A NOT-SO-STABLE RESERVOIR: HIV DRIVES INFECTED CELLS TO DIE AND DIVIDE

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Introduction

Evidence is emerging that a substantial portion of proviruses on ART are transcriptionally active and may create selection pressures on infected cells. The faster decay of intact proviruses reflects negative selection while the presence of large proviral clones integrated in cancer-related genes reflects positive selection [1-3]. We hypothesize that HIV transcription globally drives both cell death and division resulting in faster turnover of infected cells. These two forces oppose each other making it difficult to measure the extent of each [4].

Methodology

We wanted to explore if monitoring proviral orientation over time would allow us to detect the expansion force. To assess the effects of orientation, we monitored changes in integration sites in two chronically treated individuals at multiple time points over 14 years We wanted to explore if monitoring proviral orientation over time would allow us to detect the expansion force. To assess the effects of orientation, we monitored changes in integration sites in two chronically treated individuals at multiple time points over 14 years. We reasoned changes in orientation likely reflect effects by (and on) the HIV promoter.

To estimate turnover, we measured changes in clonal overlap between proviral sequences from paired time points in four chronically treated individuals (CT) and two elite controllers (EC) using the Morisita index [5].

- Integration sites were obtained from 11 timepoints in 2CT (n=4375 sites).
- We analyzed proviral orientation relative to time (Fig. 1) and clone size (Fig. 2).
- To probe mechanism, CEM-SS T cells were infected with a VSVG pseudotyped HIVDenv and single cell sorted into 696 wells and RNA-sequencing was performed on the 48 fastest and slowest clones.
- ~100 proviruses were sequenced at each timepoint in 4 CT and 2 EC
- Replicate TCR sequences were obtained at 3 time points in 2 CT and 2 EC
- Proviral and TCR clonal overlap was assessed at each pair of timepoints.
- A Morisita index was used to correct for variations in sample sizes [5].

Figure 1: Sense orientation can provide proviral clones an advantage longitudinally

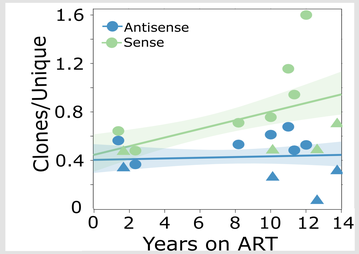
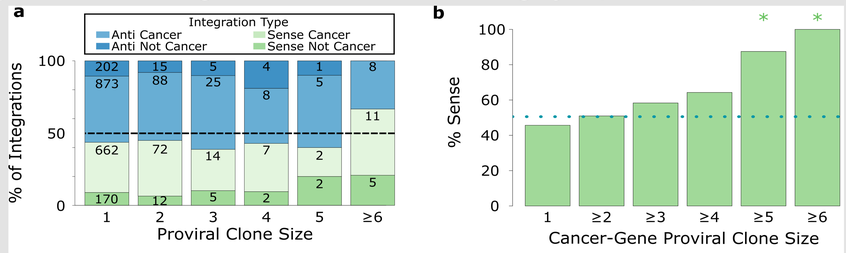


Fig. 1: The ratio of clonal to unique integrations sites are shown over time for two chronic progressors on ART, comparing those in sense (green) and antisense (blue) orientation relative to the local gene (Fig. 1A). We restricted our analysis to intronic integration sites and we define clones as integration sites detected more than once. Green and blue slopes differ with marginal posterior likelihood <0.001. The odds ratio of being a clone in sense versus antisense increased over time (p=.007).

Figure 2: Sense integration is associated with highly expanded proviral clones



c

Clone Size	1	2	3	4	5	6	7	8	9	10	11	12	15	16	18	20	21	56	89	>2	<2	Clone Odds*
Sense Cancer	170	12	5	2	2	1	1	0	1	0	0	0	1	0	0	0	0	1	0	126	170	0.7412
Anti Cancer	202	15	5	4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36	202	0.1782
Sense Non-Cancer	662	72	14	7	2	1	1	0	0	0	1	2	1	1	1	1	1	0	1	307	662	0.4637
Anti Non-Cancer	873	88	25	8	5	0	2	1	1	1	0	2	0	0	0	1	0	0	0	217	873	0.2486

Fig. 2: The stacked histogram shows the number of distinct integration sites at each clone size (Fig. 2A). Larger clones in cancer-related genes (≥ clone size 5) are mostly sense (Fig. 2B,* p<.05). The table provides the number of distinct integration sites by clone size and category (sense/antisense and cancer/non-cancer gene). The "clone odds" is the number of integration sites detected more than twice over the number detected once (Fig. 2C).

Figure 3: The HIV promoter drives host expression

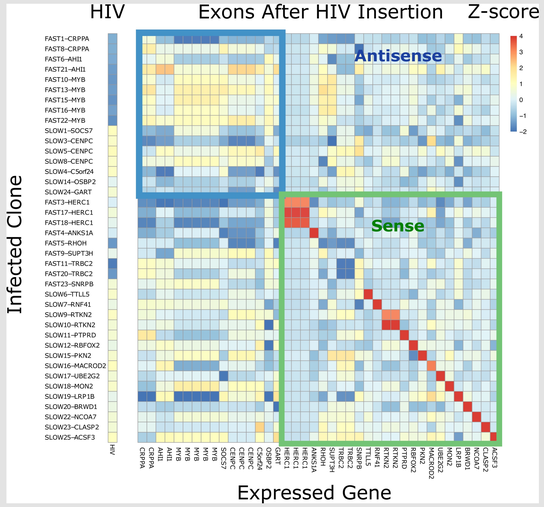


Fig. 3: RNA-seq was performed on 40 T cell clones that contained a single intronic integration site. Each row indicates the HIV integration site of the individual T cell clone, and each column indicates the expression level of a given gene in all 40 T cell clones. Z-scores represent relative expression of each gene in the 40 clones. Host gene analysis revealed 9-fold more expression (median value) when HIV inserted in sense orientation, resulting in a diagonal of red boxes. This provides a mechanism of enhanced downstream gene expression and potentially enhanced cell division (HERC1 and ANKS1A), leading to selection for HIV sense orientation. Viral gene analysis revealed low HIV expression is associated with faster cell division (p<0.001) with a trend to be in antisense.

Figure 4: Proviral turnover exceeds TCR turnover

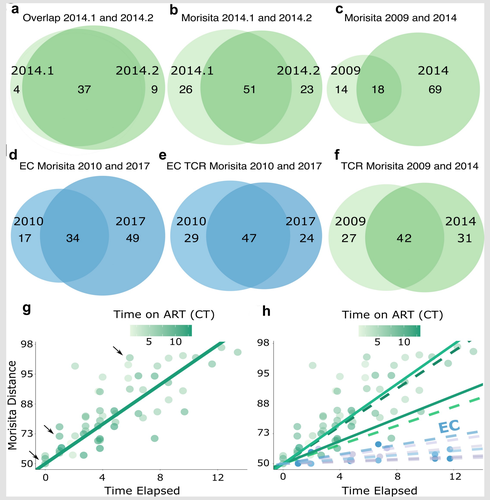


Fig. 4: Venn Diagrams were generated based on repeated proviral (A-D) and TCR sequences (E,F). In part A, we show a Venn Diagram based on repeated proviral sequences from two samples from the same timepoint of CT2. The center of the Venn diagram reflects the sum of clonal sequences present at both time points. The left flank shows there were 4 proviral clones detected just in the first sample, and the right flank that 9 proviral clones were only in the second sample from the same timepoint. A Morisita correction was applied to control for the variable sample sizes (B-F). In Part G, we plot the Morisita distance (defined as the sum of the Venn Diagram flanks) versus the time elapsed for each pair of timepoints using proviral sequences from one chronic progressor (green). In part H, we show the same analysis using proviral sequences from four CTs (green), two ECs (blue), and TCR sequences from two CT and two EC (lilac).

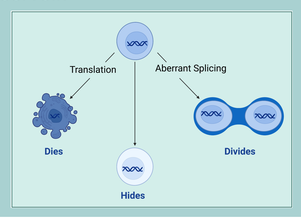
Conclusion

We were able to dissect two opposing forces that lead to reservoir contraction and expansion. The first favors antisense, while the second favors sense orientation. Both forces are initiated by LTR transcription.

Reservoir contraction occurs after expression of HIV proteins leading to cell death. This force favors antisense as it results in less HIV expression (Figs. 2-3) and is most apparent among the integration sites of clone size 1.

Reservoir expansion is due to aberrant splicing, leading to increased downstream gene expression (supported by Fig. 3). This force favors sense orientation and is most apparent among the large proviral clones (Fig. 2). Antisense orientation may also diminish HIV expression by interference, leading to selection for HIV in antisense. We conclude changes in orientation likely reflect effects due to HIV transcription.

We also provide evidence that these opposing expansion and contraction forces lead to higher turnover of infected cells. Intriguingly, proviral turnover appears faster in chronic progressors than elite controllers, consistent with their divergent proviral landscapes: specifically ECs lack proviruses in sense oriented, cancer-related genes. Thus, we provide direct evidence that HIV drives cells to die and divide leading to a "not-so-stable" reservoir.



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Acknowledgements

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