HIV Dynamics and Replication Program
NCI-Frederick

DONOR-SPECIFIC HIV-1 PROVIRAL EXPRESSION IN INFECTED T-CELL CLONES

NATIONAL CANCER INSTITUTE

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BACKGROUND

- Levels of HIV unspliced RNA (usRNA) in infected cells are typically low
- Infected T-cell clones persist on ART and are able to transcribe viral RNA
- Some transcriptionally active infected T-cell clones are present prior to ART cessation and contribute to the viral rebound population

Although some infected T-cell clones include cells that transcribe viral RNA, the fraction of cells within clones that have proviruses that are transcriptionally-active is under studied

METHODS

- Participants were on ART and enrolled in study cohorts at University of Pittsburgh, ACTG A5341, or SCOPE at UCSF and provided written informed consent (**Table 1**)
- Estimated the number of infected cells by qPCR (integrase DNA copies)
- Estimated the number of infected cells with HIV usRNA by endpoint-dilution PCR and sequencing of *gag-pol* RNA
- Calculated the fraction of infected cells (and within clones) with HIV usRNA

Table 1. Study cohorts and participant characteristics.

Cohort	Sex M/F (total)	Median CD4 count (cells/mm^3)	HIV plasma viremia (copies/mL)	Years on ART (Median [IQR])
Univ. Pittsburgh	4/2 (6)	563	<50	8.2 [2.8-13.3]
ACTG A5341	5/1 (6)	800	<50	9.0 [7.3-17.8]
SCOPE	3/1 (4)	805	<40	6.8 [4.6-11.6]
Across cohorts	**12/4 (16)**	**662**	**<50**	**8.2 [5.7-12.7]**

RESULTS

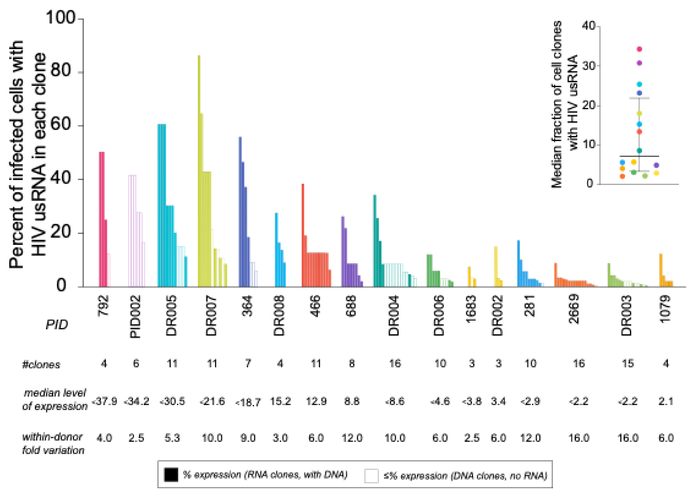


Figure 1. Plot of the fraction of cells with HIV usRNA within each of 139 different T-cell clones across 16 PWH. A total of 13,800 infected cells were assayed. Each color represents a different participant identifier (PID) and each bar a different clone. The number of clones are listed, the median level of expression, and within-donor fold variation is reported. Solid bars are exact estimates and open bars indicate the level of expression is less than or equal to the percent reported. Inset graph of the median fraction of cell clones with HIV usRNA.

In people with HIV on ART, only a small fraction of the infected cells have proviruses that are "turned on" and making viral RNA. In infected T-cell clones, this fraction varies across individuals more than across different T cell clones.

This finding suggests that host factors influence the fraction of infected cells with active proviruses more than the specific site of integration.

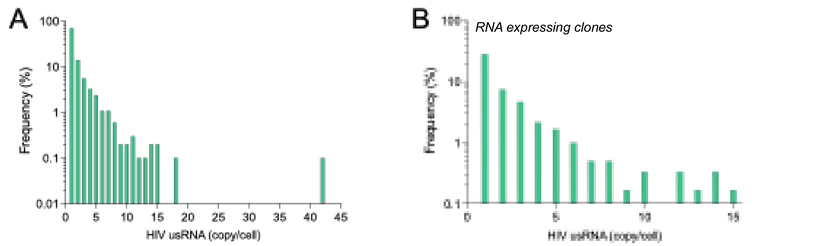


Figure 2. Distribution of the number of HIV usRNA copies per cell. (**A**) The number of HIV usRNA copies per cell across all donors on ART. (**B**) The number of HIV usRNA copies per cell in clones.

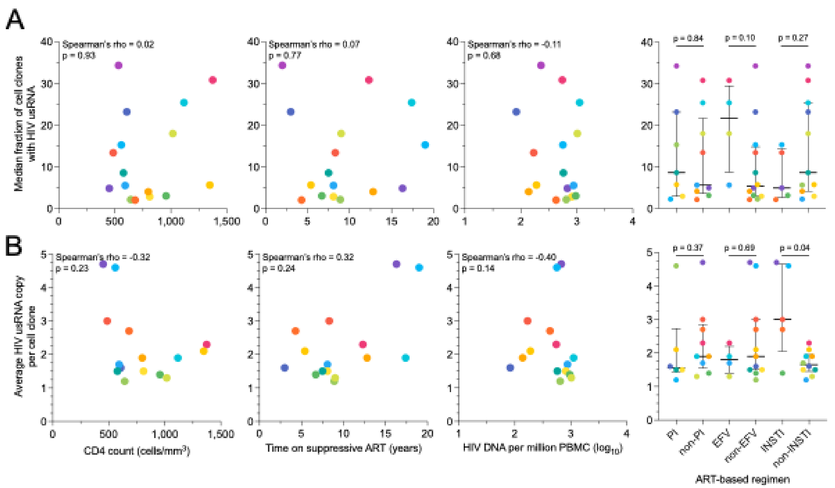


Figure 3. HIV proviral expression is not related to years on ART, CD4 count, total HIV DNA copies or ART regimen. (**A**) The median fraction of infected cell clones with HIV usRNA. (**B**) The average HIV usRNA copy per cell clone. (*Left to Right*) CD4 count, time on suppressive ART in years, $\log_{10}$ HIV DNA per million PBMC, and ART-based regimen. For ART-regimen, PI vs. non-PI, EFV vs. non-EFV, and INSTI vs. non-INSTI. Spearman correlation with associated p-value are reported. Mann-Whitney U-test for pairwise comparisons of ART-based regimens. Each color represents a different participant. *Abbreviations*: PI (protease inhibitor), EFV (efavirenz), INSTI (integrase strand transfer inhibitor).

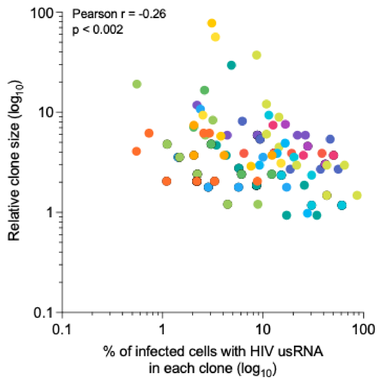


Figure 4. Association of relative clone size and the fraction of infected cells with HIV usRNA in each clone. Each color indicates a different donor and each symbol represents an individual clone. For simplicity, even if clones were identified without matching DNA sequences (open bars in **Figure 1**), they are fully colored here. Pearson correlation due to tied values was determine. Although an exact p-value was determined, it is an upper value due to uncertainty of expression in some clones, and thus set as *less than* (<).

RESULTS, findings

- **Figure 1:**
  - 139 infected cell clones were examined (13,800 single infected cells assayed)
  - Median fraction proviral expression was 8.5% (range 2.1% to 37.9%) with individual T-cell clone expression ranging from 0.6% to 86.5%
  - There was greater fold variation across donors (18.4) vs. within donor (6.0)
- **Figure 2:**
  - Most cells contained only a single detectable copy of HIV usRNA (70.5%)
  - HIV RNA expressing clones had an average of 2.2 HIV usRNA copies per cell
  - A rare, small fraction (1.3%) of expressing T-cell clones were high-expressing (>10 HIV usRNA copies/cell)
- **Figure 3:**
  - Median fraction of cells with HIV usRNA were not associated with CD4 count, time on suppressive ART, or HIV DNA
  - No differences were observed in ART-regimen, except for the average HIV usRNA copy per cell clone for INSTI vs. non-INSTI (likely due to small sample size leading to wide IQR)
- **Figure 4:**
  - As relative clone size increases, the fraction of expressing proviruses decreases

CONCLUSION

The fraction of transcriptionally active proviruses in T-cell clones had greater variation across PWH than across different clones within the same PWH. Fractional proviral expression is inversely proportional to T-cell clone size.

IMPLICATIONS

- Highlights importance of host-specific influences on proviral latency and expression rather than proviral integration site and/or sequence heterogeneity.
- Proviral expression may result in host recognition and subsequent cellular clearance for the majority of clones.

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