HIV-2 Transcription in Blood CD4+ T-Cells is Inhibited by Blocks to Elongation and Completion

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BACKGROUND:

- Compared to HIV-1 infection, HIV-2 is characterized by a significantly lower incidence and a slower progression to AIDS.[1-3]
- Like HIV-1, HIV-2 establishes a latent infection in CD4+ T-cells that prevents cure. The mechanisms of HIV-2 latency are unknown.
- To investigate whether the mechanisms that inhibit HIV-2 expression are similar to those observed for HIV-1, we quantified different HIV-2 RNA regions indicating progression through various stages of viral transcription and splicing.[4,5]

Hypothesis: HIV-2 expression is inhibited by blocks to transcriptional elongation, completion, and splicing.

METHODS:

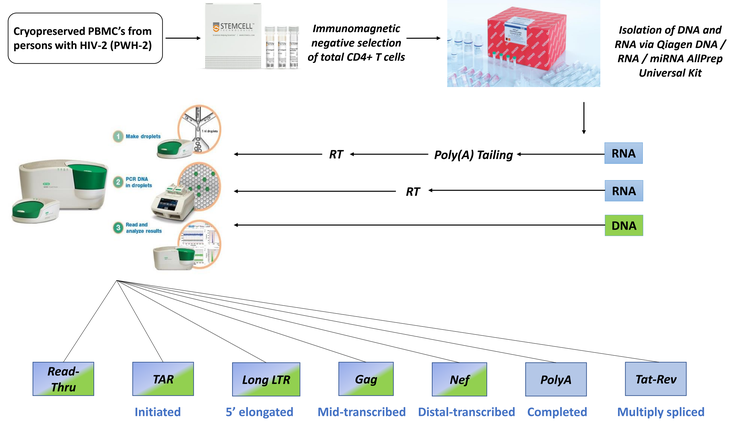


Figure 1. Schematic of the absolute quantification of HIV-2 DNA and RNA levels. Total DNA and RNA were extracted from CD4+ T cells isolated by negative selection from $3x10^7$ PBMC obtained from 6 participants enrolled across two HIV-2 cohorts (5 HEAL, 1 MIRIAM). HIV-2 DNA and RNA levels were quantified by droplet digital PCR (ddPCR). *RT = reverse transcription, "Read-Thru" = U3-U5, "TAR" = initiated, "Long LTR" = R-U5-pre-Gag (5' elongated), "PolyA" = U3-polyA (completed), "Tat-Rev" = multiply spliced Tat-Rev. Adapted from Yukl et al. 2018.*[5]

References:
1) Drylewicz et al. AIDS. 2008 Feb 9; 22(4): 457-468
2) Jaffar et al. JAIDS Hum Retrovirol. 1997 Dec 15; 16(5): 327-332
3) Marlink et al. Science. 1994 Sep 9; 265(5178): 1587-1590
4) Lu et al. PLoS One. 2022 Apr 27;17(4):e0267402.
5) Yukl et al. Sci Transl Med. 2018 Feb 28; 430(10): eaap9927

Acknowledgments:
We thank the participants of the HEAL and MIRIAM cohorts. This research was funded by the NIAID (R01AI155131, R01AI132128, P01AI169606) and NIDDK (R01DK120387).

Latent HIV-2 infection may be driven by cellular factors underlying the blocks to transcriptional ***elongation*** and ***completion.***

RESULTS:

We found a gradient in levels of different HIV-2 RNA regions: initiated (TAR) > 5' elongated (Long LTR) > mid (Gag), distal (Nef) or completed (PolyA). This gradient tends to persist after normalization to HIV-2 DNA and could indicate blocks to transcription, especially 5' elongation.

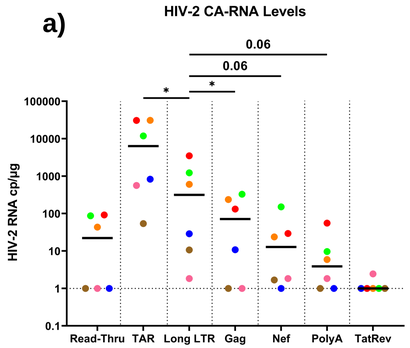
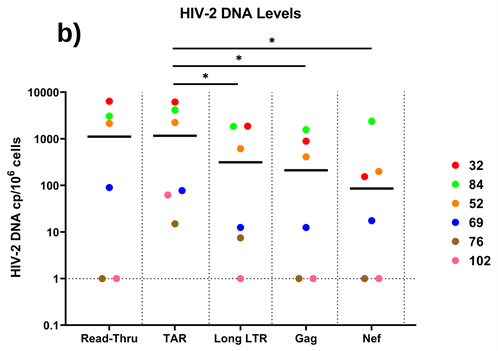


Figure 2a: HIV-2 cell-associated RNA levels in PWH-2. U3-U5 (Read-Thru), initiated (TAR), 5' elongated (Long LTR), gag, nef, completed (PolyA), and multiply spliced (Tat-Rev) HIV-2 transcripts were quantified by ddPCR in PWH-2. *Black bars indicate medians. * = p < 0.05. Transcripts that were undetected were assigned a value of 1.*

Figure 2b: HIV-2 DNA levels in PWH-2. Read-Through, TAR, Long LTR, gag, and nef HIV-2 DNAs were quantified in PWH-2. *Black bars indicate medians.* * = p < 0.05. Undetected DNAs were assigned a value of 1.*



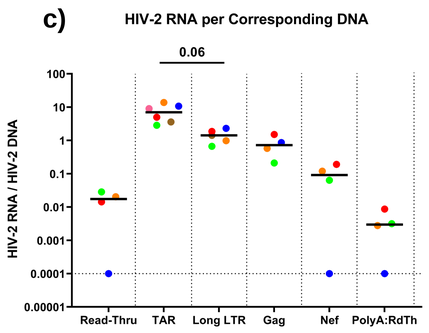


Figure 2c: HIV-2 transcripts per provirus. To determine the average level of each HIV-2 transcript per provirus, and correct for proviral mutations, we calculated the ratio of each HIV-2 RNA to its corresponding HIV-2 DNA region. *Black bars indicate medians; ratios with a value of 0 were assigned a value of 0.0001.*

RESULTS (cont'd):

HIV-2 transcript ratios suggest blocks to mid-transcription and possibly distal transcription or completion.

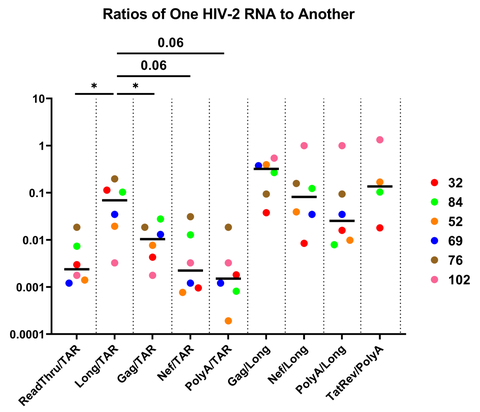


Figure 3: Progression through stages of HIV-2 transcription. Ratios of one HIV-2 RNA to another were used to measure the proportion of initiated transcripts (ratios to TAR) or 5' elongated transcripts (ratios to Long) that proceed past the 5'LTR (Long) to the mid genome (Gag), 3'end (Nef), or polyA tail (polyA). We also determined the proportion of completed transcripts that undergo multiple splicing (TatRev/PolyA). *Black bars indicate medians, * = p < 0.05.*

Our data suggest that few initiated HIV-2 transcripts become elongated, and few of those become completed.

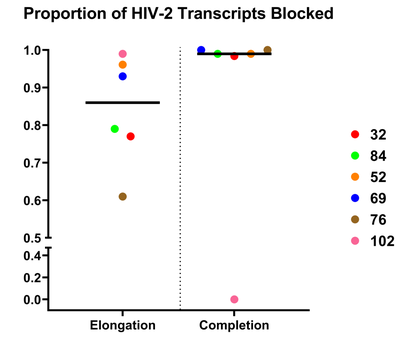


Figure 4: Proportion of HIV-2 transcripts blocked at the stages of elongation and completion. We calculated the proportion of transcripts blocked at elongation (1-[2*Long/TAR]) and completion (1-[PolyA/Long]). *Black bars indicate medians.*

CONCLUSIONS:

- **Differences in HIV-2 transcript levels and ratios suggest blocks to HIV-2 transcriptional elongation, mid-transcription, and possibly completion, as observed with HIV-1.**
- **Mechanisms underlying these blocks likely contribute to latent infection in both viruses.**
- **Future studies should investigate the cellular factors contributing to these blocks.**