Curing HIV Infection: Going Beyond N=1

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Disclosures: Dr. Siliciano is an inventor on a patent application filed by JHU and licensed by AccelevirDx. He holds no equity interest in AccelevirDx. He consults on cure related issues for Abbvie and Merck.
Establishment and maintenance of a latent reservoir

HIV gene expression depends on inducible host factors

Nabel and Baltimore, Nature 1987
Tong-Starksen et al, PNAS 1987
Duh et al, PNAS 1989
Kinoshita et al, Immunity 1997
A stable latent reservoir for HIV

Reactivation of latent HIV

Naive

Memory

Quantitative viral outgrowth assay

180-200 ml blood

Purified resting CD4+ T cells

1/1,000,000

Slow decay of the latent reservoir in resting CD4\(^+\) T cells

Half-life: 3.7 years
Time to eradication: \(> 73.4\) years

Residual viremia

Plasma HIV-1 RNA (copies/ml) vs Time (months/years)

- Archival
- Drug-sensitive
- No evolution

References:
Hermankova et al, *JAMA* 2001
Kieffer et al, *J Infect Dis* 2004
Nettles et al, *JAMA* 2005
Dinooso et al, *PNAS* 2009
Clonal nature of residual viremia

Clonal nature of residual viremia

- These sequences reflect extensive proliferation of a clone of infected cells
- Cellular sequences matching these plasma virus clones were difficult to find

Quantitative viral outgrowth assay

180-200 ml blood

PCR for proviral DNA

purified resting CD4+ T cells

T cell activation

d2: add CD4+ lymphoblasts from HIV-donors

d7: add CD4+ lymphoblasts from HIV-donors

5 x 10^6  1 x 10^6  2 x 10^5  4 x 10^4  8 x 10^3  1.6 x 10^2  Negative control

Comparison of assays for the latent reservoir

<table>
<thead>
<tr>
<th>Assay</th>
<th>Viral outgrowth</th>
<th>Total HIV DNA</th>
<th>Integrated HIV DNA</th>
<th>Total HIV DNA</th>
<th>2 LTR circles</th>
<th>Residual viremia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell/tissue</td>
<td>Resting CD4</td>
<td>PBMC</td>
<td>Resting CD4</td>
<td>PBMC</td>
<td>Rectal CD4</td>
<td>PBMC</td>
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<td></td>
<td>Chronic</td>
<td>Acute</td>
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<td>Plasma</td>
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<td>Acute</td>
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<td></td>
</tr>
</tbody>
</table>

- Infected cell frequency (per 10⁶)
- Plasmas HIV RNA (copies/ml)

Eriksson et al, PLOS Pathogens, 2013
Non-induced proviruses

Resting CD4+ T cells

PHA + irradiated allogeneic PBMC

full length, single genome analysis

Are they inducible?

d2: add CD4+ lymphoblasts from HIV-donors

d7: add CD4+ lymphoblasts from HIV-donors

Ho et al, Cell, 2013
Strategy for unbiased analysis of proviruses

Step 1: Outer PCR from U5 to U5

Step 2: *gag* and *env* inner PCRs to confirm clonal dilution

Step 3: Subject all wells to 6 inner PCRs, regardless of positivity for *gag* or *env* inner PCRs

Ho et al, *Cell*, 2013
Landscape of HIV proviruses

Landscape of HIV proviruses

Intact proviruses capable of virion production are vastly outnumbered by defective proviruses detected in subgenomic amplifications, including those used in standard PCR-based reservoir assays.
Intact, noninduced proviruses

Viral outgrowth

Infected cell frequency (per $10^6$)

Intact, non-induced

Defective

Intact, non-induced

Ho et al, *Cell* 2013

Reservoir assay

Patients
- Chronic
- Acute

Viral outgrowth

Intact by nFGS

Subgenomic PCR

Ho et al, *Cell* 2013
Reconstructing intact non-induced proviruses

Ho et al, *Cell* 2013
Replication capacity of intact non-induced proviruses

Supernatant HIV p24 (ng/ml) vs. Time post infection (days) for patients 10, 16, 17, and 20.

- NL4-3
- Rep-Comp
- Intact non-induced

Ho et al, *Cell* 2013
Non-induced proviruses have functional LTRs

Fold induction relative to NL4-3

Log transcription level in resting cells

Log transcription level in activated cells

Ho et al, Cell 2013
Some intact non-induced proviruses be induced

Ho et al, Cell 2013
Hosmane et al, J Exp Med 2017
The proviral landscape

- Each round of T cell activation induces additional proviruses
- The number of intact proviruses is more accurate measure of reservoir size
- This work redefines the target for HIV cure

Assay

- Viral outgrowth
- Intact by FGS
- Sub-genomic PCR

Intact

Ho et al, Cell 2013
Hosmane et al, J Exp Med 2017
Novel approach to reservoir measurement

Defective

1000/10^6 – 10,000/10^6

Intact

100/10^6

1/10^6

IPDA

Standard PCR
Viral outgrowth
Intact provirus
Induced RNA
Induced protein

Ho et al, Cell, 2013
Hosmane et al, J Exp Med 2017
Pollack et al, Cell Host Microbe 2017
Most proviruses are defective in most viral genes

Cells with defective and intact proviruses may be affected differently by shock and kill interventions

Bruner et al, Nature, in press
Standard PCR assays will miss effective interventions

Digital droplet assay for intact proviruses

Bruner et al, Nature, in press
Intact vs deleted proviruses

Fraction of deleted sequences correctly identified as defective

Position of 5′ amplicon vs Position of 3′ amplicon

0.2 0.4 0.6 0.8 1.0

Position of 3′ amplicon

Ho et al, Cell 2013
Bruner et al, Nature, in press
Intact vs hypermutated proviruses

Sequence conservation

Fraction mutated

Bruner et al, Nature, in press
Intact vs hypermutated proviruses

Intact proviral DNA assay

- Cell or tissue sample
- Optimized DNA extraction
- Droplet formation
- ddPCR assay for intact proviruses
- ddPCR assay for cell equivalents and DNA shearing
- Assay analytics
- Intact proviruses per 10^6 cells

- Q1: Hypermutated and/or 3' deletion
- Q2: Intact
- Q3: no provirus
- Q4: 5' deletion

Bruner et al, Nature, in press
DNA shearing reduces detection of intact proviruses

Bruner et al, submitted
Correction for DNA shearing

Bruner et al, submitted
IPDA analysis of patient samples

- Frequency per million CD4+ T cells
- QVOA (IUPM)
- Pearson r = 0.4810
  - R² = 0.2314
  - P = 0.0030
- Ratio Intact proviruses : IUPM
  - IPDA /
  - QVOA

Clonal expansion detected by integration site analysis

Maldarelli et al, Science 2014
Wagner et al, Science 2014
Cohn et al, Cell 2015
Multiple stimulation viral outgrowth assay

Time (days)

0 10 20 30 40

PHA

Ho et al, Cell 2013
Hosmane et al, J Exp Med 2017
Independent isolates of replication-competent HIV with identical sequence:

Hosmane et al, J Exp Med 2017
Proliferation of HIV-1-infected cells

- Antigen and cytokines like IL-7 drives proliferation of CD4$^+$ T cells but also induce expression of latent proviruses.
- Productively infected cells die quickly.
The latent reservoir
Clones of latently infected cells
Clones of latently infected cells
Longitudinal analysis of CD4+ T cell clones with replication-competent proviruses

Wang et al, PNAS 2018
Cellular clones producing residual viremia wax and wane
Clones of latently infected cells wax and wane
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Clones of latently infected cells wax and wane

- Is each cell in the reservoir capable of enormous clonal expansion?
- What stimuli drive the expansion?
- Do those stimuli induce viral gene expression?
- What causes the contractions?
Selection for CTL escape mutants?

Deng et al, Nature 2015
Antar et al, in preparation
Selection for CTL escape mutants?

Participant 583

Frequency of epitope variants (%)

Position of epitope in Gag

Antar et al, in preparation
Selection for CTL escape mutants?

- Participant 583
- Frequency of epitope variants (%)
- Position of epitope in Pol

Antar et al, in preparation

Selection for CTL escape mutants?
Selection for CTL escape mutants?

Participant 583

Frequency of epitope variants (%)

Position of epitope in Nef

Antar et al, in preparation
Is there selection for defective proviruses over time?

Antar et al, in preparation

2005 → 2014

Intact
Ψ deletion
5' deletion
3' deletion
Central deletion
2 deletions
Large deletion
Unmapped deletion
Hypermutation
Hypermutation + 5'
Hypermutation + 3'
Hypermutation + central
Hypermutation + unmap

Single sequences
Clones
Is there selection for defective proviruses over time?
Dynamics of latently infected CD4$^+$ T cells

- Half-life: 3.7 years
- Time to eradication: > 73.4 years

Most cells in the reservoir arise from cell proliferation, not *de novo* infection.

Infected cell clones wax and wane on a time scale of months to years.

Cells with intact and defective proviruses show *in vivo* proliferation.

*In vivo* proliferation occurs through a mechanism that does not drive high level viral gene expression.
SIV proviral landscape

Step 1: Near full genome length outer PCR at limiting dilution

Outer PCR - 9,017 bp

Step 2: gag and env inner PCRs to confirm clonal dilution

gag 1,574bp

env - 2,896 bp

Step 3: 7 inner PCRs on all wells at limit dilution

- gag 1,574bp
- A - 4,201 bp
- B - 4,073 bp
- C - 5,834 bp
- D - 4,770 bp
- NFL - 8,983 bp

Step 4: Electrophoretic separation and directly sequence PCR products without cloning

5' deletion

Intact provirus

3' deletion

DEP0_E02H

KIC_C07H

KIC_A02H

Step 5: Align sequencing reads from overlapping PCRs to obtain near full genome length sequence

Murray et al, in preparation
Proviral landscapes for SIV, SHIV, and HIV-2

• For treated SIV and SHIV infection, there is a higher fraction of intact proviruses

Murray et al, in preparation
IPDA for SIV

Murray et al, in preparation
Higher frequency of cells with intact proviruses in SIV infection

Murray et al, in preparation
Clonal sequences dominate persistent HIV

Residual viremia

Replication-competent proviruses

Simonetti et al, PNAS 2016
Lorenzi et al, PNAS 2016
Hosmane et al, J Ex Med 2017
Bui et al, PLoS Path 2017
Wang et al, PNAS 2018
SIV reservoir not dominated by clonal sequences

SIVmac251 1.8 years, ART 0.7 years

SIVmac239 >0.5 years, ART 1.1 years

Murray et al, in preparation
Clonality of persistent SIV and HIV-1

Fraction of sequences with match (%) vs. Time on ART (years)

- SIV, acute
- SIV, chronic
- HIV-1, chronic
Thanks

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Extra slides
Deletions arise during reverse transcription *in vivo*

- Not seen in limiting dilution amplification of proviral constructs by the same method
- Regions of homology at deletion junctions
- Consistent with dynamic copy choice recombination mechanism
- Non-random distribution of deletion sites
- Same deletion junction in separate amplifications from same patient (expanded clone)
- Correlation of type and frequency of defects with disease stage
- Same results with digital droplet PCR with multiple short amplicons
Mechanism of deletions

A. Wild Type
- RNA-dependent DNA synthesis and RNase H degradation
- Annealing of nascent DNA to acceptor template
- Nascent DNA extends
- Nascent DNA growing point switches template
- Deletion of directly repeated sequence

B. Slow Polymerase
- More RNA cleavage
- More template available to make switch
- Higher rate of template switching

C. Slow RNase H
- Less RNA cleavage
- Less template available to make switch
- Lower rate of template switching

Delviks-Frankenberry et al, Viruses, 2011
Mechanism of deletions
5' and 3' ends of deletions

Position (HXB2 coordinates)

Relative Frequency

5' end
3' end

Katie Bruner
Effects of hypermutation

- TGG → TAG
- TGA
- TAA

Number of internal stop codons:
- 0
- 4
- 8
- >10

Number of clones:
- 0
- 2
- 4
- 6
- 8
- 10
- 14
- 18
- 20
- 22

- gag
- gag-pol
- vif
- vpr
- tat
- rev
- vpu
- env
- nef

- No internal stop codons
- Internal stop codons

Number of internal stop codons:

Ho et al, *Cell*, 2013
Reservoir reduction vs time to rebound

Log reduction in LR

- Boston A (Transplant)
- Boston B (Transplant)
- Mississippi baby
- Berlin (Transplant CCR5^-/

Early ART
NIAID

Early ART (UCSF)

Hill et al, PNAS 2014
Effect of blocking proliferation

Reduction in proliferation rate (%)

Reservoir half-life (years)

Hill et al, in preparation
Labile infected cells populations dominate early

Blankson et al, JID 1999
Rosenbloom et al., submitted
Effects of hypermutation

Bruner et al, submitted