POST-TREAMENT CONTROLLERS LIMIT COMPLETED AND SPLICED HIV TRANSCRIPTS AFTER ATI

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00362

BACKGROUND:

- Post-treatment controllers (PTC) are a rare subset of HIV-infected individuals who can limit increases in plasma viral load after cessation of ART (1).
- The mechanisms by which PTC limit viral replication are not completely understood.
- To investigate whether specific blocks to HIV transcription contribute to post-treatment control, we measured different HIV RNA levels in PTC and noncontrollers (NC) from longitudinal samples before and after ART interruption (ATI).

HYPOTHESIS: **POST-TREATMENT CONTROLLERS WILL SHOW GREATER BLOCKS TO TRANSCRIPTIONAL COMPLETION AND MULTIPLE SPLICING.**

METHODS:

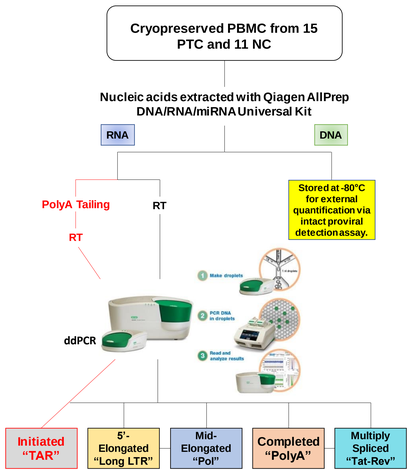

Figure 1. Quantification of HIV-1 RNA levels in PTC and NC. Longitudinal samples were obtained from 15 PTC and 11 NC while on suppressive ART (Pre ATI) and at early and late times after ART interruption (Early ATI and Late ATI). PTC were defined as having viral load≤400 RNA cp/mL at 2/3 of times for 24 weeks after ATI (2). Ten of 15 PTC rebounded and had a sample collected at the virologic failure (VF) time point. *RT = reverse transcription, ddPCR = droplet digital polymerase chain reaction. Graphic adapted from Yukl et al. 2018* (3).

RESULTS:

Viral loads (VL) increase after ATI in both PTC and NC. VL are higher in NC than PTC at both times after ATI.

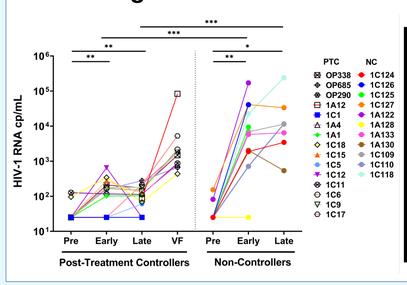

Figure 2. Plasma viral loads (VL) in post-treatment controllers and noncontrollers. VL were measured by clinical assays at Pre ATI (Pre), Early ATI (Early), Late ATI (Late), and (for some PTC) virologic failure (VF) time points. *Symbols denote individuals. p < 0.05, ** = p < 0.01, *** = p < 0.001*

Post-treatment control may be due to cell extrinsic and/or intrinsic mechanisms that limit HIV *transcriptional completion* and *multiple splicing*.

RESULTS (cont'd):

Multiply spliced HIV transcripts increase early after ATI in NC but not PTC. Completed HIV transcripts increase early after ATI in PTC and NC, but may decrease by Late ATI in PTC only.

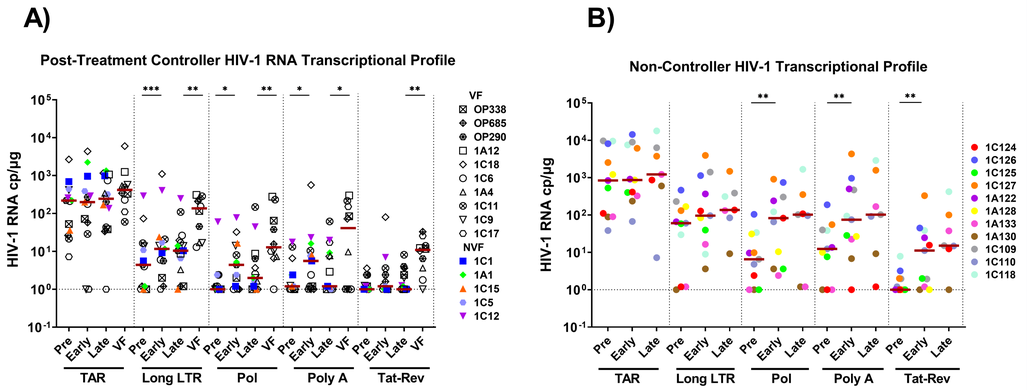

Figure 3. Longitudinal changes in HIV RNA levels in PTC and NC. Cell-associated initiated (TAR), 5'-elongated (Long LTR), mid-elongated (Pol), completed (PolyA), and multiply spliced (Tat-Rev) HIV-1 RNA transcripts were quantified in PTC (A) and NC (B) by RT-ddPCR. *Maroon bars indicate medians; * = p < 0.05, ** = p < 0.01; undetected transcripts were assigned a value of 1.*

At all time points, PTC have lower levels of initiated HIV-1 RNA / 10^6 cells (below), but not per provirus (not shown). With time after ATI, NC show larger differences in levels of completed and multiply spliced HIV RNA / 10^6 cells.

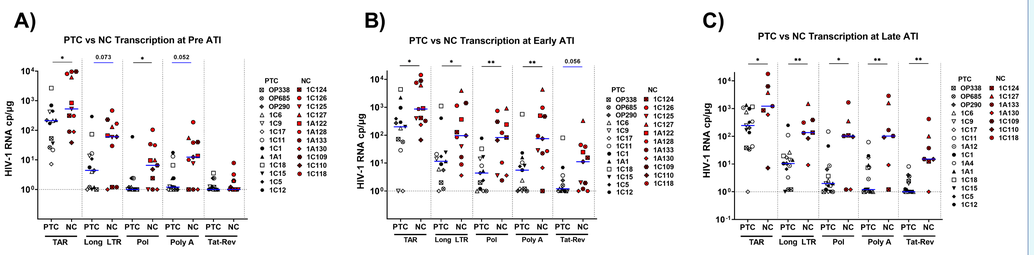

Figure 4. HIV RNA levels compared between PTC and NC. Levels of each HIV transcript per µg (~10^6 cells) were measured in PTC (black/open symbols) and NC (red) at Pre-ATI (A), Early-ATI (B), and Late-ATI (C) time points. *Blue bars within data represent medians, * = p < 0.05; undetected transcripts were assigned a value of 1.*

RESULTS (cont'd):

In NC but not PTC, HIV transcriptional completion and splicing increase after ATI. PTC show a decrease in completion from Early to Late ATI, and have less completion and splicing than NC at Late-ATI.

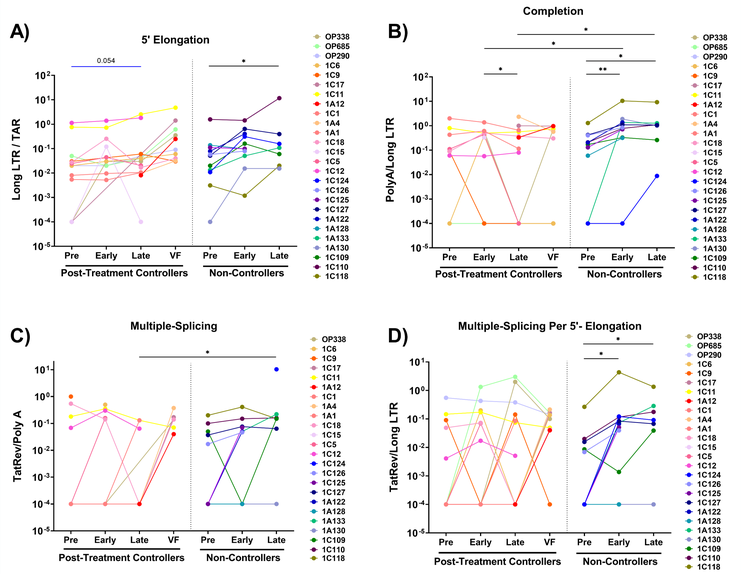

Figure 5. Progression through stages of HIV transcription. Extent of 5'-elongation (A), completion (B), multiple-splicing (C), and multiple splicing per 5'-elongation (D) were measured using the ratio of one HIV transcript to another (Y-axis titles [cp/µg / cp/µg]). * = *p < 0.05, ** = p < 0.01; ratios with a value of 0 were assigned a value of 0.0001*

CONCLUSIONS:

- In NC, viral rebound during ATI is not due to increased HIV transcription **initiation** (Fig. 3), but due to increased **completion and splicing** (Fig. 3,5).
- Unlike NC, PTC do not show increases in **multiply spliced HIV RNA** or **multiple splicing** in Early or Late ATI (Fig. 3-5).
- In PTC, **completed transcripts** tend to decline (Fig. 3) and **completion decreases** (Fig. 5) from Early to Late ATI, suggesting an **immune response** targeting cells with **completed transcripts.**

References:
1. Hocqueloux et al. AIDS. 2010 Jun 19; 24(10): 1598-601
2. Namazi et al. J Infect Dis. 2018 Dec 15; 218(12): 1954–1963.
3. Yukl et al. Sci Transl Med. 2018 Feb 28; 430(10): eaap9927

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