Immune Selection of HIV-1 Reservoir Cells After Early ART Initiation

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BACKGROUND

- Antiretroviral treatment (ART) initiation during the early stages of HIV-1 infection is associated with a higher probability of maintaining drug-free viral control during subsequent treatment interruptions[1], for reasons that remain unclear.
- Prior work demonstrated that viral reservoir cells decay faster when ART is initiated soon after viral transmission, likely due to host immune responses that may be more effective in eliminating virally-infected cell during early stages of viral infection[2,3]. However, how antiviral immune responses can interface with, engage and target viral reservoir cells following early antiviral treatment initiation is mostly unclear.

METHODS

RIVER study is a randomized-controlled human clinical trial evaluating the effect of ART-only versus ART plus kick and kill on markers of the HIV reservoir in early-treated infection.

- 10 RIVER study participants (n=5 from the ART-only group, n=5 from the treatment group) were studied using PBMC samples from randomization, 18 weeks, and 1 year time points.
- Proviral landscapes were analyzed by near full-length proviral sequencing (FLIP-seq) and matched integration site and proviral sequencing (MIP-seq).
- Phenotypic and proviral sequencing (PheP-seq) was used to investigate the phenotype of memory CD4 T cells from 3 participants.

RESULTS

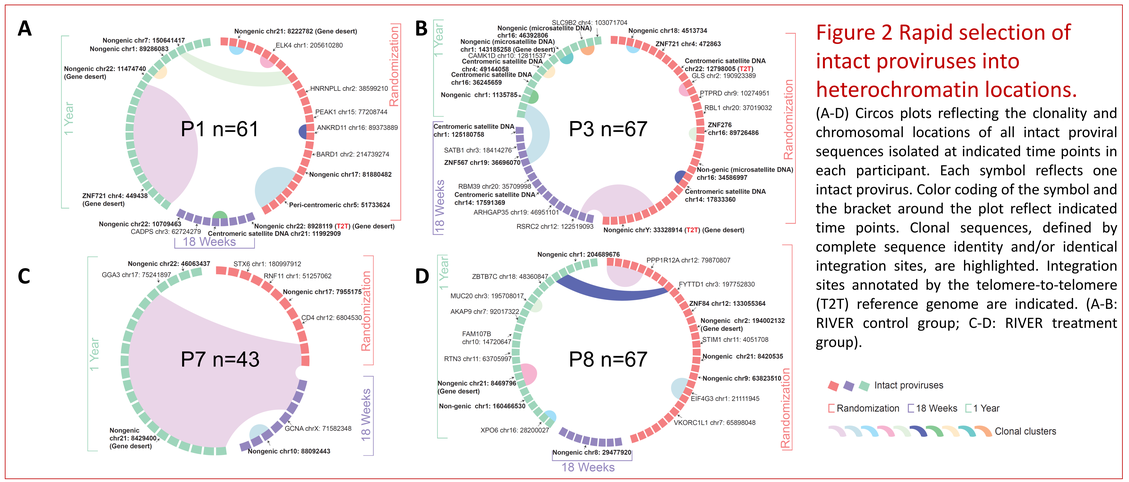

Figure 2 Rapid selection of intact proviruses into heterochromatin locations.

(A-D) Circos plots reflecting the clonality and chromosomal locations of all intact proviral sequences isolated at indicated time points in each participant. Each symbol reflects one intact provirus. Color coding of the symbol and the bracket around the plot reflect indicated time points. Clonal sequences, defined by complete sequence identity and/or identical integration sites, are highlighted. Integration sites annotated by the telomere-to-telomere (T2T) reference genome are indicated. (A-B: RIVER control group; C-D: RIVER treatment group).

Figure 3 No immune selection of defective proviruses.

(A-D) Circos plots reflecting the clonality and chromosomal locations of all defective proviral sequences isolated at indicated time points in each participant Each symbol represents one defective provirus. Color coding of symbol and the bracket around the plot reflect indicated time points. Clonal sequences, defined by complete sequence identity and/or identical integration sites, are highlighted. Color-coded arches around the plots indicate types of proviral sequences. (A-B: RIVER control group; C-D: RIVER treatment group).

Figure 1 Proviral reservoir profile in RIVER study.

(A-C) Frequencies of total (A), intact (B), and defective (C) HIV-1 proviruses in PBMCs from the control group (C) and treatment group (T) of RIVER study at indicated time points. Open circles indicate data at the limit of detection. (D) Proportions of clonal intact HIV-1 proviruses (defined as proviral sequences detected at least 2 times) within total intact HIV-1 proviruses from RIVER participants at indicated time points, and in ART and EC reference cohorts. (E) Pie charts reflecting the composition of HIV-1 DNA sequences from RIVER participants at indicated time points. (F) Average genetic distance of intact proviruses in each participant, determined by pair-wise comparisons between all unique intact proviruses within a given study participant. (G) Number of HLA-associated adapted mutations within intact proviral sequences from all subjects. Only clade B sequences were included and clonal sequences were counted once. (A-G) Data from people treated with antiviral therapy for a median of 9 years (ART) and from elite controllers (EC) are shown for reference.

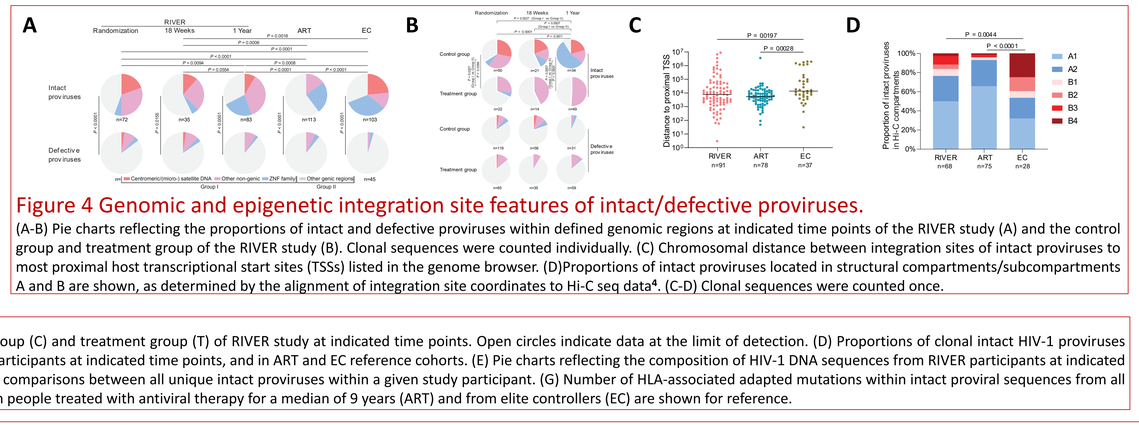

Figure 4 Genomic and epigenetic integration site features of intact/defective proviruses.

(A-B) Pie charts reflecting the proportions of intact and defective proviruses within defined genomic regions at indicated time points of the RIVER study (A) and the control group and treatment group of the RIVER study (B). Clonal sequences were counted individually. (C) Chromosomal distance between integration sites of intact proviruses to most proximal host transcriptional start sites (TSSs) listed in the genome browser. (D)Proportions of intact proviruses located in structural compartments/subcompartments A and B are shown, as determined by the alignment of integration site coordinates to Hi-C seq data[4]. (C-D) Clonal sequences were counted once.

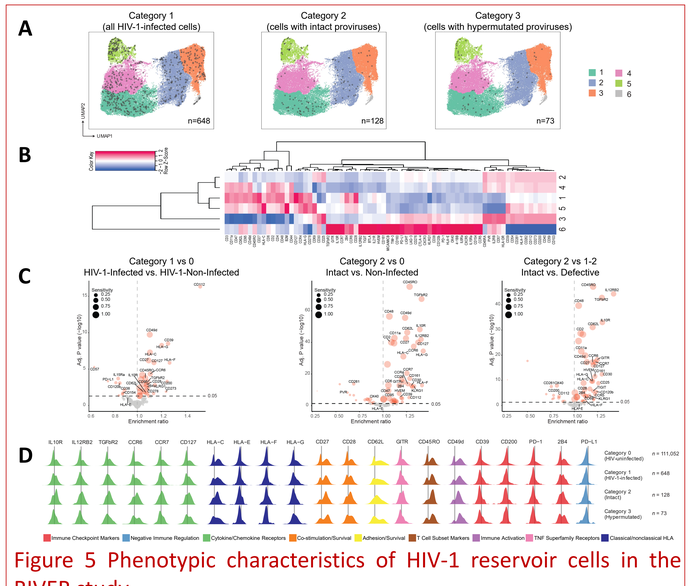

Figure 5 Phenotypic characteristics of HIV-1 reservoir cells in the RIVER study.

(A) Two-dimensional UMAP diagrams reflecting the global phenotypic profile of HIV-1 reservoir cells in memory CD4 T cells isolated from peripheral blood of three RIVER study participants. (B) Heatmap representing the normalized phenotypic profile of cells in each spherical cluster, based on 72 surface markers included in this study. (C) Volcano plots reflecting the enrichment ratio of marker-positive cells and corresponding FDR-adjusted (adj.) p-values for all 72 surface markers included in this study. Marker sensitivities, defined as the proportions of marker-positives cells in the indicated categories of cells, were indicated by dot sizes. (D) Density plots reflecting the expression of selected surface markers on indicated categories of HIV-1-infected cells from the three RIVER study participants.

CONCLUSIONS

- Early ART commencement is frequently associated with accelerated and efficient selection of genome-intact HIV-1 proviruses in repressive chromatin locations during the first year after treatment initiation.
- This selection process was unaffected by vaccine-induced HIV-1-specific T-cell responses.
- PhePseq results show that cells harboring intact HIV-1 displayed a discrete phenotypic signature of immune selection by innate immune responses, characterized by a slight but significant upregulation of HLA-C, HLA-G, HLA-F and other markers involved in innate immune regulation.
- Accelerated immune selection of viral reservoir cells already occurred during early-treated HIV-1 infection and seems partially driven by innate immune responses.

REFERENCE

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