INTACT HIV TRANSCRIPTS ARE RARE IN ART-SUPPRESSED INDIVIDUALS

00386

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BACKGROUND

- Genetically intact proviruses are the source of viral rebound after ATI.
- However, it is unclear to what degree intact viral RNA is expressed in ART-suppressed people with HIV (PWH).

METHODS

- We developed and validated a novel "intact viral RNA assay" (IVRA) that applies the primers and probes from the IPDA[1] (for the commonly mutated Psi and RRE regions).
- We measured single HIV RNA molecules in droplets (by dd-RT-PCR) to quantify HIV RNA molecules that are likely "intact" (Psi^+ RRE^+) vs 3' defective (Psi^+ RRE^-) or 5' defective (Psi^- RRE^+).
- The IPDA and IVRA were applied to DNA and RNA from 4-11 replicate aliquots of 300,000 to 1,000,000 $CD4^+$ T cells isolated from the blood of 10 ART-suppressed PWH.

RESULTS

3' defective and 5' defective proviruses were detected at similar levels and more frequently than intact proviruses.

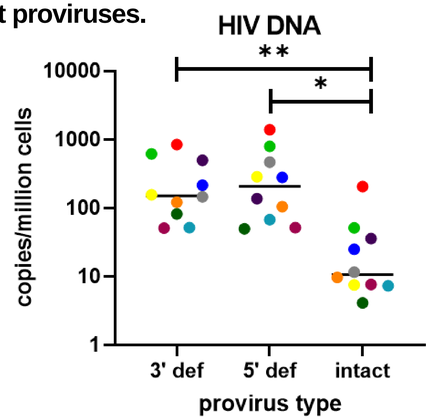


Figure 1: HIV DNA. Each color represents one individual and is consistent throughout all figures. Horizontal line represents the median. 3' def = 3' defective, 5' def = 5' defective. *=P<0.05 and **=P<0.005 (Wilcoxon signed-rank test)

- We developed a RT-ddPCR assay to detect intact HIV transcripts (Psi^+ RRE^+).
- Most transcripts from ART-suppressed individuals only contained the Psi region, likely due to transcriptional blocks.

RESULTS

3' defective HIV transcripts were in excess over 5' defective or intact transcripts.

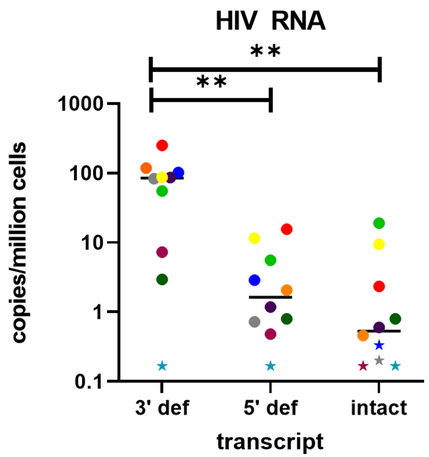


Figure 2: HIV RNA by IVRA. Each color represents one individual. Circles represent measured copies; stars represent imputed values based on 1 copy divided by the number of cells tested. Horizontal line represents the median. **=P<0.005 (Wilcoxon signed-rank test)

Almost all HIV transcripts were 3' defective.

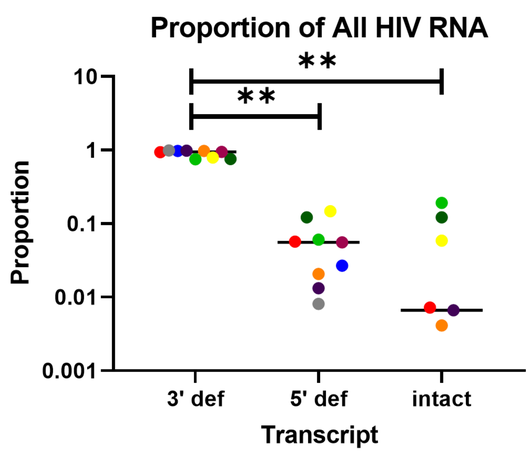


Figure 3: Transcript Proportion. Each type of HIV transcript was expressed as a proportion of the total. Each color represents one individual. **=P<0.005 (Wilcoxon signed-rank test)

RESULTS

Most proviruses do not produce intact HIV transcripts in ART-suppressed individuals.

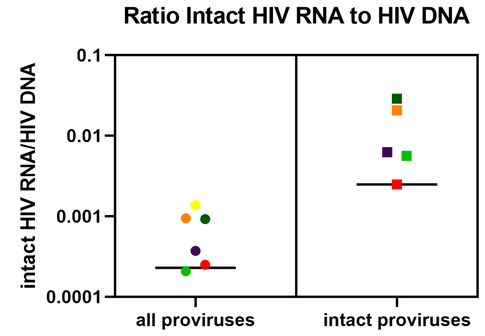


Figure 4. Average level of intact HIV transcripts per provirus. Each color represents one individual. Horizontal line represents the median.

CONCLUSIONS

- The vast excess of 3' defective over 5' defective or intact HIV RNA, which was not observed in the HIV DNA, likely represents a block to HIV transcriptional completion[2].
- A very small proportion of all HIV RNA is intact, and a very low proportion of proviruses or even intact proviruses transcribe intact HIV RNA.
- This intact HIV RNA may contribute disproportionately to immune activation on ART and may be a major source of viral rebound after ART interruption.

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REFERENCES

1- Bruner, K. M. et al. A quantitative approach for measuring the reservoir of latent HIV-1 proviruses. *Nature* 566, 120–125 (2019).
2- Yukl SA, Kaiser P, Kim P, et al. HIV latency in isolated patient $CD4^+$ T cells may be due to blocks in HIV transcriptional elongation, completion, and splicing. *Sci Transl Med 430,* (2018).

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