INTACT NON-INDUCIBLE PROVIRUSES ARE GENERATED DURING ACUTE HIV AND CAN PERSIST ON ART

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Introduction

HIV reservoirs are established during acute infection and persist during ART. However, the intactness and inducibility of these viral genomes remain poorly characterized. Here, we obtained near-full length HIV genome sequences of rare non inducible proviruses detected during acute infection and followed their fate during ART.

Methods

We collected longitudinal blood samples (pre-ART and after 96 weeks of ART) from 6 participants with acute HIV (AH, n=2 individuals/Fiebig stage [II, III and IV/V]) and 2 participants with chronic HIV (CH) enrolled in the RV254/RV304 studies (Bangkok, Thailand). We measured cell-associated viral transcripts (LTR-gag and Tat-Rev) as well as the frequency of HIV-DNA harbouring cells and capsid protein p24 expressing cells (HIV-Flow) with or without stimulation (PMA/ionomycin) pre- and post-ART (Figure 1A). Cells harbouring non-induced proviruses (p24-) were sorted for near-full length HIV genome sequencing at both time points. The phenotype of productive or induced p24+ cells was analyzed (Figure 1B).

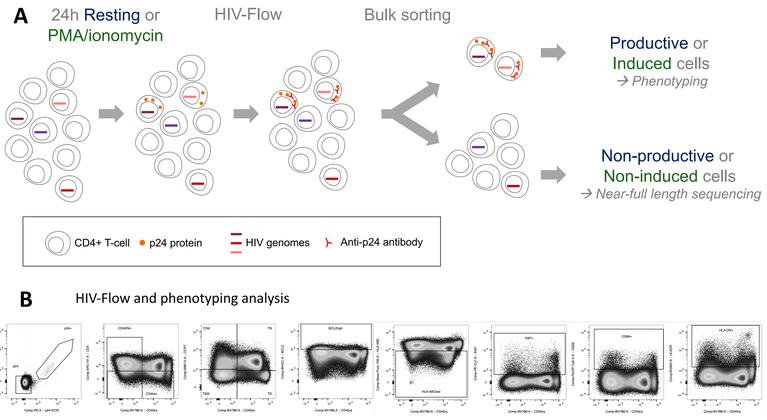

Figure 1. Study design for non-induced and non-productive HIV-infected cells analysis.
A: Overview of methods; **B:** Dot plots of HIV-Flow and the phenotyping markers analyzed (left to right: CD45RA, CCR7, Bcl2, HLA-ABC, Ki67, CD69 and HLA-DR).

Results

HIV expression in CD4+ T cells before and after ART

Participants' characteristics are presented in Table 1. Before ART, the frequency of p24+ cells (Figure 2A) and the levels of cell-associated LTR-gag or Tat-Rev HIV-RNAs (Figure 2B) did not significantly increase upon stimulation, suggesting that reactivable proviruses were rare during acute infection. After 96 weeks of ART, none of the participants who initiated ART during AH displayed detectable p24+ cells (in contrast to CH), which was consistent with low to undetectable levels of cell-associated HIV transcripts even after stimulation.

PID#	Fiebig stage	Age at Week 0 (years)	Gender	HIV subtype	Week 0 (Viremic) CD4 (/mm3)	Week 0 (Viremic) CD8 (/mm3)	Week 0 (Viremic) HIV-RNA (copies/mL)	Week 96 (ART) CD4 (/mm3)	Week 96 (ART) CD8 (/mm3)	Week 96 (ART) HIV-RNA (copies/mL)
PID26	2	26	Male	CRF01_AE/B Recombinant	249	430	736615	397	492	<20
PID27	2	22	Male	CRF01_AE	641	481	11350800	1204	1058	<20
PID28	3	22	Male	CRF01_AE	233	172	4416897	534	367	<20
PID29	3	27	Male	CRF01_AE/B Recombinant	457	640	5286145	598	781	<20
PID30	4	22	Male	CRF01_AE	571	552	14673	960	757	<20
PID14	5	39	Male	CRF01_AE	625	3936	277506	632	610	<20
PID16	Chronic	21	Male	Not Done	227	688	63588	Not Done	Not Done	<34
PID31	Chronic	45	Male	CRF01_AE	30	708	723346	267	1244	<34

Table 1. Participants' characteristics.

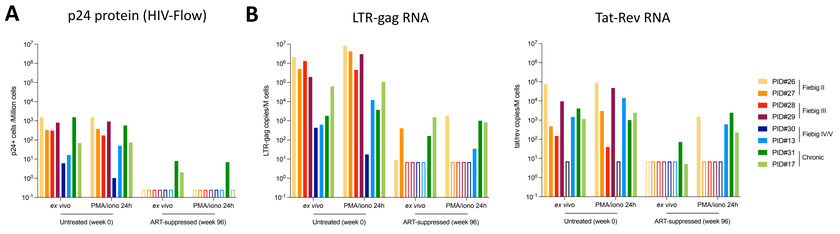

Figure 2. HIV expression in CD4+ T cells at both transcriptional and translational levels
A: Frequency of p24+ cells measured by HIV-Flow in longitudinal blood of participant initiating ART. Frequencies were measured ex vivo to detect productively infected cells and after stimulation with PMA/ionomycin for 24h to detect the translation competent reservoir. **B:** Total RNA from unstimulated or stimulated CD4+ T cells was used to measure elongated (LTR-gag) and multiply spliced (tat/rev) transcripts. Each transcript was normalized to the number of input cells.

Phenotype of p24+ cells before ART

p24+ cells displayed : a **central or an effector** memory phenotype (Figure 3A), were in a **resting state** (low to moderate levels CD69 and HLA-DR, Figure 3B), and displayed potential **escape/survival** mechanisms , (low levels of HLA class I eand standard levels Bcl2, (Figure 3C).

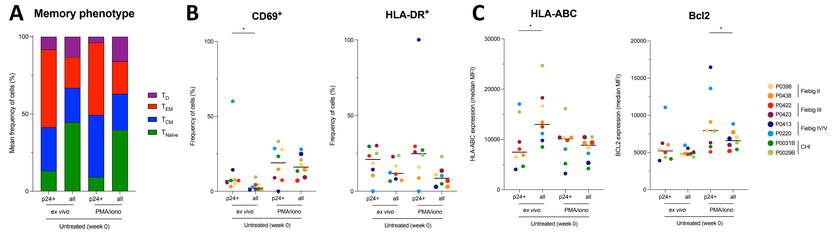

Figure 3. Phenotype of p24+ cells in acute infection
A: Proportion of each memory subset in p24+ cells and all total CD4+ T cells at the viremic time point both *ex vivo* and after stimulation. **B:** Activation status of infected cells (frequency of each subset: CD69+, HLA-DR+)
C: Survival and escape phenotype of infected cells (median fluorescence intensity [MFI]: HLA-ABC, Bcl2).

Intactness of latent reservoir before and after ART

Bulk-sorted p24- cells were analyzed for their proviral content :

- **Total and integrated HIV-DNA** were detected in almost all samples both during viremia and after 96 weeks of ART (Figure 4A) → Non-inducible (intact or defective) proviruses are archived in the viral reservoir since the earliest stages of acute infection and can persist on ART.

- **Near full-length genome amplification** (limiting dilutions / PacBio sequencing) showed 223 proviral sequences at W0 and W96 of ART (Figure 4B)
  - *ex vivo* → non-productive HIV-reservoirs
  - Stimulation (PMA/ionomycin) → non-induced HIV-reservoirs

1. *During untreated infection* : **1/3 of viral genomes were intact** (both non-induced and non-productive), indicating that deeply latent proviruses are archived since the earliest stage of infection.
2. *After 2 years of ART* : **1/10 of viral genomes were still intact** in most participants, indicating that early ART does not prevent the establishment and persistence of genetically intact genomes that are refractory to viral reactivation.

Identical sequences were rare both before ART initiation and after 2 years of suppressive therapy (median, 0 and 5%, respectively; Figure 4C), indicating that clonal expansions minimally contribute to the early dissemination and persistence of HIV genomes in p24- cells as well.

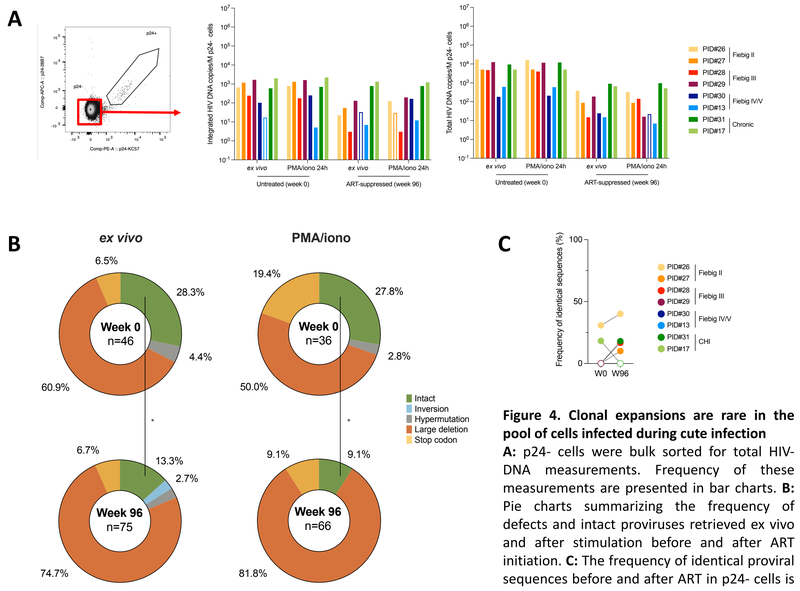

Figure 4. Clonal expansions are rare in the pool of cells infected during cute infection
A: p24- cells were bulk sorted for total HIV-DNA measurements. Frequency of these measurements are presented in bar charts. **B:** Pie charts summarizing the frequency of defects and intact proviruses retrieved ex vivo and after stimulation before and after ART initiation. **C:** The frequency of identical proviral sequences before and after ART in p24- cells is representend for each participant.

Persistence and intactness of proviral sequences in the viral reservoir

Intact proviruses were observed in 6/8 participants (PID #13, 17, 27-30; Figure 5) whereas only defective proviruses were detected in the last 2/8 (PID #26 and 31). Besides, identical and genetically intact non-induced proviruses were found both in the pre- and post-ART samples from two participants (Participants #30 and #13, who initiated ART during Fiebig stages IV and V, respectively; Figure 5).

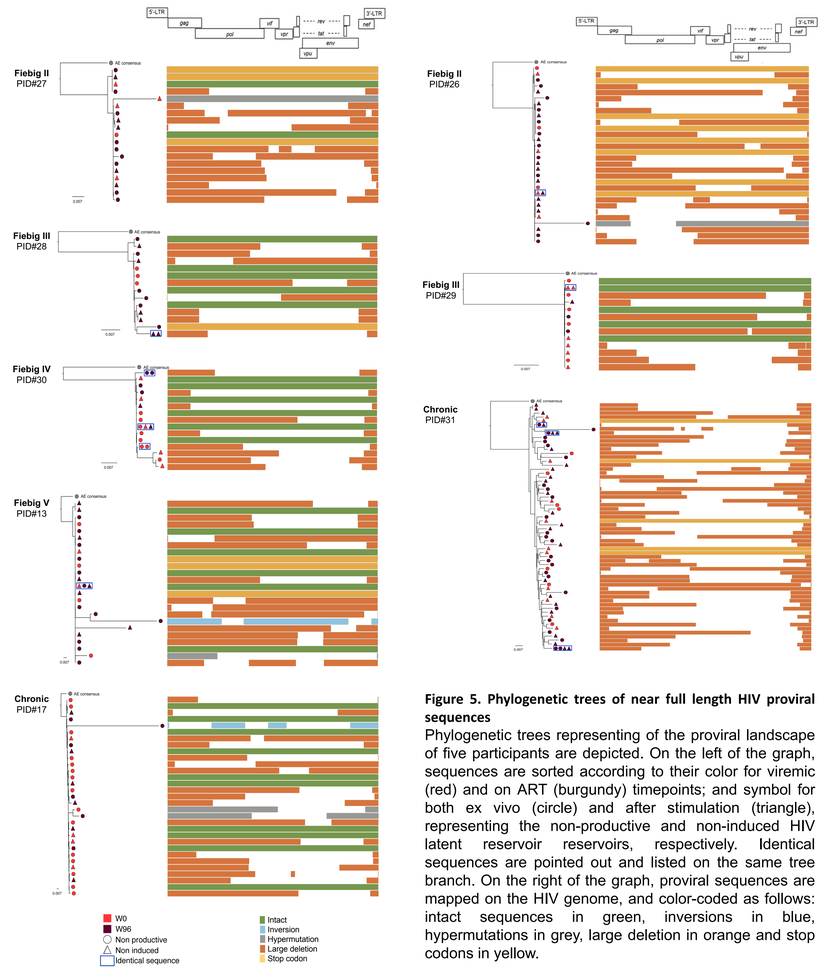

Figure 5. Phylogenetic trees of near full length HIV proviral sequences
Phylogenetic trees representing of the proviral landscape of five participants are depicted. On the left of the graph, sequences are sorted according to their color for viremic (red) and on ART (burgundy) timepoints; and symbol for both ex vivo (circle) and after stimulation (triangle), representing the non-productive and non-induced HIV latent reservoir reservoirs, respectively. Identical sequences are pointed out and listed on the same tree branch. On the right of the graph, proviral sequences are mapped on the HIV genome, and color-coded as follows: intact sequences in green, inversions in blue, hypermutations in grey, large deletion in orange and stop codons in yellow.

Conclusions

Near-full length HIV genomes sequencing in longitudinal samples from early treated individuals revealed that latent proviruse refractory to in vitro reactivation, including genetically intact genomes, are already archived early in infection.
Collectively, these data indicate that a pool of latently-infected cells harbouring intact HIV proviruses that are refractory to *in vitro* latency reversal is established early during acute infection and can persist during ART.

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Disclaimer

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