Parallel HIV RNA, Integration Site, and Proviral Sequencing in Single Reservoir Cells

Poster # 360

Kevin Einkauf[1,2], **Matthew Osborn**[2], **Ce Gao**[2], **Jane Blackmer**[2], **Chenyang Jiang**[1,2], **Eric Rosenberg**[3], **Xu G. Yu**[1,2], **Mathias Lichterfeld**[1,2]

Affiliations: [1]Brigham and Women's Hospital, Boston, MA, USA, [2]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, USA, [3]Massachusetts General Hospital, Boston, MA, USA

BACKGROUND

- The intact proviral reservoir remains the most significant obstacle to long-term HIV cure, as latently-infected cells can persist for decades despite suppressive ART
- Latency and persistence mechanisms may be influenced by the interplay of proviral genome, chromosomal integration site, and HIV transcriptional activity
- Previously, there has been no systematic approach to capture all 3 of these measures from individual reservoir cells

METHODS

- **PRIP-Seq**
  - Parallel HIV RNA, integration site, and proviral sequencing
- **PBMC from 2 HIV+ patients**
  - **P1**: Longitudinal
    - Pre-ART viremia
    - 1.3yr ART
    - 9yr ART
  - **P2**: Cross-sectional
    - 20yr ART
- **417** proviruses with matched viral seq. and HIV RNA status
  - **203** of these with integration sites

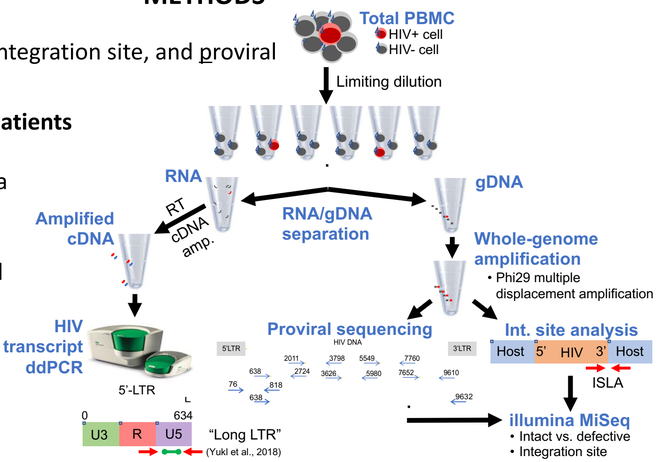


Table 1. Summary of all proviral sequences analyzed by PRIP-Seq

	P1			P2	Totals
Timepoint	Pre-ART Viremia	1.3yr ART	9yr ART	20yr ART	
Viral load (copies/mL)	87,900	< 50	< 50	< 75	
CD4 count (cells/mm³)	383	735	778	1,247	
Total PBMC assayed	220,000	2.84M	2.52M	265,496	
Total proviruses detected	62	155	133	67	**417**
Intact	10	5	7	4	**26**
% RNA+	60%	0%	14%	25%	**31%**
Defective	52	150	126	63	**391**
% RNA+	40%	42%	30%	17%	**34%**
Confirmed int. sites	23	82	59	39	**203**

Table 1. All sequences were analyzed for large internal deletions, hypermutation, out-of-frame indels, premature/lethal stop codons, internal inversions, and 5'/Psi defects. Sequences lacking all of these defects were considered genome-intact. Proviruses with detectable, paired Long LTR RNA (HXB2 522-643) were considered "RNA+".

RESULTS

Figure 1. Rapid decline in intact, RNA+ reservoir during ART in P1

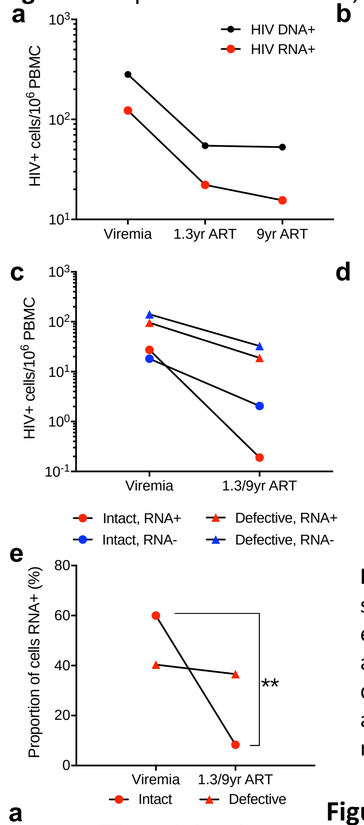


Fig. 1. **(a-b)** Longitudinal reservoir sizes and proportion of infected cells expressing HIV RNA in P1. **(c-e)** Intact and defective proviral seq.'s are distinguished. The transcriptionally-active, intact reservoir declined most rapidly during ART.

Figure 2. Distinct integration site features of transcriptionally-silent proviruses

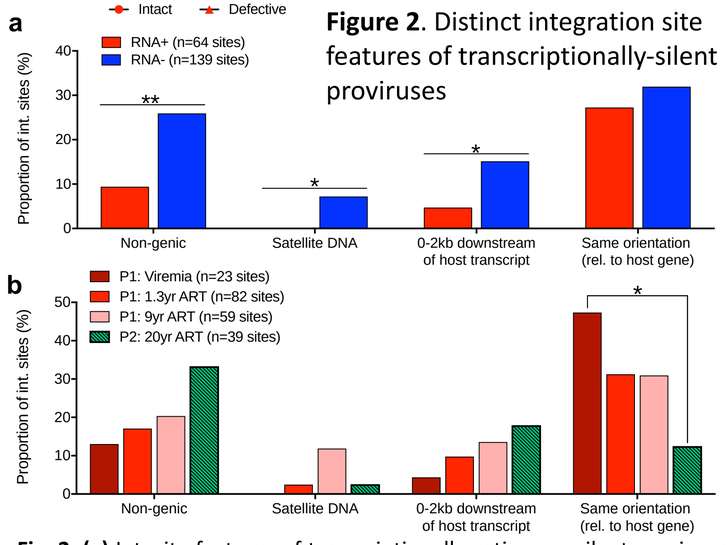


Fig. 2. **(a)** Int. site features of transcriptionally-active vs. -silent proviruses in both patients. **(b)** Longitudinal trends in int. site features. Several features were associated with HIV transcriptional silence, and these became more common after ART.

Figure 3. Proximity to same-oriented host TSS associated with HIV transcription

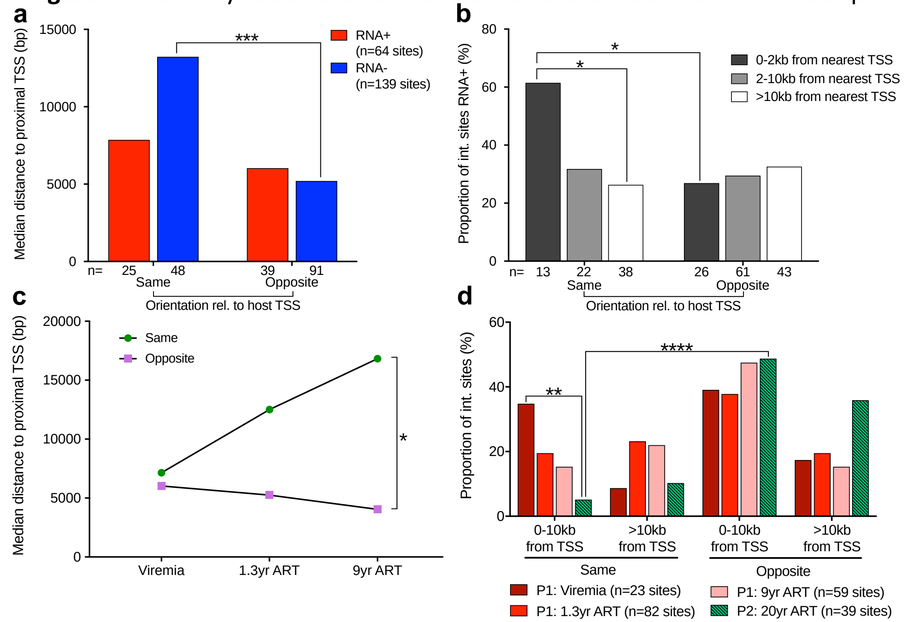


Fig. 3. **(a)** Median distances to the most proximal host transcriptional start site (TSS) for same- and opposite-oriented proviruses in both patients. **(b)** Proportion of proviruses expressing HIV RNA as a function of TSS distance and orientation. **(c)** Longitudinal trends in median TSS distance and **(d)** the proportion of integration sites within 10kb or >10kb from the nearest host TSS. Overall, relative proximity to a same-oriented host TSS increased the chance of a provirus expressing HIV RNA, and the proportion of such proviruses declined after ART.

CONCLUSIONS

- PRIP-Seq systematically links HIV transcription to proviral genomes and integration sites in single reservoir cells
  - This kind of multi-level analysis may help characterize latent reservoirs and persistence mechanisms
- In the 2 patients we studied:
  - Transcriptionally-active, intact reservoir declined most rapidly during ART
  - Silent proviruses were more likely integrated:
    - Within non-genic regions
    - Within centromeric repeats
    - Distant from same-oriented host TSS's
  - These features became more common over the course of ART
- Possible that these trends reflect immune-driven evolution of reservoirs during ART
  - During long-term ART, more transcriptionally-active proviruses may be selected against
  - This would promote progressively deeper levels of latency