Proviral Landscape in HIV-1 Post-Treatment Controllers and Non-Controllers

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BACKGROUND

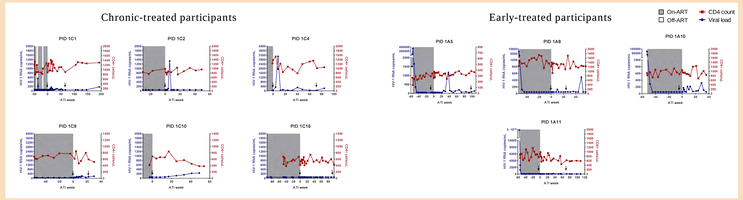
• Despite years of suppressive ART, the vast majority of HIV-infected indiviuals will experience viral rebound during an analytic treatment interruption (ATI).

• HIV post-treatment controllers (PTCs) represent a natural model of sustained HIV remission, where the virus is suppressed even after ART discontinuation. These individuals are rare and little is known about their viral reservoir.

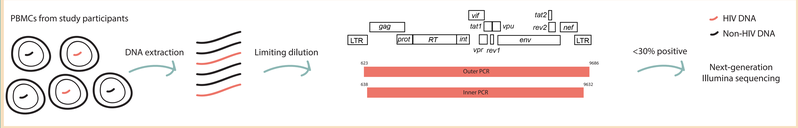
• Our aim was to survey their proviral landscape and contrast it to post-treatment non-controllers (NCs). We also assessed the relationship between different proviral species, levels of intracellular HIV RNA expression and host immune responses to define the reservoir's functional capacity.

METHODS

Ten PTCs and 16 NCs were identified from prior ACTG ATI trials. PTCs were defined as individuals who were on suppressive ART and after ATI, maintained viral loads ≤400 HIV RNA copies/mL for ≥24 weeks. The median duration of documented viral control was 63 weeks for the PTCs.

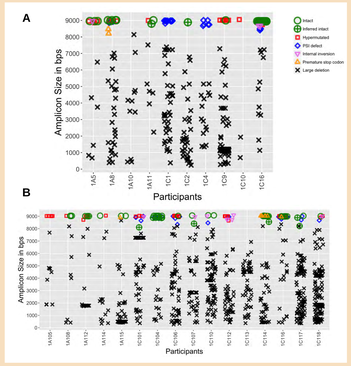


PBMCs were obtained from the pre-treatment interruption (on-ART) and near-full length proviral sequencing was performed by Illumina next-generation single-genome sequencing (NG-SGS). Unspliced CA-RNA levels were quantified by qPCR. T and NK cell phenotypes were assessed by flow cytometry and T cell intracellular cytokine staining was performed on PBMCs stimulated with an HIV *gag* peptide pool.



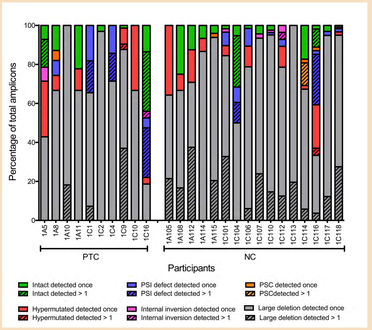
RESULTS

Overview of proviral genome dataset



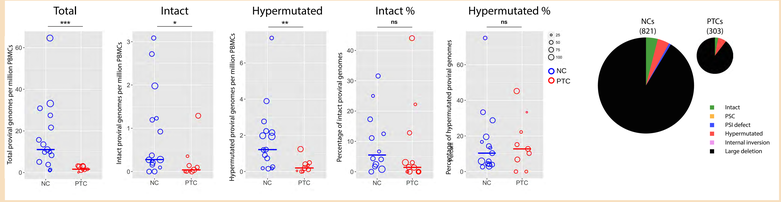
Pre-treatment interruption time point: 1124 total proviral genomes (median of 50 genomes per participant). The vast majority of proviral genomes are defective. (A) represents PTC sequences, (B) represents NC sequences.

Proportion of clonally-expanded proviruses



No significant differences between PTCs and NCs in the proportion of proviral genomes detected more than once, likely representing clonally-expanded cells. This was true regardless of whether they were harboing intact or defective sequences.

Comparison of proviral reservoir size between PTCs and NCs

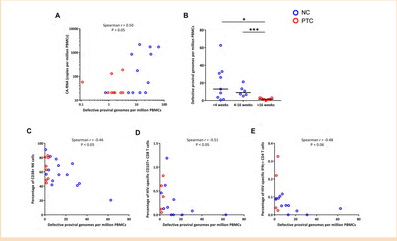


• PTCs had approximately 7-fold lower levels of total proviral genomes (TPGs, PTCs vs. NCs: median 1.6 vs. 11.1 copies/10^6 PBMCs, P<0.001).

• PTCs had lower levels of intact proviral genomes (IPGs, 0.04 vs. 0.28 copies/10^6 PBMCs, P<0.05), defective proviral genomes (DPGs, 1.5 vs 10.8 copies/10^6 PBMCs, P<0.001) and hypermutated proviral genomes (0.2 vs. 1.2 copies/10^6 PBMCs, P<0.01).

• Levels of TPGs were the best reservoir marker to differentiate between PTCs and NCs, as 81% of NCs vs. 0% of PTCs had TPGs >4 copies/10^6 PBMCs.

Correlation of reservoir size with viral rebound and immune parameters

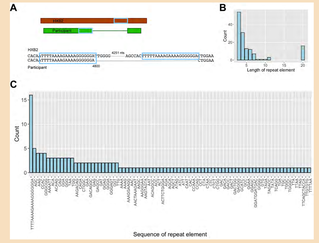


• Prior to ATI, the number of DPGs was associated with levels of unspliced CA-RNA as quantified by qPCR.

• The number of DPGs predicted the timing of viral rebound post-ATI.

• Higher CD38+ NK cell percentages, as well as levels of HIV-specific CD107+ CD8+ cells were associated with lower numbers of DPGs, but not with IPG copies.

Detection of repeat elements at HIV proviral deletion junctions



One specific repeat element (TTTTAAAAGAAAAGGGGGGA) flanked the deletion junctions in 16 different proviral sequences originating from 8 study participants (2 PTCs and 6 NCs).

CONCLUSIONS

• Quantification of the total proviral genome copy numbers before treatment interruption predicts2 which participants are likely to exhibit post-treatment control.

• The vast majority of proviral genomes amplified from both PTC- and NC-derived DNA were defective and only a small proportion were intact.

• No evidence to support that differential rates in clonal expansion of cells harboring intact proviruses mediate the PTC phenotype.

• Our results support the concept that defective HIV genomes lead to viral antigen production and interact with both the innate and adaptive immune systems.

ACKNOWLEDGMENTS

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