (cells/mm3) (n=16)	295.5 (169.5-418.8)
Short-term ART CD4 count (cells/mm3) (2-8 years, n=12)	552 (343-597)
Intermediate-term ART CD4 count (8-16 years, n=13)	697 (385-862)
Long-term ART CD4 count (16-21 years, n=12)	527 (410-600)
Suppression time on ART (to the latest sample)	18.0 (14.8-20.0)
Time from pretherapy sample to ART initiation (days)	5 (1-21)

Levels of HIV-1 DNA by LTR/*gag* assay are highly correlated with levels of intact and defective proviruses by the intact proviral DNA assay (IPDA), and still quantifiable during long-term ART when intact proviruses were below the limit of quantification. The ratios of LTR/*gag* and total HIV-DNA/intact by IPDA are highly correlated, suggesting both assays can capture the dynamics of the HIV-1 reservoir. LTR/*gag* assay complements IPDA to capture the dynamics of HIV-1 decay and is useful where intact proviruses are not detected by IPDA.

RESULTS

2) Significant reduction of HIV-DNA LTR, *gag* and IPDA intact and 5' defective proviral levels



3) LTR, *gag* and intact, defective proviral levels were highly correlated

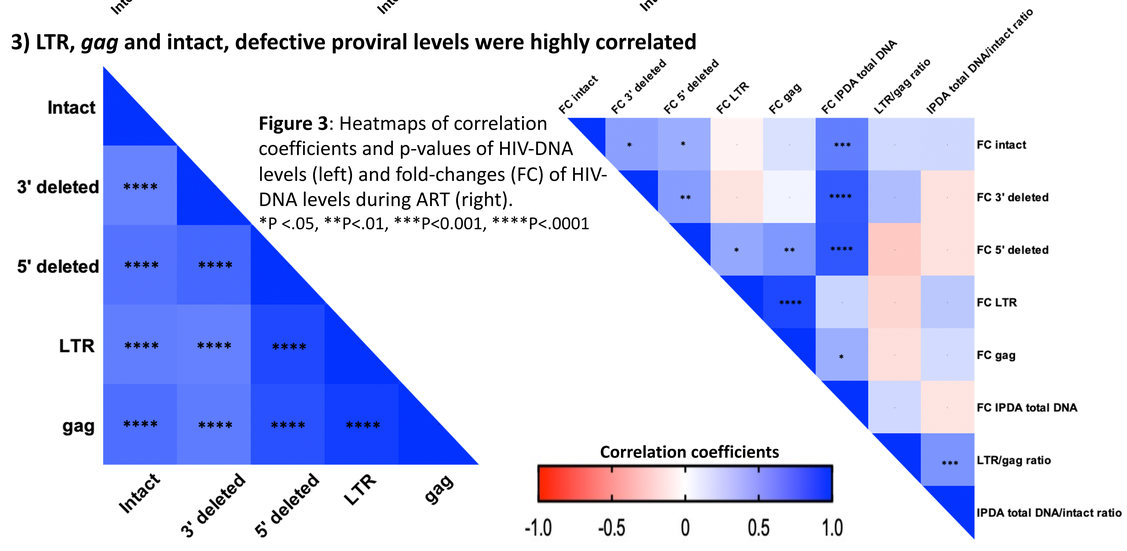


Figure 3: Heatmaps of correlation coefficients and p-values of HIV-DNA levels (left) and fold-changes (FC) of HIV-DNA levels during ART (right).
*P <.05, **P<.01, ***P<0.001, ****P<.0001

RESULTS

- Intact proviruses were not detectable in one participant (due to amplification failure of the HIV packaging signal) and were under the LLQ for 4 other participants at long-term ART and at all timepoints for one participant. *Gag* containing proviruses were not detected in one participant.

- From pretherapy to short-, intermediate-, and long-term ART, levels of intact proviruses declined 9.1, 22.9, and 45.7-fold, respectively (**Fig 2A**) while levels of 3' deleted/hypermutations were more stable, declining from 2.6-2.9-fold (**Fig 2B**) and 5'-deleted from 3.6-4.9-fold during ART (**Fig 2C**).

- LTR levels declined 1.9, 3.2, and 2.6-fold respectively (**Fig 2E**), while *gag* levels underwent greater decay (2.3, 4.5, and 5.3-fold respectively) (**Fig 2F**).

- Only the fold-change from pretherapy to ART of 5'-deleted proviral levels was positively correlated with the changes in LTR and *gag* levels.

- The ratio of IPDA total DNA/intact was significantly correlated with LTR/*gag* (p=0.0003, r =0.53) but higher than LTR/*gag* ratio, indicating substantial levels of *gag* persist during long-term ART.

CONCLUSIONS

- We observed a strong correlation of the HIV-1 DNA levels measured by the two assays at all timepoints, but the fold-changes of HIV-1 LTR and *gag* levels from pretherapy to ART were correlated only with the change of 5'-deleted proviral level by IPDA.

- LTR/*gag* assay can complement IPDA regarding the issues of primer-binding mismatch and very low frequency of intact proviruses after long-term ART, but IPDA is still a preferred choice to characterize the change of HIV-1 reservoir in interventions where participants have a substantial level of intact proviruses.

ADDITIONAL KEY INFORMATION

- The results of our study have been updated with more samples included.

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