Clonal integration site expansion of infected cells is a main contributor of HIV persistence in more differentiated T cell subsets during suppressive ART.
Background

- The drivers of HIV persistence are still not clearly understood
  - Long lived

- Proliferation
  - Homeostatic
  - Antigen induced
  - Integration site induced

- Clonal expansion of HIV infected cells have been shown *in vivo*\(^1-5\)

- Primary goal: To define the contribution of clonal expansion to HIV persistence in different T cell subsets in vivo on suppressive ART

Method

- HOPE: HOMEostasis of HIV PERSISTence
- Cross sectional study of HIV-infected individuals on ART recruited in UCSF

- Deuterated water labelling
  - Cellular turnover and half-life
  - HIV reservoir size and composition
  - HIV integration sites
  - Intact proviruses
### Baseline characteristics of cohort

<table>
<thead>
<tr>
<th>Characteristics 24 participants</th>
<th>Median [IQR]</th>
</tr>
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<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
</tr>
<tr>
<td>Age</td>
<td>51 [34-56]</td>
</tr>
<tr>
<td>Time to ART from diagnosis, months</td>
<td>17 [3.6-63.4]</td>
</tr>
<tr>
<td><strong>Time on ART, years</strong></td>
<td>3.8 [2.7-11.7]</td>
</tr>
<tr>
<td><strong>Current CD4 count, cells/mm$^3$ blood</strong></td>
<td>578 [445-745]</td>
</tr>
<tr>
<td><strong>Nadir CD4 count, cells/mm$^3$ blood</strong></td>
<td>338 [210-664]</td>
</tr>
<tr>
<td>Ratio CD4:CD8</td>
<td>1.1 [0.8-1.4]</td>
</tr>
<tr>
<td><strong>Plasma HIV RNA, copies HIV RNA/mL</strong></td>
<td>&lt;40</td>
</tr>
<tr>
<td>Integrated HIV DNA, copies/million PBMC</td>
<td>152 [52-396]</td>
</tr>
</tbody>
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HIV integration sites in T cell subsets

Subsets analysed from 24 study participants (HLA-DR-CD38-)

- Naïve
  - TN (CD45RA+CCR7+CD27+CD57-CD95-)
- Stem cell memory
  - TSCM (CD45RA+CCR7+CD27+CD57-CD95+)
- Central memory
  - TCM (CD45RA-CCR7+CD27+)
- Transitional memory
  - TTM (CD45RA-CCR7-CD27+)
- Effector memory
  - TEM (CD45RA-CCR7-CD27-)
- Terminally differentiated
  - TTD (CD45RA+CCR7-)
Methods

Genomic DNA was isolated from CD4+ T cells subsets

- **Mid-tagged sequences check:**
  - Linker, LTR and remaining LTR sequence

- **Alignment:**
  - Blat-UCSC Genome Browser (GRCH38/hg38)

- **Assignment of clonality:**
  - >2 base difference between lengths, ≥ 3 length polymorphisms present
Clonal expansion increases with differentiation

Wilcoxon signed-ranks test
Identical integration sites detected in multiple T cell subsets within a participant and larger clones are found in more differentiated cells.

Wilcoxon signed-ranks test
Gene ontology an Kegg pathway

- Gene Ontology: the framework for the model of biology. The GO defines concepts/classes used to describe gene function.

- Kyoto Encyclopedia of Genes and Genomes (KEGG)
  - KEGG pathway identifies the pathway where HIV integrates and determines enrichment by hypergeometric statistical tests with a false positive correction in a total of over 10,000 different pathways
HIV integrated genes and pathways differs in the T cell subsets

GO
- Immune function
- Epigenetic regulation
- Amino acid metabolism
- Apoptosis
- Organelle localization
- Transcription regulation
- DNA repair
- Protein processing
- Transport
- Viral replication

KEGG
- MAPK signalling/transcription
- Proliferation
- Protein transport
- Positive regulation of gene expression
- Negative regulation of gene expression
- Chemokine and interleukin signalling
- Immune cell activation and differentiation
- Migration
- Amino acid synthesis
- Apoptosis
- Cell stress and senescence
- Response to pathogens
HIV integrated genes differs in single vs clonal and large clones are enriched for cancer genes

KEGG
Chromatin features of single integration sites compared to clonal integration sites

- Compared of single vs clonal integration site location to epigenetic data available through [www.roadmapepigenomics.org](http://www.roadmapepigenomics.org)

- Single integration sites are:
  - Less commonly integrated into accessible genomes in activated and resting genes
  - More commonly integrated into repressed and quiescent genes in activated and resting genes

Single vs Clonal:

Log2 (odds ratio)

Depreciated | Enriched

- Activated transcription start site
- Bivalent transcription start site
- Flanking bivalent enhancer
- Bivalent enhancer
- Repressed polycomb
- Repressed weak polycomb
- Quiescent gene
- Flanking transcription start site
- Flanking highly transcribed gene
- Highly transcribed gene
- Weakly transcribed gene
- Genic enhancer
- Enhancer
- Zinc Finger Nuclease / repeats
- Heterochromatin

- Accessible genome
- Repressed gene

Independent fishers exact test in R
Conclusions and implications

- T cell subsets demonstrate more clonal expansion when differentiated
  - Larger clones are found in more differentiated cells and are enriched in genes associated with cancer

- Identical integration sites in multiple T cell subsets supports the idea that HIV-infected cells can differentiate and still persist on ART

- Integrated pathways between subsets and single vs clonally expanded cells are distinct
  - Integration in more differentiated cells compared to integration in less differentiated cells occurs more commonly in pathways associated with specific antigens and are often clonally expanded

- Single integration sites are in more inaccessible transcriptionally repressed genes than clonal integration sites

- Our data implies that there is a difference in potential mechanisms leading to HIV persistence in **single** and **clonal** integration sites