Intact Proviruses Persist in Expressed Genes in People with Non-Suppressible HIV on Long-term ART

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BACKGROUND

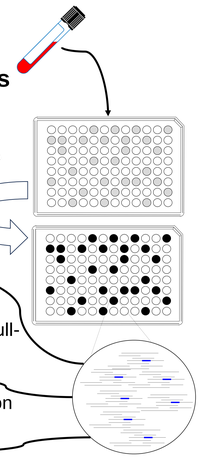
- Persistence of replication-competent proviruses during ART is the main barrier to HIV cure.
- Enrichment for intact proviruses persisting in KRAB-ZnF genes[1] and centromeric/satellite regions[2,3] on long-term ART has been reported.
- Here, we identified CD4+ T cell clones carrying intact proviruses on long-term ART, determined their integration sites, assessed their dynamics over time, measured the ability of the proviruses to be induced, and measured the levels of expression of the genes where integration occurred.

Table 1. Donor Characteristics

PID	Age*	Sex	Total Unique Integration Sites	Timepoint 1 (years on ART)	Timepoint 2 (years on ART)	Timepoint 3 (years on ART)
C-03	43	M	12,033	9	10	12
R-09	73	M	9,596	10	12	-
F-07	49	M	12,225	19	21	23

*Age at first timepoint of the study.

METHODS



1. Identify study participants and samples
- Three donors on ART for 10-20 years were sampled over 1-4 years[4,5].

2. Extract, Dilute, & Dispense PBMC DNA
- (≤1 provirus/well)

3. Perform Multiple Displacement Amplification

4. Screen for Intact Provirus
- Real Time PCR adapted from IPDA Assay[6]
  - ➢ 5' Psi / 3' RRE / LTR
- Proviruses positive for LTR, Ψ, and RRE underwent full-length HIV sequencing and integration sites analysis.

5. Map provirus integration site
- A custom pipeline was used to map discrete integration sites to hg19 and non-discrete (e.g., satellite) to T2T.

6. Identify Sequence Intact Provirus
- Near Full Length amplification sequencing[7]
- PROseq-IT (55 parameter intactness analysis)[8]

7. Post-hoc Analysis
- Full-length, intact proviral sequenced matched to autologous plasma and VOA virus sequence – either full-length of sub-genomic
- Gene expression levels in memory CD4+ T cells were determined using the Human Protein Atlas Database[9], and using RNA-seq from donor.

References

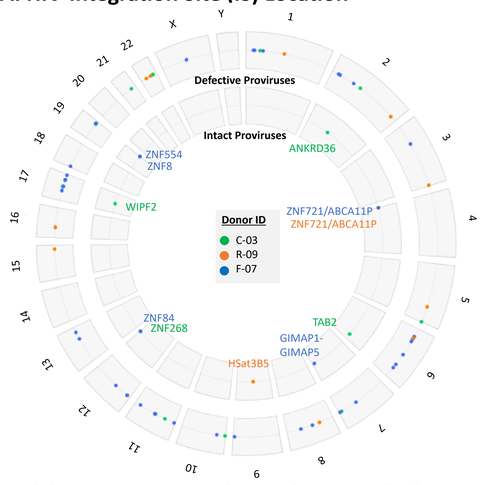
1. Einkauf KB, Lee GQ, Gao C, et al. Intact HIV-1 proviruses accumulate at distinct chromosomal positions during prolonged antiretroviral therapy. J Clin Invest. 2019;129(3):988-998.
2. Jiang C, Lian X, Gao C, et al. Distinct viral reservoirs in individuals with spontaneous control of HIV-1. Nature. 2020;585(7824):261-267.
3. Huang AS, Ramos V, Oliveira TY, et al. Integration features of intact latent HIV-1 in CD4+ T cell clones contribute to viral persistence. Journal of Experimental Medicine. 2021;218(12):e20211427.
4. Halvas EK, Joseph KW, Brandt LD, et al. HIV-1 viremia not suppressible by antiretroviral therapy can originate from large T cell clones producing infectious virus. J Clin Invest. 2020;130(11):58475857.
5. Guo S, Luke BT, Henry AR, et al. HIV infected CD4+ T cell clones are more stable than uninfected clones during long-term antiretroviral therapy. PLOS Pathogens. 2022;18(8):e1010726.
6. Bruner KM, Wang Z, Simonetti FR, et al. A quantitative approach for measuring the reservoir of latent HIV-1 proviruses. Nature. 2019;566(7742):120-125.
7. Joseph KW, Halvas EK, Brandt LD, et al. Deep Sequencing Analysis of Individual HIV-1 Proviruses Reveals Frequent Asymmetric Long Terminal Repeats. J Virol. 2022;96(13):e0012222.
8. Shao W, Shan J, Kearney MF, et al. HIV Proviral Sequence Database: A New Public Database for Near Full-Length HIV Proviral Sequences and Their Meta-Analyses. AIDS Res Hum Retroviruses. 2020 Jan;36(1):1-3. Epub 2019 Oct 29.
9. Monaco G, Lee B, Xu W, et al. RNA-Seq Signatures Normalized by mRNA Abundance Allow Absolute Deconvolution of Human Immune Cell Types. Cell Rep. 2019;26(6):1627-1640.e7.

A stable pool of intact proviruses integrated in *expressed* genes can persist on long-term ART.

RESULTS

Figure 1. Intact Proviruses Map to Genic Regions of Human Genome

A. HIV-Integration Site (IS) Location



B. Clonal Dynamics of Intact Proviruses Over 1-4 Years of Continuous ART

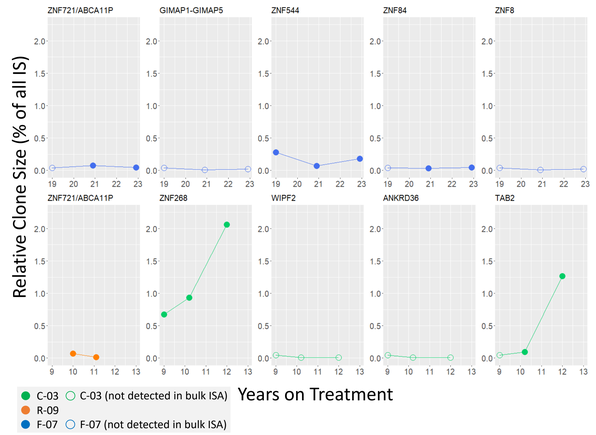


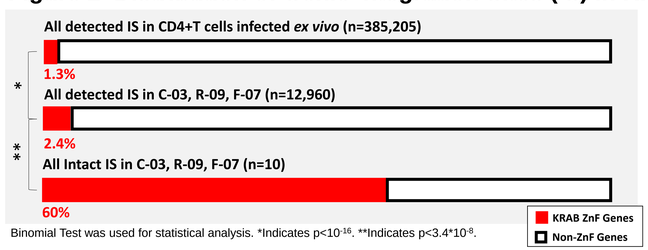
Table 2. Intact Provirus Characteristics

PID	Integration Site	Chromosome	Chromosomal Location (bp)	In/Out Gene	Provirus Orientation Relative to Gene	Intron/Exon
C-03	ZNF268	12	133777660	In	Against	Intron
C-03	WIPF2	17	38415167	In	Against	Intron
C-03	ANKRD36	2	97891945	In	Against	Intron
C-03	TAB2	6	149705538	In	Against	Intron
R-09	ZNF721/ABCA11P	4	425766	In	Against	Intron
R-09	HSat3B5	9	multimap*	Out	N/A	N/A
F-07	ZNF721/ABCA11P	4	443311	In	Against	Intron
F-07	GIMAP1-GIMAP5	7	150421195	In	With	intron
F-07	ZNF544	19	58750209	In	With	intron
F-07	ZNF84	12	133630727	In	With	intron
F-07	ZNF8	19	58792664	In	With	intron

*Centromeric/Satellite proviruses mapped to multiple locations in T2T.

- ❖ We identified 11 infected CD4+ T cell clones carrying sequence-intact HIV proviruses and 51 carrying sequence-defective HIV proviruses (**Figure 1A**).
- ❖ Two of the 10 clones carrying sequence-intact proviruses increased in size, one decreased, and seven did not change significantly over 1-4 years (**Figure 1B**).
- ❖ 10/11 intact integration sites mapped to expressed genes (**Table 2**) and 4 were in the same orientation as the gene.
- ❖ **The HIV Reservoir Remained Relatively Stable Over Time.**

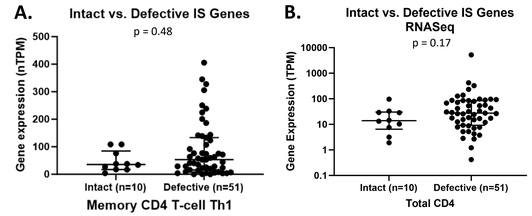
Figure 2. Distribution of Genic Integration Sites (IS) in KRAB-ZnF genes



Binomial Test was used for statistical analysis. *Indicates $p<10^{-16}$. **Indicates $p<3.4*10^{-8}$.

- ❖ Primary CD4+ T cells infected *ex vivo* were evaluated for the genic distribution of integration sites within 24 hours of infection and compared to the integration sites in all 3 donors on long-term ART.
- ❖ Compared to cells infected *ex vivo*, the donors on long-term ART had a ~2-fold enrichment for integration sites into KRAB-ZnF genes.
- ❖ Six intact clones were in **KRAB-ZnF** genes (60%), compared to the 2.4% of total proviruses (which are mostly defective) in KRAB-ZF found in all donors.
- ❖ **We observed strong enrichment of persistence in KRAB ZnF genes.**

Figure 3. No Significant Difference in Expression Levels of Genes Carrying Intact vs. Defective Proviruses



- ❖ Expression of genes containing intact proviruses was compared to genes containing defective proviruses as measured by reported Memory CD4 Th1 T cells (**Figure 3A**) and RNA-seq from donor-matched total CD4 T cells (**Figure 3B**).
- ❖ No significant difference detected between global expression of intact vs. defective-harboring genes (Mann Whitney, p = 0.48, p = 0.17).

Table 3. Intact Proviruses Match Full-length or Sub-genomic VOA and Plasma RNA Sequence

PID	Integration Site	Plasma Match (Expression)	VOA Match (Replication)
C-03	ZNF268*	+	+
C-03	WIPF2*	+	+
C-03	ANKRD36*	+	+
C-03	TAB2*	+	+
R-09	ZNF721/ABCA11P	+	+
R-09	HSat3B5	+	+
F-07	ZNF721/ABCA11P	-	+
F-07	GIMAP1-GIMAP5	-	+
F-07	ZNF544**	-	+
F-07	ZNF84	+	-
F-07	ZNF8**	-	+

*, ** Represent 1.4 kb identical sequence between 2 or more intact proviruses of distinct integration site lineage

- ❖ 10/11 intact proviral genomes match viral outgrowth assay sequence
- ❖ 11/11 intact proviral genomes match viral outgrowth assay or plasma vRNA

CONCLUSIONS

- In 3 donors with on long-term ART, we identified 11 clones carrying sequence-intact proviruses.
- Only **1/10** clones declined in size on long-term ART.
- We observed a **strong** enrichment for intact proviruses to persist in KRAB-ZNF genes.
- **10/10** intact proviruses integrated in protein-coding genes were in genes that are **expressed** in memory CD4+ T cells, including the 6 in KRAB-ZNF genes.
- Intact proviruses appear to be **inducible**.

IMPLICATIONS

- ❖ Enrichment in KRAB-ZnF genes may highlight important underlying mechanism of persistence targeted for future studies.
- ❖ These and other methods to track and eventually target reservoir in CD4 lineages (integration site, t-cell receptor specificity) will be central in the design and evaluation of functional cure interventions.

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