Intact HIV Proviruses are Unequally Distributed in T-cell Subsets During ART

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Antiretroviral therapy (ART) for the treatment of HIV-1 is highly successful in suppressing viral replication, reducing the level of virions in the blood, and increasing the life expectancy of infected individuals. Despite this, ART does not cure HIV-infected individuals, as HIV-1 remains latent in resting memory CD4+ T-cells and this dormant form of the virus is not targeted by ART or the host immune system. Latent replication-competent HIV-1 persists as viral load rapidly rebounds if ART is interrupted. A thorough understanding of the distribution of replication-competent HIV-1 within different CD4+ T-cells is required for future eradication studies.

Here, we applied near full-length sequencing to determine the potential replication-competency of HIV-1 proviruses isolated from different CD4+ T-cell subsets from participants on long-term ART.

We isolated naïve (T_N), central (T_{CM}), transitional (T_{TM}), and effector (T_{EM}) memory CD4+ T-cells from 6 participants on long term ART.

Table 1. Participant characteristics

Participant (SCOPE ID)	Viral load[a] (copies/mL)	CD4+ T cell count[a] (cells per µL)	Time of infection before initiation of therapy (months)	ART duration[a] (years)	Therapeutic regimen[b]
2302	<40	696	3.4	4.6	FPV,RTV, TDF/FTC
2115	<40	601	0.6	17.3	FTC/TDF,NVP
2275	<40	1842	0.8	15.3	FTC/TDF,NVP
2452	<40	604	24.4	3.2	MVC,RTG,ETR
2026	<40	476	117	17.7	TDF,ABC/3TC,RTV,DRV
2046	<40	1099	70	16.3	ECV, EFV/TDF/FTC

[a]At time of sampling
[b]FPV, fosamprenavir; RTV, ritonavir; TDF, tenofovir disoproxil fumarate; FTC, emtricitabine; NVP, nevirapine; MVC, maraviroc; RTG, raltegravir; ETR, Etravirine; ABC, abacavir; 3TC, lamivudine; DRV, darunavir; EFV, efavirenz; ECV, entecavir.

Full-Length Individual Proviral Sequencing (FLIPS)

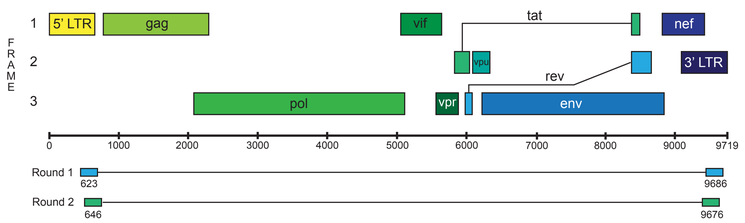
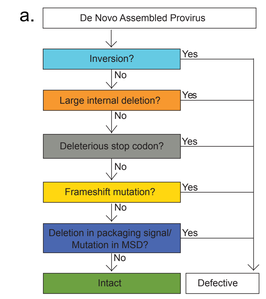


Figure 1. The FLIPS assay amplifies single, near full length (>9 kb) HIV-1 proviruses isolated from CD4+ T-cell subsets using nested PCR. Amplified proviruses are then sequenced by Next Generation Sequencing on the Illumina MiSeq Platform.

In total, we sequenced 531 proviruses. Of these, 5% were intact and potentially replication-competent.



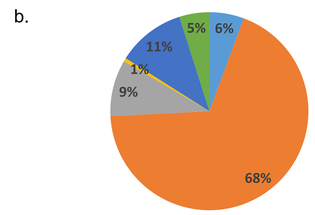


Figure 2 a. Process of elimination used to identify intact and defective HIV-1 genomes; b. Percent of intact and defective proviruses

Table 2. Number of cells and sequences obtained for each participant

Participant	Naïve			Central Memory			Transitional Memory			Effector Memory		
	Defective	Intact	# Cells analyzed	Defective	Intact	# Cells analyzed	Defective	Intact	# Cells analyzed	Defective	Intact	# Cells analysed
2302	15	4	3,287,933	35	0	704,122	31	3	820,287	32	6	728,025
2115	0	0	0	28	0	2,294,141	29	0	859,259	22	1	1,888,889
2275	0	0	0	3	0	251,852	21	0	1,057,688	33	3	1,330,640
2046	0	0	6,465	24	0	190,647	1	0	4,048	23	2	153,236
2452	35	2	641,975	34	0	3,440,763	37	0	225,734	33	3	459,055
2026	26	0	152,727	1	0	47,685	10	1	133,448	32	1	51,046
Total	76	6	4,089,100	125	0	6,929,211	129	4	3,100,464	175	16	4,610,891

Effector memory cells contain the majority of intact proviruses

The proportion of intact proviruses was significantly different across the cell subsets (P < 0.001)

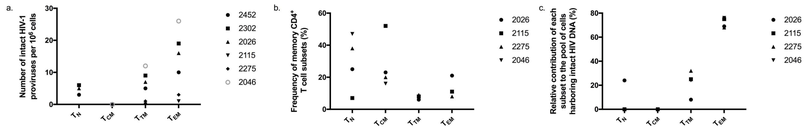


Figure 3. a. Number of intact proviruses per million cells for each CD4+ T-cell subset; b. Total frequency of each memory CD4+ T-cell subset; c. Relative contribution of each subset to the pool of cells harboring intact HIV

- In all participants we identified expansions of identical proviral sequences (≥ 2 identical sequences)
- 92% of expansions were of defective proviruses

Table 3. Number of expansions of identical proviruses identified in each participant

	2452	2302	2026	2115	2275	2046
Defective	5	7	7	7	5	4
Intact	0	0	1	0	1	1

- Identified 3 participants with expansions of genetically intact proviruses
- Identical intact proviruses suggest a role for proliferation in the maintenance and stability of the replication-competent reservoir

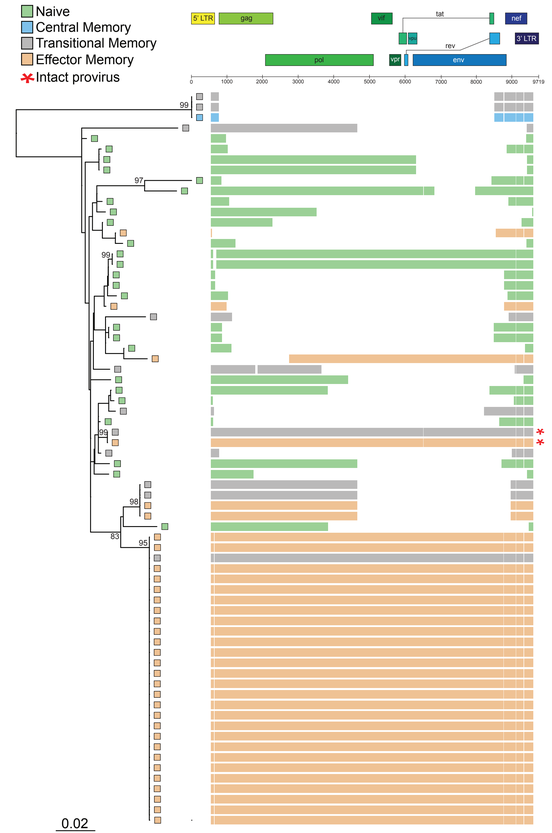


Figure 4. Phylogenetic tree and sequence representation of sequences obtained for participant 2026

Conclusions

- 5% of proviruses sequenced from T_N, T_{CM}, T_{TM}, and T_{EM} cells of participants on long-term ART are intact
- T_{EM} contain the most intact HIV-1 proviruses
- Identified expansions of intact proviruses in 3 participants
- Role for proliferation in the maintenance of the replication-competent reservoir

Acknowledgments

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