Short-term ART Interruption has Little Effect on Levels of Integrated Proviral DNA

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Background

- Analytic treatment interruption (ATI) trials are necessary to assess strategies for sustained ART-free remission.
- The impact of ATI on reservoir size after resumption of ART is unclear.
- Levels of integrated HIV DNA can be used to evaluate the effect of interventions on the size of the proviral reservoir.

Objectives

- To validate a novel integrated HIV DNA assay, termed the HIV Integrated DNA size-exclusion (HIDE) assay
- To assess the impact of short-term ATI on the size of the total proviral reservoir

Methods

Assay Validation

- Genomic DNA size-selected for >20kb fragments by the automated BluePippin pulsed-field gel electrophoresis system to eliminate unIntegrated-DNA species\(^1\).
- Proviral DNA levels were measured by qPCR and normalized to cellular input by CCR5 qPCR.
- HIV-negative DNA was spiked with either linear near-full-length HIV amplicons or a 12kb HIV-encoding plasmid to confirm elimination of unIntegrated DNA.
- HIV DNA levels were assayed before and after size-selection in three replication-incompetent HIV-infected cell lines: J-Lat, 8E5 and an integrated DNA standard for the Alu-gag assay.
- HIV-infected cell lines and participant samples were measured by the standard Alu-gag assay for comparison.

Study Samples

- Cryopreserved PBMCs were obtained from 12 participants from 4 previously completed ACTG ATI trials.
- Participants samples were obtained prior to ATI, during ATI and a median of 27 weeks after ART reinitiation and assayed for levels of integrated HIV DNA as described above.

Results

- A median 28% of total input DNA was recovered after size-selection for >20kb.

- BluePippin eliminated 99% of unIntegrated HIV DNA species spiked into HIV-negative DNA.

- Levels of HIV DNA did not change after size-selection for genomic DNA in cell lines containing integrated, replication-incompetent HIV DNA (mean ratio total:size-selected 1.02).

- Levels of HIV DNA before and after HIDE

- Levels of HIV DNA after ART Resumption

Conclusions

- The HIDE assay measures integrated HIV DNA levels using an automated size-selection system that is less sample and labor-intensive than current assays.
- Despite an increase in integrated DNA levels during the ATI, the viral reservoir subsequently was reduced to pre-ATI levels after approximately 6 months of ART.

Implications

- The HIDE assay is a useful technique that can be applied to quantify levels of total integrated HIV DNA.
- Short-term ART can be conducted without causing a significant, long-term effect on the size of the total proviral reservoir.

Acknowledgements

- We are grateful for the contributions of the participants who made this study possible. We thank the staff and principal investigators of the ACTG studies A371, A5068, A5170, and A10197. We thank Ronald Bosch and Eugenia Agha at the Harvard School of Public Health for their help and advice. We appreciate the support of the MIT BioMicrocenter and the Tabora and Kuritzkes labs for their valuable feedback. This work was supported in part by National Institute of Allergy and Infectious Diseases of the National Institutes of Health grants AI03194 (to J.Z.L), AI019004 (Statistical and Data Management Center of the AIDS Clinical Trials Group), AI088350 (AIDS Clinical Trials Group), UM1 AI047701, and a subcontract from AI003830 to the Harvard Virology Support Laboratory (to D.R.K.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. J.Z.L. has received research support and served as a consultant for Gilead Sciences and Merck.

References


Table 1: Median Participant Characteristics

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<tr>
<th>ART Duration prior to ATI (years)</th>
<th>ATI Duration (weeks)</th>
<th>ATI Duration post-ATI (weeks)</th>
<th>Plasma viral load prior to ATI (c/mL)</th>
<th>CD4+ count prior to ATI (per mm(^3))</th>
<th>Spearman ( r = 0.94 )</th>
<th>( P = 0.02 )</th>
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<td>3.9</td>
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<td>27</td>
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Statistical Analysis

- Wilcoxon matched-pairs signed rank test was used in analysis of pre- and post-ATI integrated DNA levels.