The HIV Latent Reservoir in Ugandans: Implications for HIV Cure

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Acknowledgements

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Andrew Redd
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Adam Capoferri
Robert Siliciano
Janet Siliciano
Aggrey Anok
Jingo Kasule
Taddeo Kityamuweesi
David Serwadda

And the Rakai Health Sciences Team and Participants!!
Dynamics of HIV-1 Replication in Patients on ART Therapy

- **Start Rx**
- **First phase** ($t_{1/2} = 1$ day)
- **Second phase** ($t_{1/2} = 2$ weeks)

**Limit of Detection**: 50 copies/ml

1) Viral replication not suppressed by HAART
2) Viral reservoirs

**x-axis**: Time on HAART (days)

**y-axis**: Plasma HIV-1 RNA (copies/ml)
Latent Reservoir Poses the Greatest Barrier to Cure

$t_\frac{1}{2} = 44.2$ months
73.4 years

Latent Viral Reservoir (LVR) in Sub-Saharan Africans

- HIV cure research is dependent on accurate measurements of the LVR. However, no studies had previously quantified LVR in sub-Saharan Africans.

- High burden of endemic infections and other regional differences (viral subtype) may affect size of the LVR and efficacy of cure strategies.

- Quantitate the LVR size and dynamics over time, and measure correlates of immune parameters in SSA in order to tailor cure strategies as they develop.
Study Populations

• Rakai, Uganda:
  – 70 HIV+ individuals on ART; >two VL <40 copies 12-18 months apart.
    • LVR quantification: Quantitative viral outgrowth assay (Q-VOA)
    • Retested annually for 5 years to determine decay curves

• Baltimore, USA:
  – 51 Moore Clinic patients studied using same techniques (Q-VOA)
    • Decay curves already calculated
Frequency of Resting CD4+ T cells Infected with Latent, Replication-Competent HIV-1 in Americans and Ugandans as Measured by QVOA

• Isolates were sequenced in gp41 and pol using MiSeq NGS sequencing protocol
• No difference in IUPM between A, D, recombinants (p=0.3)
  – A: median = 0.46 IUPM (IQR: 0.21 – 1.55 IUPM)
  – D: median = 0.34 IUPM (IQR: 0.15 – 0.79 IUPM)
  – Recombinants: 1.10 IUPM (IQR: 0.24 – 2.20 IUPM)
• Continuing to sequence additional outgrowth wells from all participants for clonality

Prodger et al., Clin Infect Dis. 2017
Direct Correlation of Reservoir Size (IUPM) with Set-point Viral Load and Inverse Correlation with Time Virally Suppressed

Slopes not significantly different, p=0.3
Latent Reservoir Size by Gender

• In this original study, Ugandan women had a much smaller reservoir size than American women, but the difference was not significant due to the few women in the US study.

• Thus we expanded the study to include a total of 90 Ugandans (57 women and 33 men).

• Ugandan women had a significant lower median reservoir size (0.53 IUPM) compared to men (1.01 IUPM) (p<0.01).
# Ugandan Study of HIV Latent Reservoir by Sex

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Females (n = 57)</th>
<th>Males (n = 33)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41.1 (37.4, 47.2)</td>
<td>44.2 (40.3, 47.1)</td>
<td>0.15</td>
</tr>
<tr>
<td>Subtype, n (%)</td>
<td></td>
<td></td>
<td>0.76</td>
</tr>
<tr>
<td>A</td>
<td>9 (15.8)</td>
<td>5 (15.2)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2 (3.5)</td>
<td>1 (3.0)</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>26 (45.6)</td>
<td>17 (51.5)</td>
<td></td>
</tr>
<tr>
<td>A/D</td>
<td>7 (12.3)</td>
<td>3 (9.1)</td>
<td></td>
</tr>
<tr>
<td>A/F</td>
<td>1 (1.8)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>A/C</td>
<td>0</td>
<td>1 (3.0)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>12 (21.1)</td>
<td>6 (18.2)</td>
<td></td>
</tr>
<tr>
<td>Pre-ART Viral Load ($\log_{10}$ copies/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females (n = 47); Males (n = 28)</td>
<td>4.62 (3.88, 4.93)</td>
<td>4.72 (4.17, 5.22)</td>
<td>0.18</td>
</tr>
<tr>
<td>Nadir CD4+ T cell count (cells/μL)</td>
<td>180 (109, 232)</td>
<td>168 (129, 238)</td>
<td>0.92</td>
</tr>
<tr>
<td>Time on ART (years)</td>
<td>7.0 (5.3, 8.5)</td>
<td>6.9 (3.3, 9.3)</td>
<td>0.86</td>
</tr>
<tr>
<td>CD4+ T cell count at QVOA (cells/μL)</td>
<td>594 (461, 740)</td>
<td>458 (380, 559)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CD4+/CD8+ T cell ratio at QVOA</td>
<td>0.89 (0.65, 1.12)</td>
<td>0.63 (0.56, 0.84)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Viremic time (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females (n = 16); Males (n = 16)</td>
<td>6.1 (4.2, 10.2)</td>
<td>5.6 (3.7, 7.8)</td>
<td>0.46</td>
</tr>
</tbody>
</table>
Frequency of Viral Outgrowth by Sex

The graph illustrates the frequency of viral outgrowth (IU/PM) by sex. The x-axis represents sex (Female vs. Male), and the y-axis represents the frequency of viral outgrowth on a logarithmic scale. The data points indicate a statistically significant difference between the sexes, as indicated by the p < 0.01 notation.
Measurement of HIV DNA gag per million cells
Proportion of Reactivated HIV per DNA

The figure shows the proportion of reactivated HIV per DNA for females and males. The x-axis represents sex (Female and Male), and the y-axis represents the proportion of reactivated HIV virus on a logarithmic scale. The p-value is 0.06.
Summary and Future Studies

- HIV latent reservoir was smaller in Ugandans compared to Americans, and differed significantly by gender among Ugandans, but not by subtype.

- Further studies on gender differences in latent reservoir activation.
  - As shown by others, estrogen receptor-1 is a key regulator of HIV-1 latency that imparts gender-specific restrictions on the latent reservoir (Das et al, PNAS 2018; Scully et al, JID 2019).
  - Accurate measurements of intact, replication-competent virus, total integrated viral DNA and intact proviral DNA (IPDA).

- Sequencing of the viral outgrowths for clonality and timing with ARV use.