Formation of the Long-Lived, Early-Forming Reservoir is Punctuated by Episodic Seeding

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BACKGROUND

- Much of the long-lived reservoir is formed around the time of ART initiation. However, there is a portion of the reservoir that forms earlier in untreated infection.
- The kinetics behind the formation of the "early-forming" reservoir have not been elucidated.

METHODS

- MiSeq with PrimerID was used to generate pre-ART RNA sequences (rainbow arrows, **Figure 1**).
- DNA reservoir amplicons were generated at end-point dilution and sequenced using PacBio with barcodes.
- DNA reservoir sequence entry into the long-lived reservoir was estimated using the phylogenetic relationship between pre-ART RNA sequences and DNA reservoir sequences (**Figure 2**).
  - DNA reservoir sequences dating to the year before ART were excluded from further analysis (blue shading in Figures 1-3).
- Generated a model of continuous reservoir formation. Compared the number of reservoir sequences observed to date to each pre-ART time point to the estimate (**Figure 3B**).

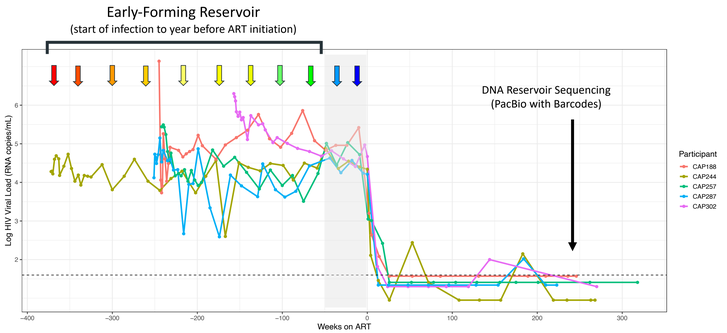

Figure 1. Sampling intervals and methods used to generate pre-ART RNA, and on-ART DNA reservoir sequences. Pre-ART RNA sequences were generated from an average of 10 pre-ART time points (rainbow-colored arrows). The early-forming reservoir is defined as the portion of the reservoir that dates to time points between enrolment and 2 years before ART. After an average of 5 years on suppressive ART, the DNA reservoir was sequenced using PacBio with barcodes.

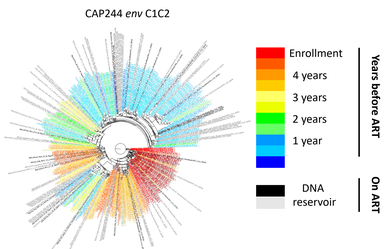

Figure 2. Dating the entry of DNA reservoir sequences into the long-lived reservoir. Phylogenetic trees are constructed containing pre-ART RNA sequences (color-coded by pre-ART time point) and DNA reservoir sequences. Black DNA reservoir sequences represent non-hypermutated sequences, grey; hypermutated sequences with the putative hypermutated positions masked.

Periods of enhanced reservoir seeding drive the formation of a large portion (41%) of the early-forming reservoir.

RESULTS

- A median of 100 unique DNA reservoir sequences were obtained for each participant (range: 46 – 133)
- All sequences dating to the year before ART were removed. A median of 46 DNA reservoir sequences (range: 27 – 64) remained for subsequent analyses (**Figure 3A**).
- In 4 of 5 participants, there were distinct pre-ART time points that contained significantly more reservoir sequences than expected under the continuous seeding mode (**Figure 4**).

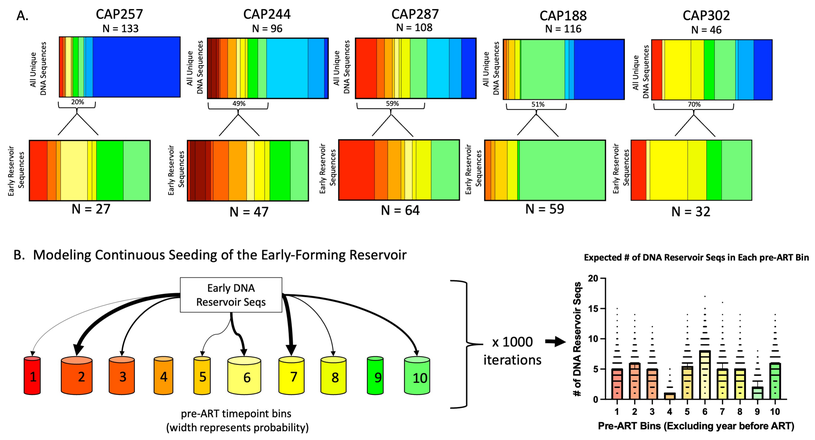

Figure 3. Examining the kinetics of the formation of the early-forming reservoir. A) Unique DNA reservoir sequences from each participant are shown on the top row. Underneath are graphs depicting the number of unique DNA reservoir sequences contained in the early-forming reservoir. B) In the model of continuous seeding, early DNA reservoir sequences are randomly distributed 1000x into pre-ART time point bins based on the duration of untreated infection represented by each bin. These data points are used to generate graphs that depict the expected number of DNA reservoir sequences that date to each pre-ART time point (B, right panel). The observed number of DNA reservoir sequences dating to each time point is then compared to this estimate to evaluate the fit of the model.

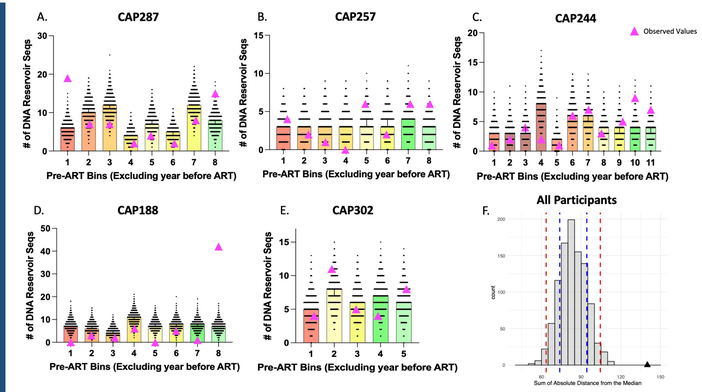

Figure 4. Periods of significantly enhanced reservoir seeding are observed in the background of low-level continuous seeding. A-E) Continuous seeding model results for each participant. Modeled data are shown in black, observed values in pink. F) Combined analysis of the fit of the continuous seeding model in all five participants (black triangle). One and two standard deviations from the median are depicted in blue and red lines, respectively.

- In 4 of 5 participants with observed periods of enhanced reservoir seeding (relative to our continuous seeding model), an average of 41% of the early-forming reservoir was formed during these episodic seeding events.

CONCLUSIONS

- This study examined the formation of the early-forming reservoir to understand whether this portion of the reservoir is formed continuously or episodically.
- Using a model of continuous reservoir formation, we observed that 4 of 5 participants had distinct pre-ART time points that contained significantly more DNA reservoir sequences than expected under this model.
- Overall, 41% of the early-forming DNA reservoir was established during these episodic seeding events.
- Elucidating the mechanisms that drive these periods of enhanced reservoir formation will enable the design of interventions to block them, thus reducing the size of the reservoir.

ACKNOWLEDGEMENTS

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