HIV TRANSCRIPTS DURING ART ARE PREDOMINANTLY DERIVED FROM DEFECTIVE PROVIRUSES

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BACKGROUND

Low levels of HIV cell-associated RNA (caRNA) and proteins are detectable in many PWH even after years of ART. Here, we report a novel viral transcript sequencing method (TranSeq) and performed in-depth longitudinal characterization of the dynamics and interplay between HIV proviral DNA reservoirs and caRNA in a single PWH over 7 years of ART.

METHODS

- The study participant is a Latin-American male with subtype B HIV who began ART during chronic infection.
- DNA and caRNA were extracted from peripheral blood CD4⁺ T cells at multiple time points over 7 years of ART.
- HIV proviral reservoir size and caRNA expression were quantified by digital droplet PCR.
- Near-full-length (NFL) HIV proviral genomes (FLIPseq) and polyadenylated viral transcripts (TranSeq) were recovered.

TransSeq-SCA

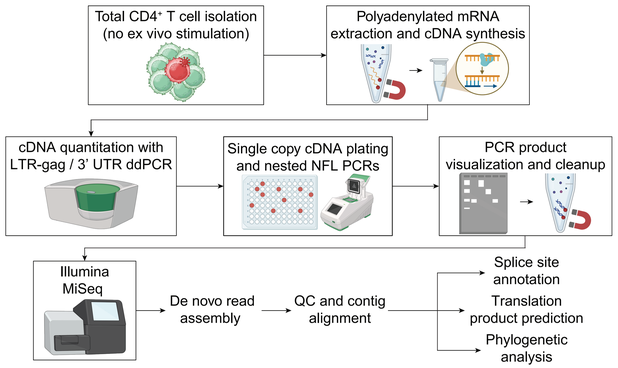
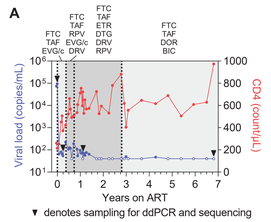
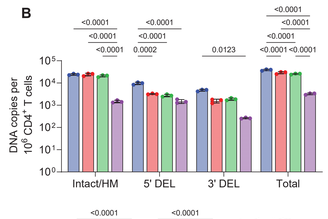


Fig. 1: Clinical characteristics and ddPCR-based viral reservoir quantitation spanning 7 years of ART.

A) Viral loads declined rapidly upon ART initiation,however low level viremia (<200 copies/mL) was detectable until 1.4 years.
B) Intact and defective proviruses decayed slowly over the first year of ART and had markedly decreased by 7 years.
C) Viral caRNA levels declined rapidly following ART initiation and then remained relatively stable until 7 years of ART.





	PreART	3 months ART	1 year ART	7 years ART
Viral load (copies per mL)	96125	54	<40	<40
CD4 (count per μL)	214	372	722	974

Fig. 2: The majority of HIV transcripts during ART contain deletions and/or early stop codons in essential genes.

A and B) Near-full-length sequences of proviral DNA (A) and viral caRNA (B) categorized by presence of APOBEC3-mediated hypermutation, point mutations that introduce premature stop codons in coding regions, and deletions localized to the 5' leader (5' leader DEL) or coding regions (large DEL). For caRNA only (B), sequences are additionally classified by presence of aberrant splice junctions that would not permit translation of full-length proteins.

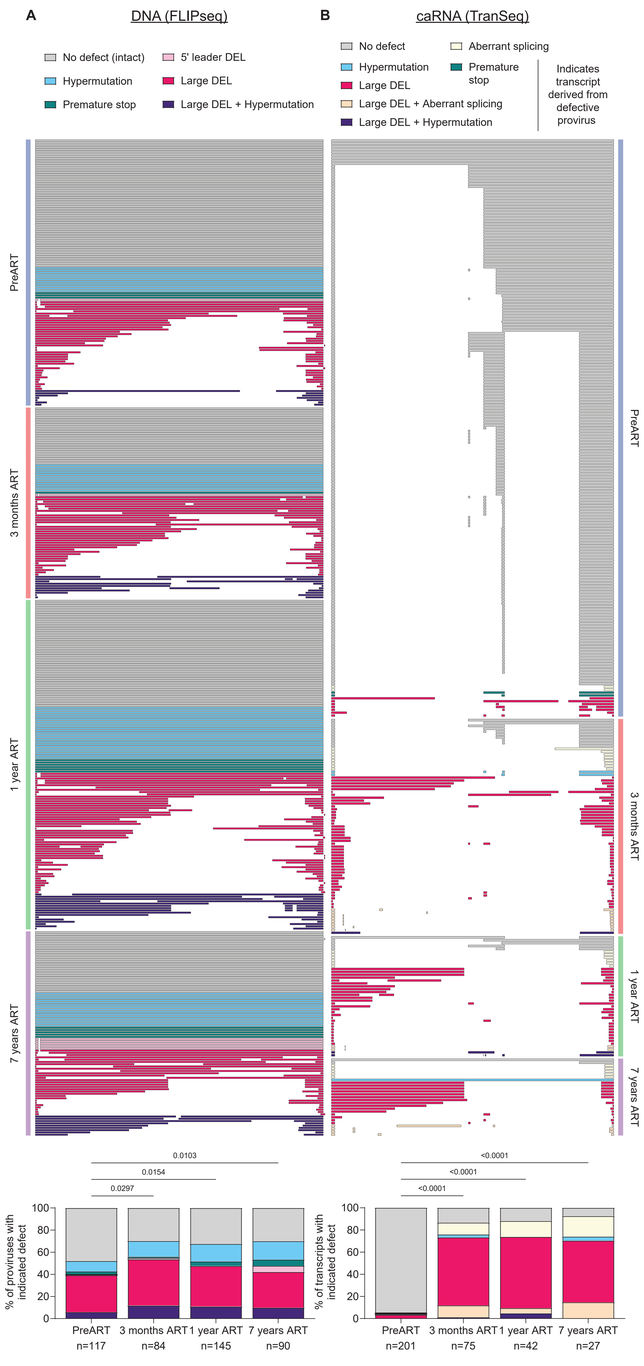


Fig. 3: Noncanonical splice acceptor usage by D1 detected during ART.

A) Spliced transcripts recovered during ART frequently contain junctions from D1 to noncanonical A8 sites or to putative acceptor sites containing AG-dinucleotide motifs.
B) A8 sites are 3' splice acceptors located within *nef* and therefore transcripts with direct D1-A8 splicing are not predicted to encode any full-length protein. 8/11 putative acceptors are located within *gag* or *pol* and only occur before large 3' deletions (≥4.7kb). 1/11 and 2/11 putative splice acceptors are located within *env* and *nef*, respectively. No transcripts associated with putative splice acceptors are predicted to encode full-length proteins.

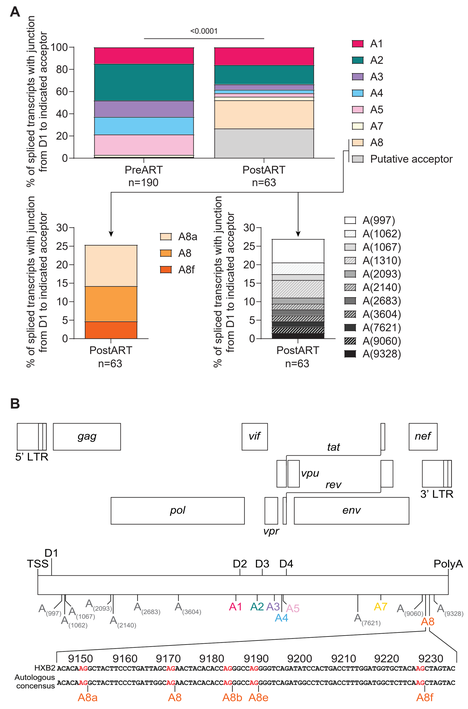


Fig. 4: HIV transcripts encoding full-length proteins are rare during ART.

A) Large deletions and premature stop codons in HIV transcripts recovered during ART can result in ORFs encoding various truncated protein forms. Deletions can additionally result in ORFs consisting of fusions between two or more proteins and/or extended amino acid sequence from noncanonical reading frames. Aberrant splicing or extremely large deletions can result in transcripts that do not contain any canonical HIV translational start codons.
B) Of the small fraction of post-ART transcripts with full-length ORFs, those encoding Vpr and Rev are underrepresented and those encoding Vif and Nef are overrepresented.

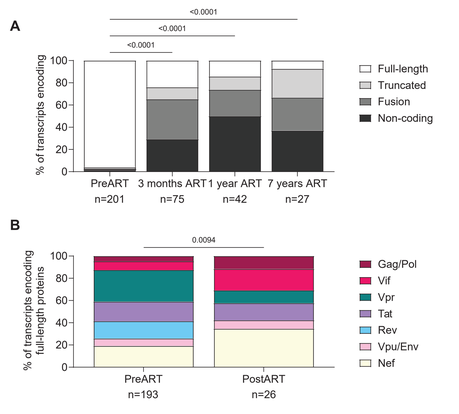
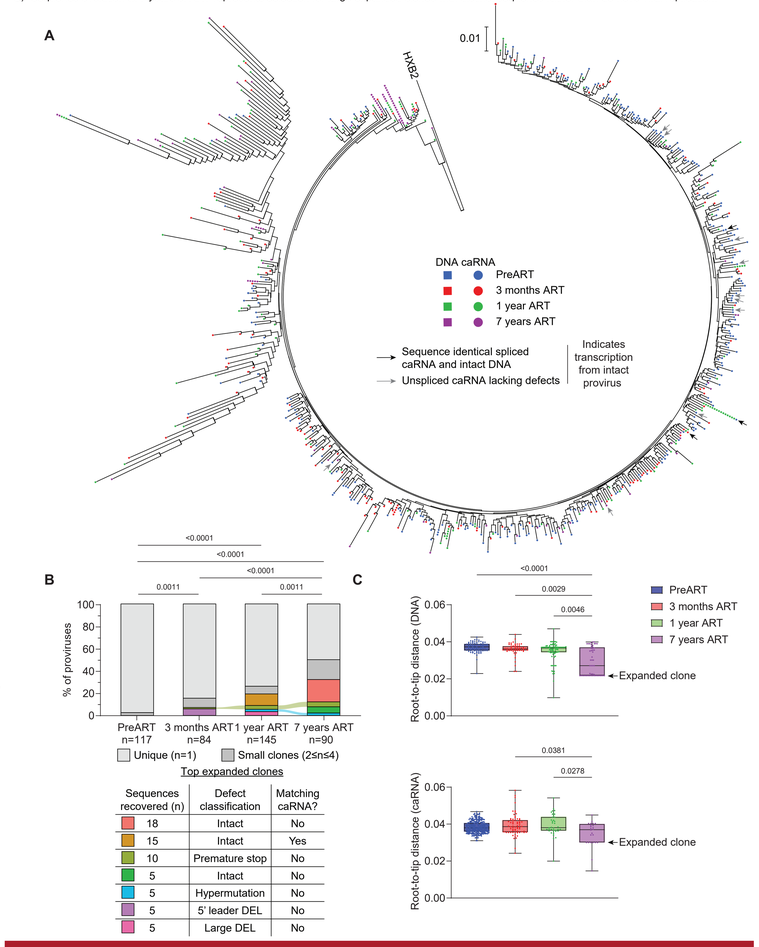


Fig. 5: Phylogenetic analysis of proviral DNA and viral caRNA sequences reveals distinct transcriptionally-active clonal populations.

A) Spliced transcripts with identical sequence to intact proviruses and unspliced transcripts lacking defects are detectable up to 7 years after ART initiation.
B) Large expanded proviral clones with transcriptional activity are detectable after 1 year of ART.
C) Sequence evolution at 7 years of ART represents selection for large expanded clones with distinct sequence rather than de novo viral replication.



Sequences recovered (n)	Defect classification	Matching caRNA?
18	Intact	No
15	Intact	Yes
10	Premature stop	No
5	Intact	No
5	Hypermutation	No
5	5' leader DEL	No
5	Large DEL	No

CONCLUSIONS

- During ART, most HIV transcripts originate from defective proviruses.
- Ongoing low-level expression of translation-competent transcripts from intact and defective proviruses occurs even after years of ART.
- Chronic HIV antigen presentation from defective proviruses during ART may shape HIV-specific immune responses and contribute to inflammation.

ACKNOWLEDGEMENTS

Funding: This work is supported by NIH NIAID R01-AI162221 (GQL) and UM1-AI164565 (RBJ).
We sincerely thank the study participant for their invaluable contribution to this research.

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