Dynamics of Different Proviruses and HIV Transcripts After Acute ART Treatment

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BACKGROUND

- Different subsets of HIV-transcribing cells may differ in their persistence on ART, contribution to immune activation, and rebound potential
- We investigated the dynamics of intact proviruses and different HIV transcripts after initiation of ART during acute infection
- We hypothesized that completed, multiply spliced, and intact HIV RNA would decay at a faster rate than incomplete or defective HIV RNA

METHODS

- CD4+T cells were isolated from blood before ART **(T1)**; and after 6 mo **(T2)** on suppressive ART from 16 PWH (Treat Acute Cohort)
- **Different HIV DNA regions** (U3-U5, TAR, R-U5/Gag and Pol) as well as **intact** (Psi+RRE+) and **defective** (Psi−RRE+; Psi+RRE-) **HIV DNA** were measured by ddPCR
- **Different HIV RNA regions** (total initiated [TAR], 5'elongated [R-U5/Gag], mid-transcribed [Pol, unspliced], completed [U3-polyadenylated], multiply spliced [TatRev]) as well as **intact** (Psi+RRE+) and **defective** (Psi−RRE+; Psi+RRE-) **HIV RNA** were measured by dd-RT-PCR

ACKNOWLEDGMENTS

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ART appears to select for cells with lower levels of completed and intact HIV transcripts

From T1 to T2, we observed progressive reductions in initiated, 5'elongated, mid-transcribed, completed, and multiply spliced HIV transcripts

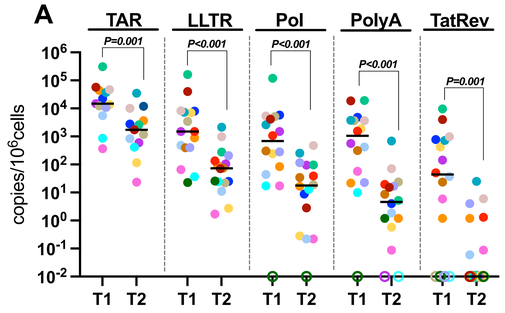
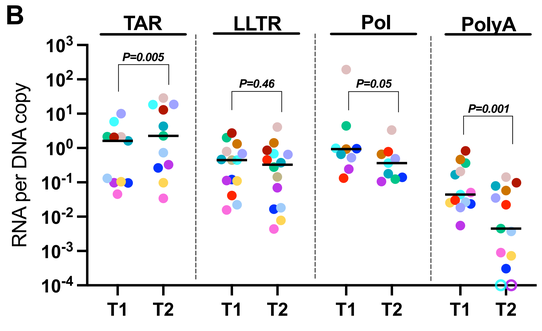


Figure 1A. Cell-associated HIV transcripts before ART (T1) and after 6 mo on ART (T2). Initiated (TAR), 5' elongated (LLTR), Pol (unspliced, mid-transcribed), completed (PolyA), and multiply spliced (TatRev) HIV transcripts were quantified by dd-RT-PCR. **Median fold decline from T1 to T2**: 10 (TAR); 13 (LLTR); 20 (Pol); 184 (PolyA); and 162 (TatRev). *Bars indicate medians. Open symbols denote undetectable levels and were assigned a value of 0.01.*

After normalizing to the corresponding HIV DNA, only mid-transcribed (Pol) and completed (PolyA) RNA transcripts decreased significantly on ART

Figure 1B. The level of HIV transcripts per provirus before ART (T1) and after 6 mo on ART (T2). We normalized each RNA transcript to the corresponding HIV DNA region to correct for infection frequency and proviral mutations. Transcripts per provirus of **i)** initiated (TAR); **ii)** 5' elongated (LLTR); **iii)** unspliced (Pol); and **iv)** completed (PolyA) HIV RNA. *Bars indicate medians. Open symbols denote undetectable levels and were assigned a value of 0.0001.*



From T1 to T2, there was a significant reduction in HIV 5' elongation, mid-transcription, and completion, suggesting selective decay or immune selection

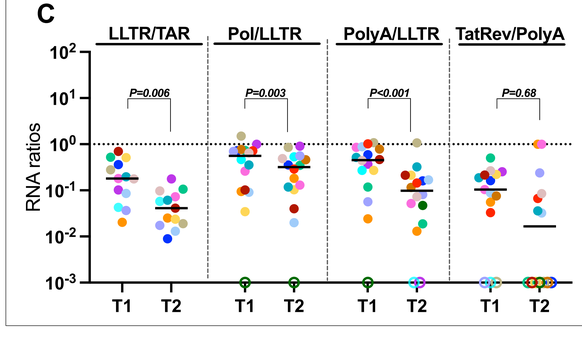


Figure 1C. Ratios of one HIV RNA to another to measure progression through HIV transcription at T1 and T2. Ratios are independent of HIV infection frequency. The proportion of **i)** all HIV transcripts that are elongated [LLTR/TAR]; **ii)** elongated HIV transcripts that are mid-transcribed [Pol/LLTR] or **iii)** completed [PolyA/LLTR]; and **iv)** completed transcripts that are multiply spliced [TatRev/PolyA]. *Bars indicate medians. Open symbols denote undetectable levels and were assigned a value of 0.001.*

The median fold decline in intact HIV RNA was ~10x greater than either type of defective RNA, even after correcting for HIV DNA

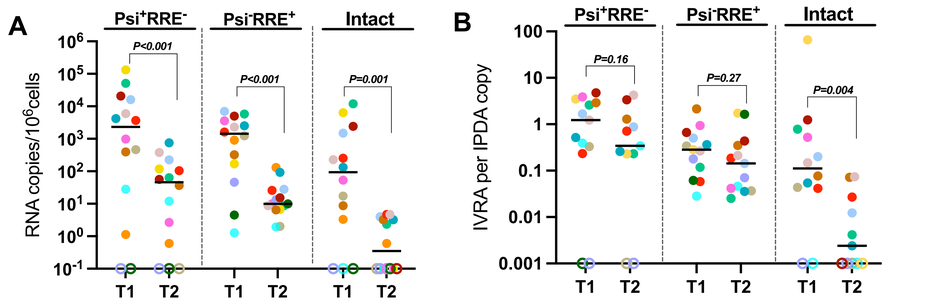


Figure 2A-B. Intact vs. defective HIV RNA (2A) and ratio of HIV RNA to the corresponding HIV DNA (2B). 3'defective (Psi+RRE-), 5'defective (Psi-RRE+), and intact (Psi+RRE+) HIV transcripts were measured by dd-RT-PCR (IVRA). **Median fold decline from T1 to T2:** HIV RNA: 47 (3'defective), 56 (5'defective), and 372 (intact; Figure 2A); RNA/DNA: 2.0 (3'defective), 2.5 (5'defective), and 22.6 (intact; Figure 2B). *Bars indicate medians. Open symbols denote undetectable levels and were assigned a value of 0.1 (2A) and 0.001 (2B).*

Only 1% of all HIV RNA was intact at T1 (median ~0.01), while intact HIV DNA was more common (~0.1). The changes from T1 to T2 seemed different for RNA and DNA

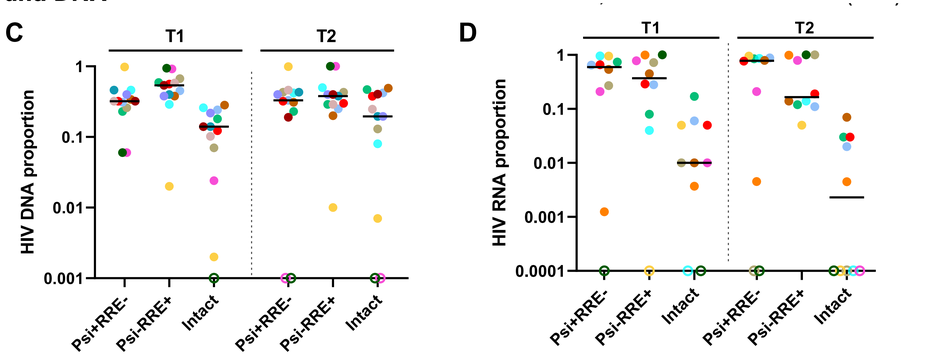


Figure 2C-D. The proportion of 3'defective (Psi+RRE-), 5'defective (Psi-RRE+), and intact (Psi+RRE+) HIV DNA (2C) and HIV RNA (2D). *Bars indicate medians. Open symbols denote undetectable levels and were assigned a value of 0.001 (2C) and 0.0001 (2B).*

CONCLUSIONS

- **After 6 months of ART, mid-transcribed and completed HIV RNA decayed faster than initiated or 5'elongated HIV RNA (Fig. 1A-B), while intact HIV transcripts tended to decay faster than defective ones (Fig. 2A-B).**
- **Since ART does not target HIV transcription, these findings suggest differences in the lifespan and/or immune clearance of cells making different HIV transcripts.**