Barriers to HIV Cure

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Financial Relationships With Commercial Entities

- Dr Siliciano has no relevant financial affiliations to disclose. (Updated 04/14/16)

Learning Objectives

After attending this presentation, participants will be able to:
- Describe how the latent reservoir for HIV arises
- List current approaches to curing HIV infection
- Describe how these approaches will be evaluated clinically
How does early ART affect likelihood of cure?

13% 1. Smaller latent reservoir
18% 2. More rapid reservoir decay
3% 3. No effect

How is the latent reservoir best measured?

27% 1. Viral outgrowth assay
27% 2. DNA PCR assays
27% 3. Plasma HIV RNA
3% 4. Western blot

Viral dynamics in patients on ART

- Start ART
- $t_{1/2} = 1$ day
- $t_{1/2} = 14$ days
- Activated CD4+ T cells
- Eradication in 2-3 years
- Limit of Detection (50 copies/ml)
Physiology of resting and activated CD4+ T cells

Establishment of immunologic memory

HIV infection of activated and resting CD4+ T cells
Establishment of the latent reservoir in resting CD4+ T cells

NFκB sites in the HIV LTR

Reactivation of latent HIV
An assay for latently infected cells

Purified resting CD4+ T cells
PHA + irradiated allogeneic PBMC
p24 Ag
180-200 ml blood

Chun et al., Nature, 1997
Finzi et al., Science, 1997

Slow decay of latently infected CD4+ T cells

Time to eradication > 73.4 years

Finzi et al., Nature Med., 1999

Slow decay of the reservoir

Time to eradication > 73.4 years

Crook et al, JID 2015
Latency results from infection of memory precursor cells

- Resting
- Fully activated Memory precursor
- Resting memory

% Relative
permissiveness

Day 0 Day 3 Day 6 Day 9 Day 12

Deng et al., submitted

- Residual viremia
- Sensitive to current regimen
- Archival
- Non-evolving

Residual viremia

- Start Therapy
- HAART

Plasma HIV RNA (copies/ml)

0 100 1000 10000 100000

0.001 0.01 0.1 1 10

Limit of Detection (50 copies/ml)

1 copy/ml

Time on HAART (days/years)

Hermankova et al., JAMA, 2001
Persaud et al., J Virol., 2003
Kafarri et al., J Inlan Dis., 2004
Nathoo et al., JAMA, 2005
Baley et al., J Virol., 2005
Brennan et al., J Virol., 2009

- Residual viremia cannot be reduced by treatment intensification

Dinoso et al., PNAS, 2009

Many later raltegravir intensification studies

Add 4th drug

Dinoso et al., PNAS, 2009
Mary later reintegrated intensification studies
The first cure

- Host immune system, including latently infected cells, largely eliminated by condition regimen (chemo + irradiation and by graft vs host disease.
- Donor cells protected from HIV infection due to absence of CCR5

“Boston Patient B”

Plasma HIV RNA (copies/ml)

Time after Rx interruption (months)

Matched allogeneic HSCT

Stop ART

Henrich et al, JID, 2013

The Mississippi baby

These delayed rebound cases prove that HIV can persist in a latent form for years and then begin to replicate

Persaud D et al., NEJM 2013
**Approaches to HIV cure**

- **Gene Rx**
  - Prevent reactivation
  - Induce elite control

**Other approaches to HIV cure**

**Gene Editing Strategies used in Cure Research:** target integrated provirus with engineered nucleases (ZFN, TALENS, or CRISPR/Cas9)

**Problems**
- No way to deliver enzymes into every infected cell in vivo
- Off-target effects

**Other approaches to HIV cure**

Gene Rx — ZFN targeting CCR5 gene in patient CD4+ T cells or HSC. Reinfuse engineered, HIV-resistant cells back into patients

**Problems**
HIV can still replicate in non-engineered cells. (In Berlin patient, CCR5+ host cells eliminated by conditioning regimen and graft vs. host effects)
Fundamental approach to HIV cure

- How do we identify latency reversing agents?
- Will cells be eliminated following reversal of latency?
- How do we measure the reservoir in eradication trials?

Current status of LRA trials

- Numerous LRAs identified in studies with transformed cell lines and primary T cell model systems
- Few shown to work ex vivo with cells from patients
- In clinical trials, no reduction in the reservoir yet demonstrated
- In clinical trials, evidence for increases in cell-associated HIV RNA (Archin et al.)
- Some evidence for slight transient increases in plasma HIV RNA after LRA treatment (romidepsin, panobinostat, TLR7 agonist)

Assay for reversal of latency using patient resting CD4+ T cells

- Measure intracellular HIV RNA and virion release
- 5 x 10^6 cells/well
- 500 x 10^6 resting CD4+ T cells
- TCR agonist
- Positive control

- Laird et al, PLOS Pathogens, 2013
Induction of HIV RNAs by LRAs

Bullen et al, Nat Med 2014

Induction of HIV RNAs by combinations of LRAs

Laird et al, in preparation

Fundamental approach to HIV cure

*How do we measure the reservoir in eradication trials?*

*How do we identify latency reversing agents?*

*Will cells be eliminated following reversal of latency?*

*How do we measure the reservoir in eradication trials?*
Fate of infected CD4 cells after latency reversal *in vivo* is unknown

CTL killing of latently infected cells treated with SAHA

CTL killing of latently infected cells treated with SAHA

E:T = 1:1

Shan et al., *Immunity*, 2012
**Fundamental approach to HIV cure**

- How do we identify latency reversing agents?
- Will cells be eliminated following reversal of latency?
- How do we measure the reservoir in eradication trials?

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**An assay for latently infected cells**

- 180-200 ml blood
- Final testing CD4+ T cells
- 1/1,000,000
- 5' and 3' probe
- Chun et al., Nature, 1997
- Fassi et al., Science, 1997
**Assays for latent HIV**

- **Viral outgrowth assay (VOA)**
- **DNA PCR**
- **Induction of HIV RNA**
- **Induction of virion production**

**Viral outgrowth vs PCR assays**

- **Eriksson et al., PLOS Pathogens, 2013**

**Non-induced proviruses**

- **Ho et al., Cell, 2013**

**Non-induced proviruses**

- **Ho et al., Cell, 2013**
Non-induced proviral clones (n=213)

32.4% of non-induced proviruses have lethal G→A hypermutation

45.5% of non-induced proviruses have large internal deletions
Deletions and hypermutation

Bruner et al, submitted

Non-induced proviruses

Ho et al, Cell, 2013

Replication capacity of intact non-induced proviruses

Intact non-induced proviruses
Size of latent reservoir

62 fold

Ho et al, Cell, 2013

Can intact non-induced proviruses be induced?

180-200 ml blood

Purified resting CD4+ T cells

All rest., lymane blasts from rest. disem.

Recover cells from negative wells

d2: add CD4+ lymphoblasts from HIV- donors

d7: add CD4+ lymphoblasts from HIV- donors

Ho et al, Cell, 2013

Can intact non-induced proviruses be induced?

Resting CD4+ T cells

PHA+ allo PBMC

47% 53%

PHA+ allo PBMC

39% 61%

PHA+ allo PBMC

30% 61%

Ho et al, Cell, 2013

Nina Hosmane
**Take home points**

- There is no clinical assay for the latent reservoir
- DNA PCR assays widely used for reservoir analysis mainly detect grossly defective proviruses
- The quantitative viral outgrowth assay remains the best available assay for the latent reservoir, but better assays are urgently needed.
- Other approaches: transient blips following LRA administration, time to rebound after ART interruption

**Predicting time to rebound after reservoir reductions**

Hill et al, PNAS 2014

**Time to rebound**

Hill et al, PNAS 2014
What will cure look like?

Time Post Infection

Plasma HIV RNA (copies/ml)

What will cure look like?

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