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

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.
- Proportional 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 ampiclons 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, BES 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.

Table 1: Median Participant Characteristics

<table>
<thead>
<tr>
<th>ART duration prior to ATI (years)</th>
<th>ATI Duration (weeks)</th>
<th>ART duration post-ATI (weeks)</th>
<th>Plasma viral load prior to ATI (cp/mL)</th>
<th>CD4+ count prior to ATI (per mm³)</th>
<th>Spearman r</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.9</td>
<td>12</td>
<td>27</td>
<td>&lt;50</td>
<td>852</td>
<td>0.94</td>
<td>0.02</td>
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</tbody>
</table>

Statistical Analysis

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

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 were higher in total (red) vs. size-selected (black) DNA across all timepoints, especially during the treatment interruption (representative participant shown).

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 quantifying levels of total integrated HIV DNA.
- Short-term ATI can be conducted without causing a significant, long-term effect on the size of the total proviral reservoir.

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References