HIV-1 latency reversal augments persistent HIV-1 transcription during ART

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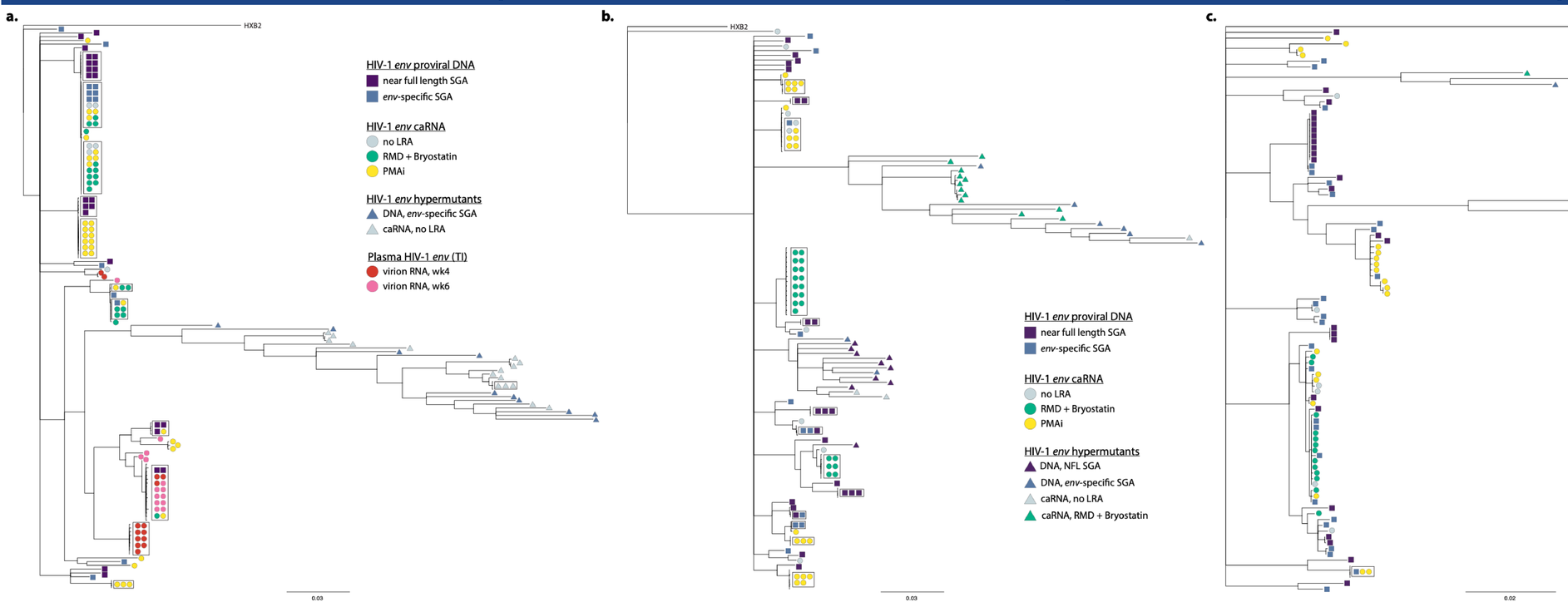


Figure. HIV-1 phylogenies during latency reversal. Proviral DNA and HIV-1 caRNA at baseline are shown with induced caRNA after 24hr PMAi or romidepsin 20nM in combination with bryostatin 1nM LRA boosting for three ART-treated suppressed participants. (a) HEAL047, (b) HEAL055, (c) HEAL066. Neighbor-joining trees with the Tamura-Nei model using HXB2 as an outgroup, 1,000 bootstrapped replicates, and a support threshold of 70% are shown.

Background

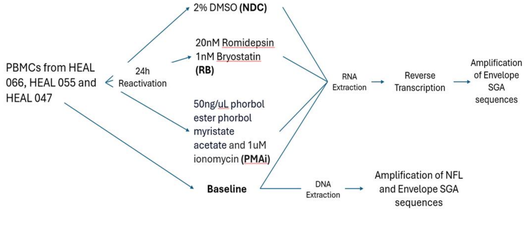
- Antiretroviral therapy (ART) reduces but does not eliminate intracellular HIV-1 transcription in people with HIV (PWH).
- We previously found that combination latency reversal agents (LRA) Romidepsin and Bryostatin results in similar levels of HIV-1 unspliced cell-associated RNA (caRNA) reactivation to PMA in combination with ionomycin (PMAi).
- Qualitatively, the sequences of HIV-1 caRNA that are induced LRAs are less well studied than their quantitative levels.

Aim

To understand the diversity of HIV-1 RNA transcripts seen after LRA treatment and if similar parts of the HIV-1 proviral landscape are transcribed during LRA exposure across treatments

Methods

Cryopreserved PBMC from three PWH on ART were used to amplify near-full length (NFL) and *env*-specific HIV-1 proviral DNA and caRNA single genome amplicons (SGA) before and after a 24-hour exposure to PMAi or the LRA boosting combination of romidepsin 20nM and bryostatin 1nM. Amplicons were sequenced, trimmed and assessed for APOBEC hypermutation using the Los Alamos Hypermut web tool. Neighbor-joining trees with the Tamura-Nei model using HXB2 as an outgroup. A convenience sample size of 20 SGAs each for HIV-1 NFL DNA, *env*-specific DNA, and env caRNA for each condition was used.



Results

- An average of 100 SGA were generated per participant.
- HIV-1 *env*-containing caRNA were amplifiable from all three participants' PBMC before LRA exposure.
  - SGAs represent unspliced HIV-1 caRNA spread across the phylogenies and included hypermutated sequences.
- The choice of primers, NFL vs env-specific, affected the detection of hypermutated proviral DNA.
- LRA boosting and PMAi can activate HIV-1 transcription from similar parts of the virus reservoir.
- LRA boosting stimulated hypermutated HIV-1 caRNA transcription in some participants, whereas PMAi did not.
- LRA boosting and PMAi can induce identical HIV-1 mRNA transcripts as occurs when ART is interrupted.

Conclusions

- Unspliced HIV-1 caRNA of 8-9kb in length were detected during ART and originated from >1 transcriptionally active cellular clone.
- HIV-1 latency during treated infection is best described a period of inefficient, but not absent, virus mRNA production in blood.
- HIV-1 caRNA hypermutants may be generated during latency reversal.
- Treatment with HIV-1 LRA boosting regimens can induce virus RNA transcription identical in sequence to that produced during PMAi and a treatment interruption.

Acknowledgements

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