The Challenge of an HIV Cure

HIV provirus integration and expression on long-term suppressive therapy

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Objectives

1. To understand the role of HIV in causing AIDS

2. To understand how antiretroviral drugs control, but do not cure HIV infection

3. To understand the role of persistently infected, dividing, cells in HIV persistence
Disclosures

1. Financial:
   Tocagen, Inc.  SAB member and shareholder

2. Off-label or unapproved drug recommendations:
   None
Global Burden of HIV Infection

Total: 36.7 million [34.0-39.8 million]

People undergoing antiretroviral therapy: 17 million

UNAIDS, 2015

WHO, 2015
Challenges of HIV Infection

• Current antiviral therapies that can fully suppress viral replication and allow infected people to live a mostly normal life, but there are still both individual and global challenges.
• Only about half the affected population has access to drugs, particularly in the worst-hit areas.
• Effective means of blocking transmission (PREP) are known, but not widely used. A vaccine is still a dream.
• Development of resistance to antiviral drugs is still a major issue, particularly in poorly-resourced areas.
• Antiviral drugs effectively suppress HIV infection, but will never cure it.
Timothy Brown was cured with a bone marrow transplant.

Why can’t we cure HIV Infection in everyone?
HIV-1 DNA Decays Much Less Than Plasma Virus RNA after Initiating ART

- **Phase I**: t½ = 1.5 days
- **Phase II**: t½ = 28 days
- **Phase III**: t½ = 273 days
- **Phase IV**: t½ = ∞

Plasma HIV-1 RNA (copies/ml) vs. Years on ART

- **Clinical LOD**: (50 copies/mL)
- **Interrupt therapy**

HIV DNA in PBMC (relative to start of ART)

HIV-1 DNA decays much less than plasma virus RNA after initiating ART.

Courtesy Ben Hilldorfer, UPitt
Two Features of HIV Biology are Helpful in Understanding Persistence

• Generation of genetic diversity during ongoing virus replication.
• Stable integration of DNA copy (provirus) of the viral genome at one of millions of sites in the host cell DNA
• The first important point is to distinguish between two models: ongoing low-level replication and latent proviruses in long-lived cells.
Inferring Virus Replication from Genetic Diversity
Clonal Expansion of RNA and DNA Sequences During Suppressive ART (NO Evidence for Evolution)

Different clonal populations of RNA and DNA appear after years of therapy.

*Highly variable from patient to patient
No Evolution from Pre-therapy in Rebound Viremia After Long-term ART

PT 3
- pre- Rx (0.7%)
- 7 yr rebound (0.8%)

divergence = 0.2%

Pt 4
- pre- Rx (1.0%)
- 5 yr rebound (0.6%)

divergence = 0.01%
Is There Ongoing HIV Replication on ART?

• The problem with the previous studies was that the high diversity of HIV in chronically-infected individuals made it difficult to detect additional diversification on ART.
• Therefore we studied HIV populations in a set of infected people who were diagnosed and started on antiretroviral therapy (ART) within a few weeks of infection, when the virus population has had very little time to evolve.
• Although very difficult to identify, these patients provide a much stronger signal to detect HIV evolution.
No Evidence for Ongoing HIV Replication on ART?  
(At least in blood)

CH 62-1  
APD=0.001%  
Hypermunets:  
7Δ  7Δ  

CH 68-5  
APD<0.001%  
Hypermunets:  
0Δ  4Δ  

CH 84-4  
APD<0.001%  
Hypermunets:  
5Δ  4Δ  

CH 91-4  
APD=0.001%  
Hypermunets:  
1Δ  5Δ  

△ Pre-ART  
△ 2.8 – 3.7 years on ART
Summary and Implications

• No evidence for on-going cycles of viral replication in individuals fully suppressed on ART
• Implies that the HIV reservoir is not maintained by on-going cycles of viral replication and therefore developing more potent ART will not cure HIV infection
• Others have proposed that ongoing replication during ART is not seen in blood, but does occur in the lymphoid compartment

• What’s going on in lymph nodes?
Does HIV Replication Persist in Lymphoid Tissues During ART?

• Sequenced paired lymph node and PBMC samples in four suppressed individuals and compared to pre-ART populations

• Sequenced longitudinal lymph node samples from individuals on ART
Proviral Populations Are Not Different in PB and LN After 5 Years of Suppression on ART

Diversity:
- Pre ART PB: 0.5%
- Long-term ART PB: 0.1%
- Long-term ART LN: 0.3%
No Difference in HIV Populations Across Lymph Nodes

Panmixia: $p=0.6$
Diversity: Right LN: 1.6%
Left LN: 1.3%
Summary and Implications

• No evidence for on-going cycles of viral replication in lymph nodes of adults fully suppressed on ART

• No evidence for “compartmentalization” of infected cells among lymph nodes and peripheral blood

• The HIV reservoir is not maintained by on-going cycles of viral replication and therefore developing more potent ART will not cure HIV infection

• Are identical sequences expanded clones or are they identical HIV variants (founder virus maybe?) with different integration sites?
NO Evidence for a Role of HIV Replication in Maintaining the True Reservoir

What does maintain the reservoir?
The Persistent Steady State

Pre-ART

Short - lived cells

Long - lived cells

ART

Erosion

Proliferation

The number of infected cells remains about the same, but clonal populations appear
Integration Site Preferences 1.

• HIV DNA can irreversibly integrate at many millions of possible sites in the cell genome, and sites of integration can uniquely “tag” single infected cells and their progeny.

• In long-lived HIV-infected cells, sites of integration are determined both by initial preferences (i.e., “hot spots”), by selection after integration, and by chance.

• The assay we used (thanks to Rick Bushman and Charles Bangham) involves shearing infected cell DNA, ligating linkers, PCR amplification with LTR and linker-specific primers, and paired-end Illumina sequencing.

• Integration site is adjacent to LTR primer, and breakpoint is net to linker-specific primer.

• Multiple sequences with the Identical integration site and multiple breakpoints imply clonal expansion of the cell after infection.
Multi-Scale Analysis of HIV Integration in and ex Vivo

• We have assessed integration site distribution in cultured cells (PBMC, stem cells, various cell lines (>150,000 sites each) or in HIV infected patients during suppressive ART, and compared with gene expression (RNA-seq).

• In the next slides, cumulative integration sites in each interval of chromosome 16 are shown in red, and the expression level of each gene or region in blue.

• As you will see, the distribution of integration sites is highly similar, even between freshly isolated PBMCs and highly aneuploid epithelial cell lines, like HeLa.
A. Whole Chromosome 16 (350 kb/bin)
B. Chromosome 16 ca 10X from A (40 kb/bin)

Jurkat Chromosome 16, 9263001-19263000

HeLa Chromosome 16, 9263001-19263000

HEK 293 Chromosome 16, 9263001-19263000

PHA+ PBMC Chromosome 16, 9263001-19263000

Patient 1 Chromosome 16, 9263000-19262999

All Patients Chromosome 16, 9263000-19262999
C. Chromosome 16 10X from B (4kb/bin)
Integration Site Preferences 2. Selection for Specific Regions

• In long-lived HIV-infected cells, sites of integration are determined both by initial preferences (i.e., “hot spots”) and by selection after integration.

• Integrations in MKL2 (and a couple of other genes) in patient 1 are clearly due to selection for preferential growth or survival due to effects of provirus on host gene expression, reminiscent of well-known models of retroviral oncogenesis.

• Only a very small fraction of proviruses seem to be involved in such effects. What does the overall distribution look like?
Integration Site Analysis Identifies Highly Expanded Clones in Patient 1

N=1726

Expanded clones with fewer than 7 integrants

<table>
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<tr>
<th>Gene</th>
<th># of integrants</th>
<th>% of total integrants</th>
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<tr>
<td>Ambiguous</td>
<td>55</td>
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<tr>
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<tr>
<td>MKL2b</td>
<td>22</td>
<td>1.3</td>
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<td>ATP6V1G3</td>
<td>17</td>
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<tr>
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<td>DDX6</td>
<td>11</td>
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<tr>
<td>FSIP1</td>
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<tr>
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<tr>
<td>STAT5B</td>
<td>7</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Maldarelli et al. Science 2014
Are **all** HIV Integrations in Patients on ART in Expanded Clones?

- We think it likely that they are, but we sample only about $10^{-6}$ of the CD4+ T cell population.

- We might be able to figure it out if we knew the underlying distribution of clones, but this is a very difficult problem...
What does the City Look Like?
Oh
Clonal Expansion of Infected Cells can Arise in Several Ways

- Direct effects of the integrated provirus on cell multiplication or survival
- Chance outgrowth of a memory cell during homeostatic replacement
- Antigen driven expansion during an active immune response

The latter two effects have nothing to do with the provirus, but it serves to mark all descendants of an originally infected cell.
Case Report: Patient 1:
Persistence and Rebound of HIV Viremia on cART
HIV Viremia: Persistence and Rebound on cART

Prior to change in cART

After change in cART

Therapy switch

WT Virus

WT Virus ONLY

M184V K103N Resistance Mutations Present

HIV RNA

CD4 (cells/ul)

Prior to change in cART

After change in cART

100

100

100

100

1x10^5

1x10^6

1x10^7

7/10/11
9/09/11
11/07/11
1/06/12
3/06/12
5/05/12
7/01/12
AMBI-1 Full Provirus is Intact

Amplify in overlapping fragments and sequence

Encodes Infectious virus!
The Predominant Virus in Plasma is Produced by the AMBI-1 Provirus

P6-RT Sequences
- AMBI-1 provirus
- HIV RNA 12.14.09 Plasma
- HIV DNA 12.09.11 CD4 DNA
- HIV RNA 12.09.11 Plasma
- HIV RNA 07.23.12 Plasma
- HIV RNA 08.31.12 Plasma

*Bootstrap values > .85

Log copies/ml

Date

Predominant Plasma Clone (PPC)

*Bootstrap values > .85
A Highly Expanded HIV Clone Carries an Infectious Provirus

This is the first known case where we could identify and characterize a clone of latently-infected cells responsible for infectious virus in blood.

Only a small fraction of cells express virus RNA (not shown). What is the epigenetic state of this provirus? (We don’t know yet. It’s not DNA methylation)

What is driving clonal expansion? (We know a little bit)
Despite the potential for viral cytopathic effect and immune-mediated killing of expressing cells, cell clones can both expand and harbor intact proviruses that produce infectious virus over time.

Ca 1% of cells with the AMBI-1 provirus express small amounts of RNA at any one time.

(M. Kearney)
Summary

• We found no evidence for either ongoing replication or compartmentalization of HIV in blood or lymph nodes during suppressive ART, implying that infection is primarily maintained as long-lived cells infected prior to ART.

• Integration site may influence persistence and clonal expansion and therefore, the virus that rebounds

• Expanded clones CAN contain infectious proviruses, and are almost certainly the source of rebound virus when therapy is stopped.

• Curing HIV infection will require completely new strategies for eliminating or otherwise dealing with this expanding persisting reservoir, which must be very large, and is highly variable from one patient to the next.
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