Transcriptionally Active Defective HIV-1 Proviruses Persist in All CD4 T Cell Subsets during ART

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BACKGROUND

More than 95% of HIV-1 proviruses detected are defective but often associated with RNA transcripts during antiretroviral therapy (ART). These transcriptionally active defective proviruses may contribute to persistent immune activation seen in people with HIV (PWH) receiving ART (Imamichi et al., 2016 and 2020 PNAS; Singh et al, 2023 AIDS). The distribution and persistence of these transcriptionally active defective proviruses has not been described.

METHODS

11 PWH originally enrolled in the Strategic Timing of Antiretroviral Therapy (START) trial were studied. Levels of HIV-DNA and total HIV-RNA in frozen whole blood were determined at the baseline and 4-11 years later by real-time PCR. Also, at the 2nd visit, CD4 cell subsets were isolated by flow cytometry sorting with CD45RO and CD27 antibody staining to identify naïve (NA), central memory (CM) and effector memory (EM) subsets. Sequences of HIV-DNA and cell-associated (CA) HIV-RNA in CD4 subsets were characterized by 5'LTR-to-3'LTR PCR single-genome amplification and direct amplicon sequencing. Levels of HIV-DNA and CA HIV-RNA in the CD4 subsets were determined per-cell as well as per 1mL of blood.

RESULTS

Table 1. Characteristics of study participants

	Archival time point (ART naïve)		Contemporary time point (on ART)		
Pt ID	Plasma HIV-RNA (copies/mL)	CD4+ count (cells/μL)	Plasma HIV-RNA (copies/mL)	CD4+ count (cells/μL)	Time (yrs) on ART
1	9909	513	<40	582	6.2
2	22668	545	<40	694	9.8
3	7341	792	<40	1417	8.9
4	18500	1026	<40	789	4.2
5	77400	645	<40	745	4.0
6	9076	524	<40	861	10.6
7	12700	638	<40	1096	8.0
8	21600	658	<40	791	6.8
9	19500	547	<40	1173	7.8
10	12756	794	<40	908	4.6
11	7870	719	<40	1062	4.5

All participants were male. Median age (IQR): 41 (26, 49), Race: 55% white, 18% black, 27% others.

RESULTS

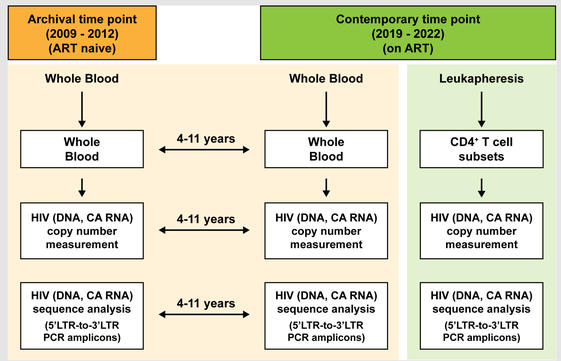


Fig. 1. Study design. 11 PWH originally enrolled in the Strategic Timing of Antiretroviral Therapy (START) trial were studied.

Frozen whole blood were analyzed at the archival and the contemporary time points. At the 2nd visit, CD4+ T cell subsets: naïve (Na), central memory (CM) and effector memory (EM) were analyzed from leukapheresis.

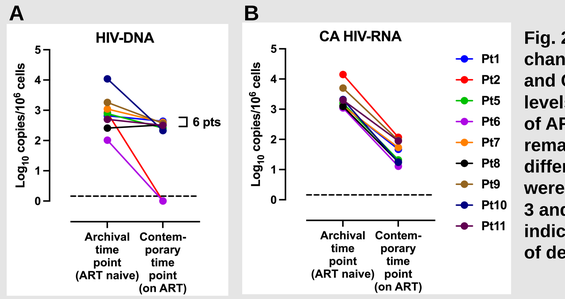


Fig. 2. Longitudinal changes in HIV-DNA (A) and CA HIV-RNA (B) levels over 4 to 11 years of ART. HIV-DNA levels remained stable (<1 log difference) in 6 pts. Data were unavailable for Pts 3 and 4. The dotted line indicates the lower limit of detection.

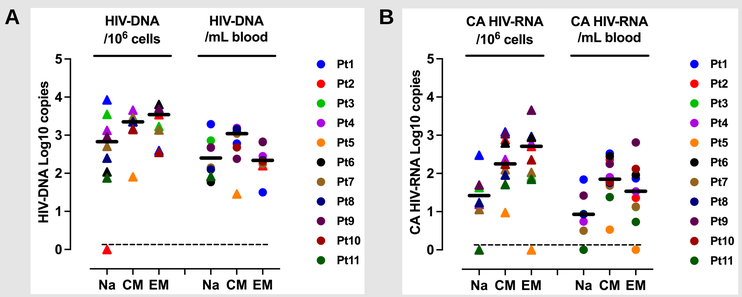


Fig. 3. Levels of HIV-DNA (A) and CA HIV-RNA (B) in naïve (Na), central memory (CM) and effector memory (EM) CD4+ T cell subsets after 4-11 years of ART. Transcriptionally-active HIV-1 proviruses can be found in all three CD4+ T cell subsets. The dotted line represents the lower limit of detection. HIV-1 copy numbers are expressed as copies per million cells and milliliter of blood.

RESULTS

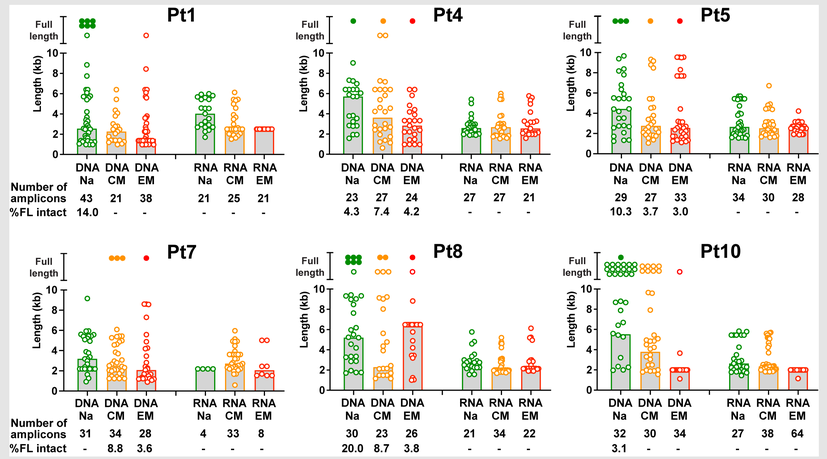


Fig. 4. Representative patterns of HIV-DNA and CA HIV-RNA sequence length distributions in naïve (Na), central memory (CM), and effector memory (EM) CD4+ T cell subsets from six individuals after 4–11 years of ART. Full-length intact HIV-1 proviruses can be found in all three CD4+ T cell subsets, with no evidence of transcription from these intact proviruses.

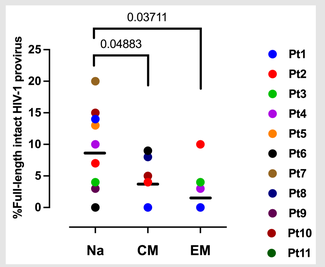


Fig. 5. The frequencies of full-length intact HIV-1 proviruses were measured in naïve (Na), central memory (CM) and effector memory (EM) CD4+ T cell subsets with a median 9% of proviruses in a full-length intact from in Na cells, 4% in CM cells, and 2% in EM cells. Statistical significance was assessed using the Wilcoxon matched pairs test.

RESULTS

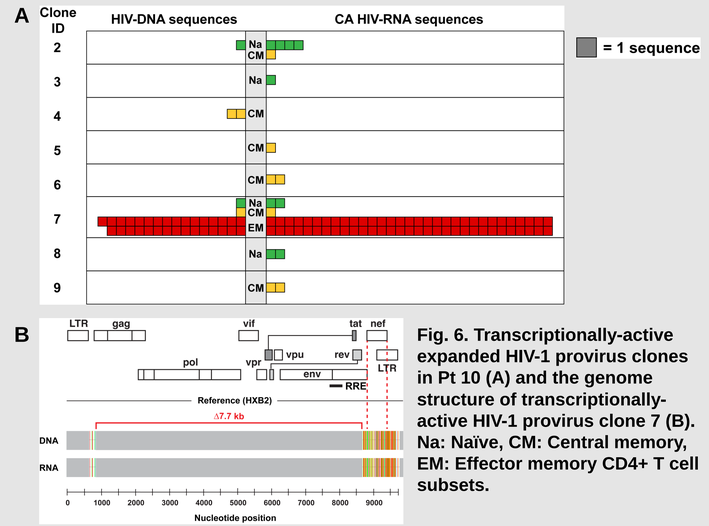


Fig. 6. Transcriptionally-active expanded HIV-1 provirus clones in Pt 10 (A) and the genome structure of transcriptionally-active HIV-1 provirus clone 7 (B). Na: Naïve, CM: Central memory, EM: Effector memory CD4+ T cell subsets.

SUMMARY

Transcriptionally active "defective" HIV-1 proviruses were present in all naïve, central memory and effector memory CD4+ T cell subsets in a cohort of 11 pts after 4-11 yrs of ART. The majority resided in central memory (CM) cells, identifying the CM cell pool as an important source for long-term HIV-1 transcriptional persistence on ART. This has implications for the mechanism of persistent immune activation among ART-treated participants.

REFERENCES

(1) Imamichi et al., 2016, PNAS 113(31): 8783-87
(2) Imamichi et al., 2020, PNAS 117(7): 3704-10
(3) Singh et al., 2023, AIDS 37(14): 2119-30

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