Establishment of the HIV-1 DNA Reservoir Mirrors the Replication-Competent Reservoir

Abstract # 0328

CAPRISA

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Background

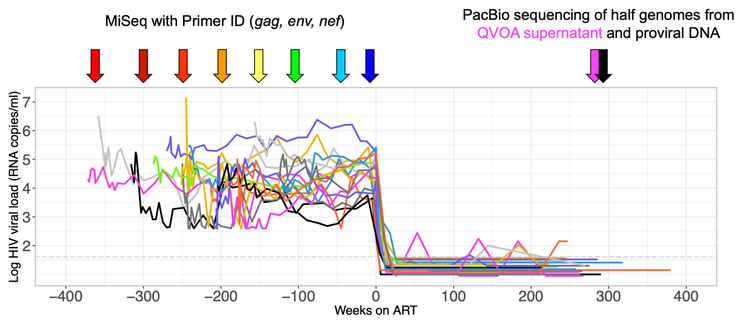
- All individuals living with HIV-1 on antiretroviral therapy (ART) have a long-lived reservoir[1].
- As this reservoir is one of the main barriers to cure, understanding the principles that govern its formation may inform strategies to limit its size, thus lowering the barrier to a cure.
- We recently demonstrated that the replication-competent portion of the long-lived reservoir originates from viruses circulating near the time of ART initiation[2].
- In the current study, we examine the formation of the viral DNA reservoir and replication-competent reservoir in the same set of participants.

Methods

- Plasma was collected longitudinally from 17 women in the CAPRISA-002 cohort an average of 9 times prior to ART initiation.
- After an average of 5.1 years on ART, PBMCs were collected.
- Subgenomic pre-ART HIV-1 RNA sequences were generated using MiSeq with Primer ID.
- On-ART HIV-1 RNA sequences were generated by PacBio sequencing of the outgrowth virus produced by performing a quantitative outgrowth virus assay (QVOA) on resting CD4+ T cells isolated from the PBMCs.
- On-ART HIV-1 proviral DNA sequences were generated by PacBio sequencing of PBMC-derived viral DNA amplified at limiting dilution.
- Viral evolution prior to ART was used to date when both replication-competent and proviral DNA sequences were seeded into the long-lived reservoir[2].

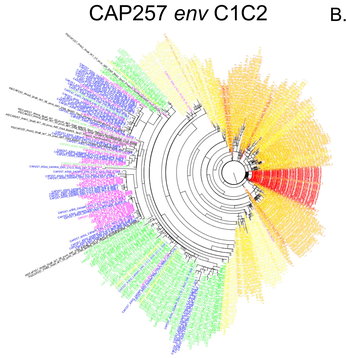
Results

Viral Loads and Sampling Strategy



The Primarily Defective HIV-1 DNA Reservoir Clusters Phylogenetically with the Replication-Competent Reservoir

A.



B.

Participant	# of QVOA-derived sequences	# of DNA-derived sequences	Total # of reservoir sequences analyzed	Percent Late
CAP336	11	7	18	94
CAP257	45	8	53	92
CAP316	12	9	21	86
CAP288	7	12	9	84
CAP333	1	5	6	50
CAP222	2	11	13	38
CAP244	1	8	9	33
CAP302	6	10	16	31
CAP277	1	8	9	11
CAP287	6	0	6	67
CAP206	19	0	19	63
CAP372	10	0	10	40
CAP217	16	0	16	38
CAP380	0	4	4	N/A
CAP268	5	0	5	N/A
CAP280	5	0	5	N/A
Average			**10.8**	**55.9**

Figure 1. Summary of sequences analyzed. A) A partial *env* tree containing pre-ART RNA sequences, QVOA-derived reservoir sequences (pink) and DNA-derived reservoir sequences (black). Reservoir sequences were dated using distance and tree-based measures[2]. B) A summary table showing the number and origin of the reservoir sequences analyzed, as well as a summary of what percent of reservoir sequences date to the year prior to ART initiation (percent late). We did not calculate a percent late for individuals with fewer than 6 reservoir sequences.

The Long-lived HIV-1 DNA and Replication-Competent Reservoirs are Seeded at Similar Times

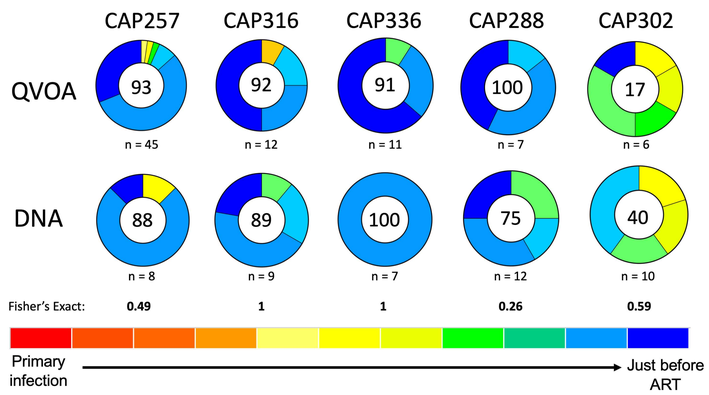


Figure 2. Comparison of the timing of the formation of the replication-competent and primarily defective DNA reservoirs. In the five participants for whom we had both QVOA-derived and DNA-derived reservoir viruses, we compared the timing of reservoir formation using phylogenetic methods[2]. The percent of reservoir sequences that date to the year before ART was initiated is shown in the center of each pie chart. Fisher's exact test was used to generate the indicated p value. In these five participants, the QVOA-derived estimates of the timing of reservoir formation do not differ from the DNA-derived estimates, even though the DNA reservoir is largely defective.

The Majority of the Long-lived Reservoir is Established in the Year Before ART Initiation

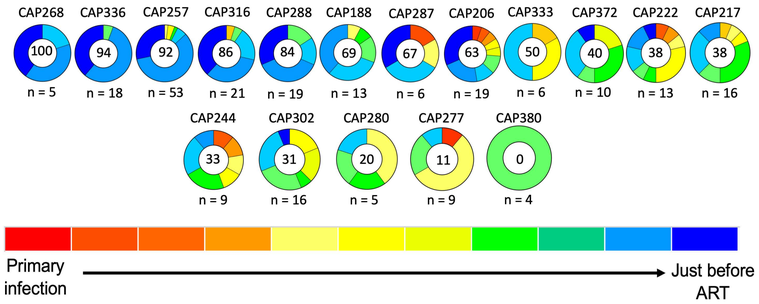


Figure 3. Dating the long-lived reservoir in 17 women from the CAPRISA-002 cohort. Pie charts depict the entry of sequences into the reservoir. The percent shown in the center of each chart represents the proportion of sequences that entered the reservoir in the year before ART. The number of reservoir sequences examined is shown below each chart. Analyzing participants with at least six reservoir sequences, we determine that 55.9% of the reservoir is seeded in the year prior to ART initiation.

Deeper Sampling of the DNA Reservoir Reveals a Minority of Early Variants

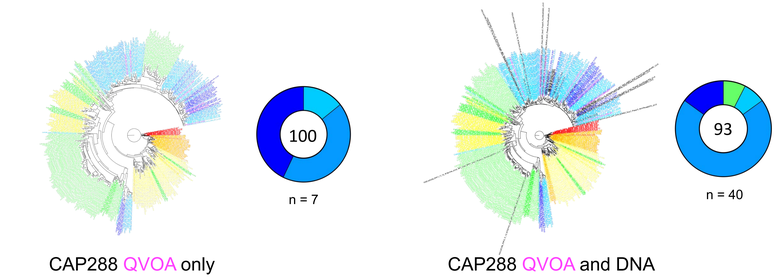


Figure 4. Deeper sampling of the DNA reservoir reveals a minority of early variants in an individual with only late QVOA-derived reservoir sequences. A) Phylogenetic tree containing pre-ART RNA sequences and QVOA-derived reservoir sequences along with a pie chart depicting when the reservoir sequences were seeded. B) Phylogenetic tree containing pre-ART RNA sequences, and both QVOA-derived and DNA-derived reservoir sequences. Deeper sampling reveals that a minority of reservoir sequences were seeded earlier.

Conclusions

- In this small study of n = 5 participants, we did not detect a difference in when the replication-competent and DNA reservoirs are formed.
- The majority of the long-lived reservoir was seeded in the year prior to ART initiation.

References /Acknowledgements

1. Chun TW et al. PNAS.1997.
2. Abrahams M-R et al. Sci Trans Med. 2019.

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