Selective decay of different HIV transcripts after ART initiation in chronic infection

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Background

- In the first 6 months after ART initiation in acute infection, completed and intact HIV RNA decay faster than incomplete or defective HIV RNA (1).
- To investigate the impact of starting ART in chronic infection, we measured different HIV DNA and RNA levels in longitudinal samples obtained during untreated chronic infection (T1) and up to 4 times after ART suppression (T2-T5).

Hypothesis: Completed and intact HIV RNA will decrease faster than incomplete or defective transcripts but will reach a plateau.

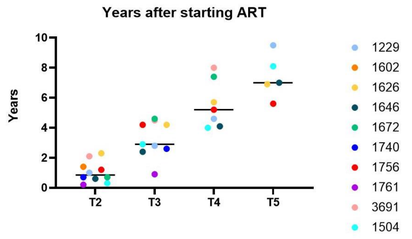


Figure 1. Sampling intervals between timepoints. The y-axis shows the time in years after the start of ART, and the x-axis indicates the timepoint (T2, T3, T4, or T5).

Methods

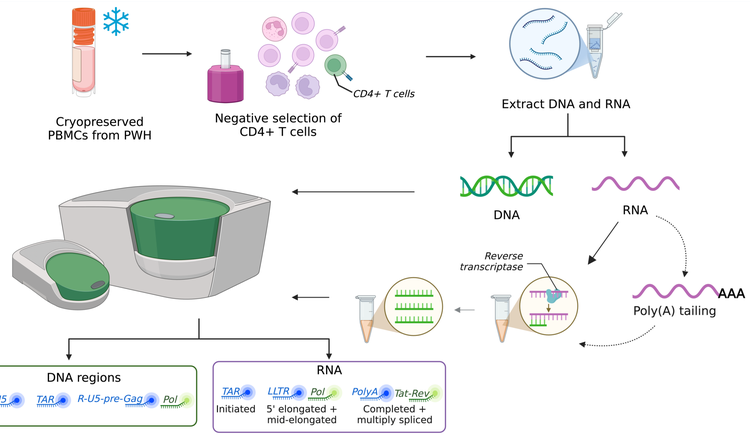


Figure 2. Quantification of HIV-1 DNA and RNA levels. Longitudinal samples were obtained from 10 PWH during untreated chronic infection (T1) and up to four timepoints on suppressive ART (T2-T5). Eight PWH had samples available from T4 and five had samples from T5.

Results

In untreated chronic infection (T1), levels of initiated HIV RNA were greater than 5' elongated HIV RNA, which were greater than completed HIV RNA, but no difference was observed between completed and multiply spliced HIV RNA.

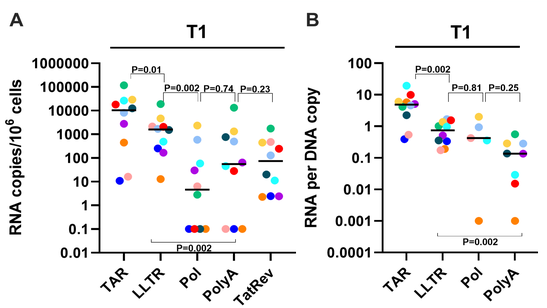


Figure 3. Transcriptional blocks before initiation of ART. A) Cell-associated initiated (TAR), 5' elongated (LLTR), mid-transcribed/unspliced (Pol), completed (PolyA), and multiply spliced (TatRev) HIV transcripts were quantified by RT-ddPCR during untreated chronic infection. **B)** Levels of each HIV RNA were normalized to the corresponding HIV DNA regions. *Horizontal lines indicate medians. Colors indicate individual study participants. P-values (two-tailed) were calculated using the Wilcoxon signed rank test.*

- After **ART** initiation during **chronic infection**, some HIV proviruses (5'LTR) and HIV transcripts (multiply spliced) showed a more sustained decay.
- With more **time on ART**, we observed no further **changes in HIV DNA or RNA**.
- These findings suggest the need for new therapies that can target the remaining proviruses/transcripts and/or **modulate expression** of more immunogenic HIV RNA and protein.

After ART initiation, TAR and R-U5-pre-Gag HIV DNA levels decreased from T1 to T2, but only R-U5-pre-Gag levels continued to decrease between T2-T3 and T3-T4 (not shown). After T4, we observed no further decreases in HIV DNA.

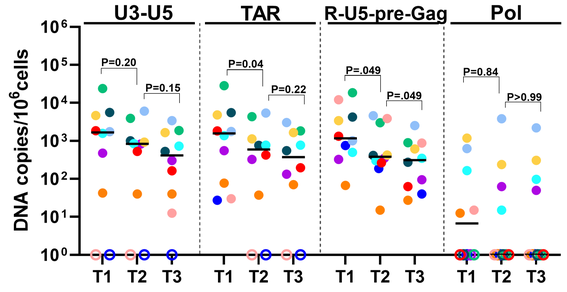


Figure 4. HIV DNA levels before and after initiation of ART during chronic HIV infection. Levels of U3-U5, TAR, R-U5-pre-Gag, and Pol HIV DNA were measured by ddPCR before (T1) and after initiation of suppressive ART (T2 and T3). *Undetected transcripts were assigned a value of 1. Open circles indicated undetectable values.*

From T1 to T2, we observed decreases in all HIV RNA levels except initiated, and in completed HIV RNA per provirus. From T2 to T3, only multiply spliced HIV RNA continued to decrease. After T3, no changes were observed.

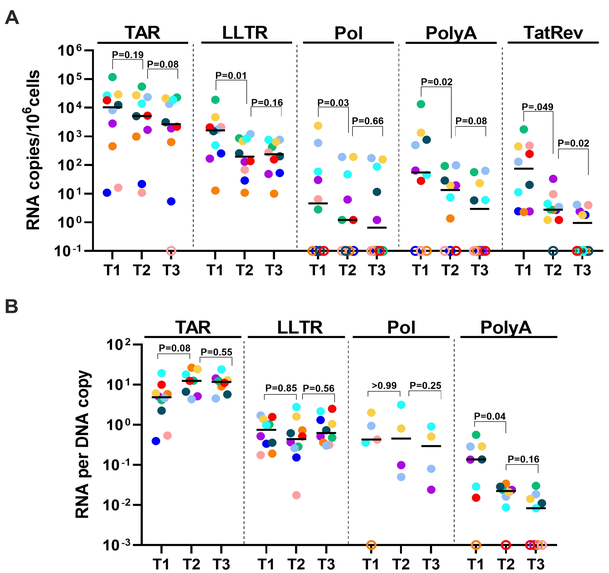


Figure 5. HIV RNA levels before and after ART initiation. A) Levels of initiated (TAR), 5' elongated (LLTR), mid-transcribed/unspliced (Pol), completed (PolyA), and multiply spliced (TatRev) HIV RNA per µg (~10^6 cells) were measured before (T1) and after initiation of ART (T2-T3). **B)** HIV transcript levels were normalized to the corresponding HIV DNA regions.

Results (continued)

From T1 to T2, we observed decreases in intact and defective HIV DNA, and in intact and 3' defective HIV RNA. After T3, no further changes were observed.

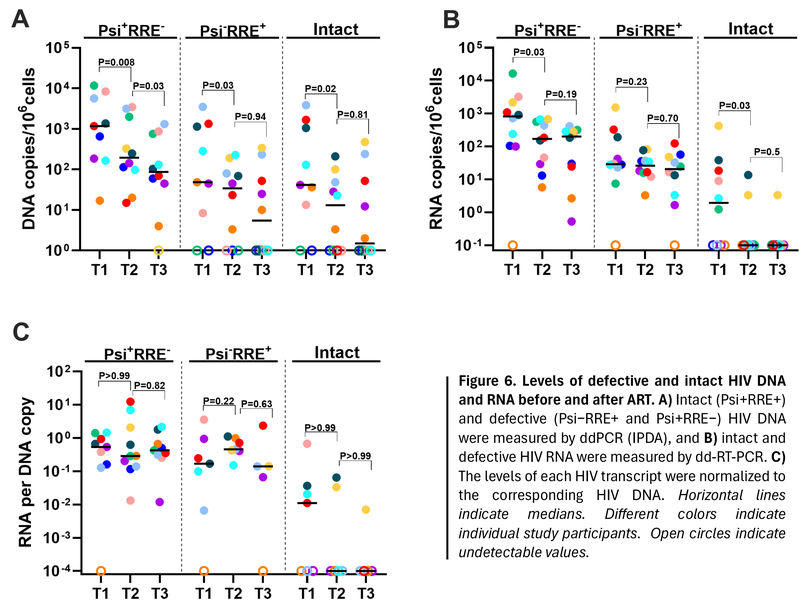


Figure 6. Levels of defective and intact HIV DNA and RNA before and after ART. A) Intact (Psi+RRE+) and defective (Psi−RRE+ and Psi+RRE−) HIV DNA were measured by ddPCR (IPDA), and **B)** intact and defective HIV RNA were measured by dd-RT-PCR. **C)** The levels of each HIV transcript were normalized to the corresponding HIV DNA. *Horizontal lines indicate medians. Different colors indicate individual study participants. Open circles indicate undetectable values.*

Conclusions

- During untreated chronic infection, differences between the levels of initiated, 5' elongated, and completed HIV RNA/DNA suggest blocks to HIV transcription (elongation, completion) and/or immune selection against cells making elongated and completed transcripts (Fig. 3).
- After ART, we found evidence for more prolonged decay of certain proviruses (R-U5-pre-Gag HIV DNA declined through T4; Fig. 4) and HIV transcripts (multiply spliced HIV RNA declined through T3; Fig. 5), indicating that their clearance may be dictated by different or more sustained immune mechanisms.
- However, levels of all proviruses and HIV transcripts appeared to reach an equilibrium after a median of 5 and 3 years (respectively) on ART.

Acknowledgments

References

(1) Janssens et al. Journal of Virology. 2025 Feb 5. 0:e01431-24

Acknowledgements

The authors acknowledge all the study participants who contributed to this work, as well as the clinical research staff of the UCSF Treat Acute HIV and SCOPE (Observational Study of the Consequences of the Protease Inhibitor Era) cohorts. This work was supported by the National Institute of Allergy and Infectious Diseases (P01AI169606, R01AI132128) and the National Institute of Diabetes and Digestive and Kidney Diseases (R01DK120387), and investigator-initiated research grants from ViiV Healthcare (A126326) and Gilead Sciences (IN-US-236-1354).

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