No Evidence Of Ongoing Replication In Tissue Compartments During cART

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Background: Understanding HIV Persistence is Key to Curative Strategies

- Combination antiretroviral therapy (cART) controls but does not cure HIV
- Mechanisms responsible for persistence may be diverse

Proposed Mechanisms Contributing to HIV Persistence During cART

**PRIMARILY VIRAL**
- Proviral integration into long lived cells
- Cycles of viral replication

**PRIMARILY HOST**
- Immune response
- Cycles of cell replication (clonal expansion)
- Sanctuary
Background: HIV Genetic Diversity Increases Over Time

Overall diversity (APD) = 0.35%

Early Diagnosis

APD = 1.1%

+ 32 Months

Composite

0.005 6 nt

0.005 6 nt

0.005 6 nt
Hypothesis and Objective

HYPOTHESIS

Ongoing cycles of HIV replication are detectable in tissues by accumulation of genetic change

OBJECTIVE

Analyze HIV in tissue and blood compartments in individuals undergoing long term cART
Methods

Colonoscopy, healthy
[N = 2]

- Plasma, PBMC (longitudinal) and Gut-Associated Lymphoid Tissue (GALT) nucleic acid extraction
- Single Genome Sequencing (SGS, pro-pol, c. 1200 nt)
- Phylogenetic analysis (Neighbor Joining)
- Population genetics analysis

Autopsy, Primary Effusion Lymphoma
[N = 1]

- PBMC (longitudinal) and tissue nucleic acid extraction
- Quantification of HIV DNA (RT-PCR, ddPCR)
- Single Genome Sequencing (SGS, pro-pol, c. 1200 nt)
- Phylogenetic analysis (Neighbor Joining)
- Population genetics analysis
### Study Population

<table>
<thead>
<tr>
<th>PID</th>
<th>Age (y)</th>
<th>Gender</th>
<th>Duration of infection (y)</th>
<th>Duration suppressed prior to tissue sampling (y)</th>
<th>Baseline HIV RNA (Log₁₀ copies/ml)</th>
<th>HIV RNA at tissue sampling (Log₁₀ copies/ml)</th>
<th>CD4 nadir (cells/µl, %)</th>
<th>CD4 at Tissue sampling (cells/µl, %)</th>
<th>cART at tissue sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>M</td>
<td>16</td>
<td>16</td>
<td>5.4</td>
<td>&lt;1.6</td>
<td>242 (25)</td>
<td>560 (42)</td>
<td>TDF/FTC/RPV</td>
</tr>
<tr>
<td>2</td>
<td>58</td>
<td>M</td>
<td>16</td>
<td>14</td>
<td>5.5</td>
<td>&lt;1.6</td>
<td>253 (13)</td>
<td>280 (30)</td>
<td>TDF/FTC/EFV</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>M</td>
<td>8</td>
<td>8</td>
<td>5.2</td>
<td>&lt;1.6</td>
<td>50 (12)</td>
<td>50 (12)</td>
<td>ABC/3TC/DTG</td>
</tr>
</tbody>
</table>

**cART initiated early after diagnosis**

**Prolonged viral suppression by cART**
No Evidence of Molecular Evolution in GALT over 14-16 years cART

PID1

APD = 0.4%

PID2

APD = 0.2%

No Evidence of Molecular Evolution in GALT over 14-16 years cART
Participant 3: Case Summary

2003: HIV negative
11/2007: Diagnosed with HIV (RNA log 5.2, CD4 538 cells/µl), Kaposi Sarcoma (KS)
12/2007: Started cART with TDF/FTC/EFV, consistently < 50 c/ml plasma
05/2008-2015: Chemotherapy for KS, Castleman’s, Primary Effusion Lymphoma (PEL)
HIV DNA is Detectable in Most Tissues 12 Hours *Post Mortem*

### HIV Recovery

<table>
<thead>
<tr>
<th>Location</th>
<th>HIV DNA (c/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal Lobe</td>
<td>0</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0</td>
</tr>
<tr>
<td>Spinal Cord</td>
<td>1.8</td>
</tr>
<tr>
<td>KS skin lesion</td>
<td>1.9</td>
</tr>
<tr>
<td>Lymph node</td>
<td>75</td>
</tr>
<tr>
<td>Spleen</td>
<td>12</td>
</tr>
<tr>
<td>Jejunum</td>
<td>54</td>
</tr>
<tr>
<td>Ileum</td>
<td>90</td>
</tr>
<tr>
<td>Colon</td>
<td>2</td>
</tr>
<tr>
<td>Lung</td>
<td>52</td>
</tr>
<tr>
<td>Effusion cells</td>
<td>6</td>
</tr>
<tr>
<td>Kidney</td>
<td>7</td>
</tr>
<tr>
<td>Testes</td>
<td>3</td>
</tr>
</tbody>
</table>

### Genetic Analysis

<table>
<thead>
<tr>
<th>Single Genome Sequences</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph node</td>
<td>30</td>
</tr>
<tr>
<td>Spleen</td>
<td>38</td>
</tr>
<tr>
<td>Jejunum</td>
<td>12</td>
</tr>
<tr>
<td>Ileum</td>
<td>23</td>
</tr>
<tr>
<td>Colon</td>
<td>5</td>
</tr>
<tr>
<td>Lung</td>
<td>3</td>
</tr>
<tr>
<td>Effusion cells</td>
<td>10</td>
</tr>
<tr>
<td>Kidney</td>
<td>5</td>
</tr>
<tr>
<td>Testes</td>
<td>1</td>
</tr>
<tr>
<td>Total Tissue</td>
<td>127</td>
</tr>
<tr>
<td>PBMC</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>234</td>
</tr>
</tbody>
</table>
No Evidence Of Molecular Evolution In Tissue Compartments

NO EVIDENCE OF POPULATION SHIFTS OR COMPARTMENTALIZATION

▲ PBMC (6/2013)
△ PBMC (4/2015)
◆ LYMPH NODE
◆ SPLEEN
◆ JEJUNUM
◆ ILEUM
◆ COLON
◆ EFFUSION
◆ KIDNEY
◆ LUNG
◆ TESTES

APD = 0.3%
HIV Infected Cells Undergo Clonal Expansion In PBMC And GALT

Josefsson et al., PNAS 2013
HIV Infected Cells Undergo Clonal Expansion In PBMC And Tissues
Summary

- No evidence of ongoing cycles of HIV replication in tissue compartments and PBMC in study participants undergoing long term cART
  - 8-16 years suppression
  - 450 single genome sequences
- Clonal expansion of HIV infected cells can take place across multiple tissue compartments
  - Strategies to eradicate HIV reservoirs will require elimination of clonally expanded populations
- HIV DNA was recovered from most, but not all, tissues post mortem
  - HIV persistence studies will benefit from autopsy analyses
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– R. Dewar
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