Solo LTR Formation Promotes Elimination of Proviruses in Persons with HIV

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BACKGROUND

A major obstacle for curing persons with HIV (PWH) is persistence of HIV-infected cells during combination antiretroviral therapy (ART). Most persistent cells harbor defective proviruses, including solo LTRs, which are the result of cellular homologous recombination during DNA replication and clonal expansion; the recombination event excises the entire coding sequence of HIV, leaving only a solo LTR provirus. Solo HIV LTRs are reported (Anderson et al., 2019; Botha et al. 2023) and may dominate proviral populations, but little is known about their formation, frequency, and persistence during ART. We combined a new screen for solo LTR with single genome sequencing and integration site analysis (ISA) to identify and characterize solo LTR in PWH.

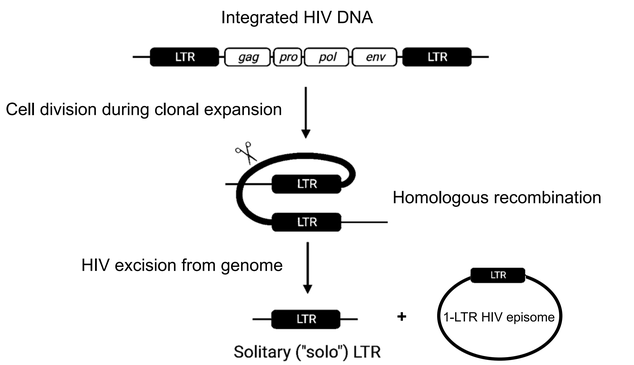


Figure 1 . Structure of retrovirus and formation of solo LTR. The structure of a typical HIV provirus includes gag, pro, pol, and env encoding regions with flanking identical LTRs. Due to the homology between the flanking LTRs, they can undergo homologous recombination and form solitary ("solo") LTR. The excised provirus (1-LTR episome) is gradually lost during cell division.

METHODS

Study Participants

Individuals Peripheral blood lymphocytes (PBLs) from adults undergoing ART in clinical protocols at NIH were screened using multiplexed droplet digital PCR (ddPCR) that targets LTR and sequences downstream from the 5' LTR. A high ratio of LTR to non-LTR sequence implies an excess of LTRs and the potential presence of solo LTR proviruses. We developed specific ddPCR to characterize and quantify levels of HIV clones, and designed bridging PCR strategies that amplify solo LTR to validate provirus structure.

Table 1

Participant	Age at enrollment (y)	Race /Ethnicity	Risk	RNA Pretherapy (Log_{10} copies/ml)	Nadir CD4 (cells/µl)	Nadir CD4 (%)	Peak CD4 (cells/µl)	Peak CD4 (%)	ART
AVBIO2-21	51	Not Black/ Hispanic or Latino	MSM	5.46	23	2	539	25	TDF- FTC-r/ATZ
AVBIO2-02	46	Not Black/ Hispanic or Latino	MSM	5.58	22	3	285	13	Abacavir-3TC-Efavirenz

Solo HIV LTR Proviruses Persist for Years on ART and Undergo Clonal Expansion, Even in Immune Non-responders"

RESULTS

Highly Expanded Clone with a Solo LTR in *HORMAD2* Gene

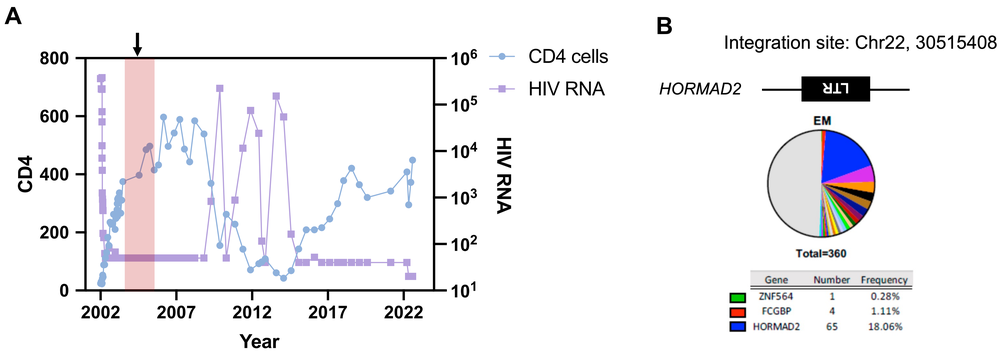


Figure 2. (A) A participant (AVBIO2-21) underwent antiretroviral therapy during HIV infection. The number of CD4 cells/ul and viral RNA copies were monitored from 2002 until 2023. (B) Previous study (Anderson *et al.*) discovered a solo-LTR provirus inserted into intronic sequence in the *HORMAD2* gene on chromosome 22. Integration site analysis revealed that the *HORMAD2* provirus exhibits clonal expansion I the effector memory (EM) cells (Data from Anderson et al., 2019).

Dynamics of Clonal Expansion of Cells Harboring a Provirus in the *HORMAD2 gene*

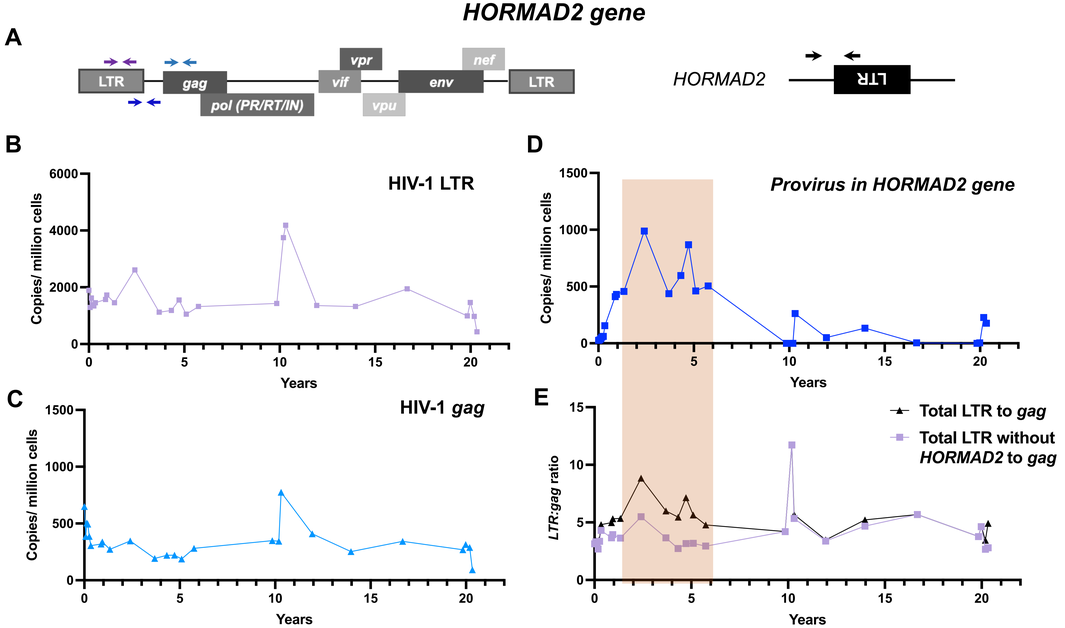


Figure 3. (A) HIV-1 provirus map showing positions of primers amplifying LTR, *gag* and LTR/*HORMAD2* junction. (B-D) HIV-1 DNA copies per million PMLs were examined by ddPCR and shown in light purple (LTR), light blue (*gag*) and blue (*HORMAD2* provirus). (E) HIV-1 LTR with and without *HORMAD2* LTR to *gag* ratio at different time points during cART. The shaded area represent the stage of clonal expansion of solo-LTR provirus in the HORMAD2 gene.

The Provirus in the *HORMAD2* Gene Remains Detectable After Years of cART

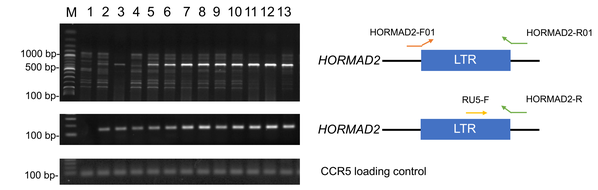


Figure 4. The right diagram indicates the positions of primers amplifying the *HORMAD2* solo LTR and total HIV-1 LTR. The left gel shows the levels of *HORMAD2* provirus and HIV-1 LTR in the PMLs from the early cART. Sample 1 to 13 represents time points collected from year 1 to 3. Analysis samples obtained pre- and post ART have not detected the parent provirus that gave rise to the solo LTR (limit of detection approximately …)

Clonal Expansion of a Solo HIV Provirus in Immune Non-Responder

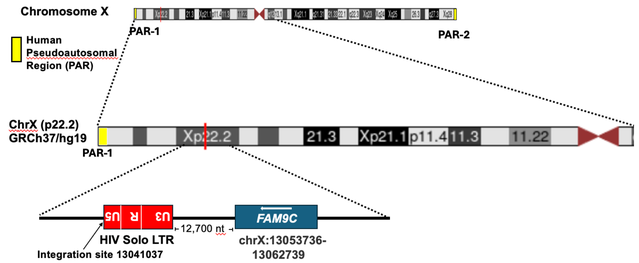


Figure 5. Characterization of HIV provirus located on Chromosome X. 3% of 1726 integration sites were present in a non-genic region near *FAM9C* on the X chromosome in PBMC of participant AVBIO2-02 after undergoing ART for >10 y. The provirus was mapped to the short arm of the X chromosome, outside of the two regions of known homologous recombination, denoted pseudoautosomal regions PAR-1 and -2 located at the ends of the short and long arms respectively, of chromosomes X and Y. During meiosis, corresponding PAR regions on the X and Y chromosome pair, and undergo recombination. Homologous recombination in PAR-1 during meiosis is essential for proper gametogenesis. Recombination between X and Y chromosomes outside PARs in males is poorly understood but highly infrequent. The identification of a solo LTR demonstrates that homologous recombination may take place in somatic cells outside of PARs.

CONCLUSIONS

- **Homologous recombination in cells infected with HIV can excise the entire coding sequence of HIV provirus, leaving only a solo LTR**
- **A large HIV-infected clone with a solo provirus integrated into the *HORMAD2* gene was characterized in study participant AVBIO2-21**
  - ***HORMAD2* functions in the pathway of meiosis and mainly expressed in testis.**
- **The cell harboring the *HORMAD2* provirus underwent a rapid expansion shortly after cART was initiated**
- **Abundance of the clone with solo HIV LTR waxed and waned, as shown for replication-competent HIV clones (*Simonetti et al., 2016; Siliciano and colleagues 2018*)**
- **The expansions of the cells containing *HORMAD2* provirus is associated with the increased LTR:*gag* ratio**
- **Homologous recombination can take place on the X chromosome outside of PARs**

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