The Challenge of Curing HIV

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Northwestern
21 October 2014
Major Challenges Facing the HIV Field

HIV chemotherapy has been a major medical accomplishment over the past 2 decades and has dramatically reduced the morbidity and mortality of those with access to care.

I see two major scientific challenges for the future of the management of HIV, neither of which may be achievable:

- For HIV uninfected people, develop an HIV vaccine.
- For HIV infected people, develop approaches to cure the latent reservoir.
Why “Cure” HIV?

- Relieve treatment fatigue
- Reduce drug costs
- Reduce drug toxicity
- Reduce the morbidity and mortality associated with viral persistence and immune activation (e.g., malignancies, cardiovascular disease, CNS impairment)
- Reduce drug resistance
- Reduce transmission
HAART does not eradicate HIV

HIV infection is characterized by high levels of circulating viruses in the blood. Antiretroviral drugs are capable of suppressing HIV to undetectable levels. However, the virus rebounds after cessation of therapy.

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The definition of **viral latency**: Virus infectivity that can be induced from a cell that is not replicating virus.

Examples:
- Lambda (λ) phage
- Herpes simplex virus (HSV)
- Varicella-zoster virus (VZV)
Questions (Research Opportunities) to address the HIV latent reservoir and eradication strategies

1. How is latency established and maintained?
2. What cellular targets are candidates for drug discovery to activate latent virus safely?
3. Do our current regimens completely suppress replication?
4. What is the composition of the latent reservoir (size, cell types)?
5. Are there anatomic sanctuaries (CNS, genital tract)?
6. Will drugs that induce HIV transcription result in cytolytic death or will an additional factor be required?
   • induction of translation
   • enhanced T cell immunity
7. Will gene therapy or immunologic interventions contribute to the approach?
8. How do you measure the latent reservoir and the impact of candidate eradication strategies?
Two big challenges to identifying an effective eradication intervention

1. Drug discovery
   • What cellular targets are candidates for drug discovery to activate latent virus safely?
   • How do you identify compounds with few well characterized targets?

2. How do you measure the latent reservoir and the impact of candidate eradication strategies?
Strategies for cure

- Eliminate residual virus replication and then eliminate latently infected cells
  - Cytolysis mediated by virus replication
  - T cell killing of cells expressing viral antigens

- Enhance HIV-specific immunity
  - “therapeutic” vaccine
  - anti-PD1/anti-PDL1

- Make cells “resistant” to HIV
  - Target HIV provirus (Zinc finger, CRISPR)
  - Target CCR5
  - Target infected cells with toxin conjugates
Richman et al., Figure 2

Prostratin → HDAC Inhibitor → IL-7 → HMBA → Purging Latent Reservoir

Latently Infected CD4 T Cell

Productively Infected CD4 T Cell

HIV RNA → Budding → Assembly → 1–2 Days HIV-induced Cell Death

HIV → Uninfected CD4 T Cells

Science 323, 1304 (2009)
HDACi turn HIV-1 transcription “on”

Slide courtesy of Javier Martinez-Picado/Sharon Lewin

SAHA to reactivate HIV

Adminstration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy

N. M. Archin¹, A. L. Liberty¹, A. D. Kashuba¹, S. K. Choudhary¹, J. D. Kuruc¹, A. M. Crooks¹, D. C. Parker¹, E. M. Anderson², M. F. Kearney², M. C. Strain³, D. D. Richman³, M. G. Hudgens¹, R. J. Bosch¹, J. M. Coffin², J. J. Eron¹, D. J. Hazuda⁵ & D. M. Margolis¹
Clinical studies with HDACi

<table>
<thead>
<tr>
<th></th>
<th>Vorinostat (Merck)</th>
<th>Romidepsin (Celgene/Gilead)</th>
<th>Panobinostat (Novartis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex-vivo HIV-1 reactivation</td>
<td>weaker</td>
<td>strong</td>
<td>strong</td>
</tr>
</tbody>
</table>
| Dose, dosing schedule and formulation | 400mg p. o.  
• Single-dose (Archin et al, Nature 2012)  
• three consecutive doses per week for eight weeks (Archin et al, JID 2014)  
• daily for 14 days (Elliott et al, CROI 2013) | 5mg/m² i. v. x3 (Aarhus)  
• 0.5/2/5mg/m² single-dose RCT (ACTG) | 20mg p. o.  
TIW (M, W, F)  
QOW  
• Rasmussen et al, Lancet HIV 2014 |
| Pilot clinical trials in HAART-treated HIV patients | completed (UNC, Melbourne) | in process (ACTG, Aarhus) | completed (Aarhus) |
The factors modulating HIV activation and transcription are complex and incompletely understood.
Ultra high throughput screen to identify new molecules, mechanisms and combinations

- Latent Jurkat T-cell model
- Screened 2.9 million compounds in the presence of 250 nM vorinostat
- All hits titrated alone or with 250 nM vorinostat
- Identified HDAC inhibitors and 2400 unique hits

Barnard, Hazuda, CARE CROI 2013
FTIs Synergize with HDACi’s in a Jurkat HIV Latency Model system

FTi’s from different structural classes synergize with Vorinostat

FTi’s synergize with HDAC inhibitors from different HDACi selectivity profiles

![Graph 1](image1)

![Graph 2](image2)
Immune strategies for a functional cure

- **HIV Vaccine**: Stimulating HIV-1-specific CTLs prior to reactivating latent HIV-1 may be essential for successful eradication efforts (Shian Immunity 2012, Archin Nature 2012)

- **Blocking PD-1** may functionally restore CD4+ and CD8+ T cells, allowing control of the viral reservoir (Day Nature 2006, Trautmann Nat Med 2006, Porichis Blood 2011)

- **IL-7 therapy** induces a sustained increase in T cell numbers (Levy JCI 2009, Sereti Blood 2009) and may purge the latent reservoir by inducing viral production in latently infected cells (Scripture Adams JVI 2002, Wang JCI 2005)

What is the impact of these strategies on the mechanisms of HIV persistence during HAART?
Immune strategies for a functional cure

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- **Anti-PD-1 L1** given to SIV-infected macaques during ART delayed rebound in some animals after treatment interruption, but the effect was modest (Mason et al, 2014 CROI).

- **IL-7 induced proliferation of T cells** but the total reservoir of HIV-infected lymphocytes increased with this increase (ERAMUNE01, Pogliaghi et al, CROI, 2014).
Strategies for cure

- Eliminate residual virus replication and then eliminate latently infected cells
- Cytolysis mediated by virus replication
- T cell killing of cells expressing viral antigens
- Enhance HIV-specific immunity
- Make cells “resistant” to HIV

### Table 1

<table>
<thead>
<tr>
<th>Mechanism of anti-viral</th>
<th>Name</th>
<th>Target</th>
<th>Delivery of genes</th>
<th>Cell transplantation</th>
<th>Company</th>
<th>Phase</th>
<th>Status of clinical</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>ZFN</td>
<td>SB-728-T</td>
<td>Host (CCR5 DNA)</td>
<td>Adenoviral vector</td>
<td>Autologous CD4+ T cells</td>
<td>Sangamo Biosciences</td>
<td>I–II</td>
<td>Ongoing</td>
<td>[18]</td>
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<tr>
<td>C46 peptide</td>
<td>M870</td>
<td>Viral (env protein)</td>
<td>Retroviral (MMLV)</td>
<td>Autologous CD4+ T cells</td>
<td>University Medical Center Hamburg-Eppendorf</td>
<td>I</td>
<td>Ongoing</td>
<td>[27]</td>
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<tr>
<td>Ribozyme</td>
<td>RRz1 (OZ1)</td>
<td>Viral (tat/vpr mRNA)</td>
<td>Retroviral (MMLV)</td>
<td>Autologous CD4+ T cells</td>
<td>Janssen-Cilag Pty Ltd., UCLA</td>
<td>I–II</td>
<td>Ongoing</td>
<td>[44,45]</td>
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<tr>
<td>Ribozyme</td>
<td>MY-2</td>
<td>Viral (U5 and pol mRNA)</td>
<td>Retroviral (MMLV)</td>
<td>Autologous CD4+ T cells</td>
<td>UCSD</td>
<td>I</td>
<td>Completed</td>
<td>[40,42]</td>
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<tr>
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<td>RRz1</td>
<td>Viral (tat/vpr mRNA)</td>
<td>Retroviral (MMLV)</td>
<td>Syngeneic CD4+ T cells</td>
<td>Johnson &amp; Johnson, St. Vincent’s Hospital</td>
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<td>[41,43]</td>
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<td>Ribozyme</td>
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<td>Viral (tat/rev mRNA)</td>
<td>Retroviral (MMLV)</td>
<td>Autologous CD34+ HPC</td>
<td>Ribozyme, City of Hope</td>
<td>II</td>
<td>Completed</td>
<td>[67]</td>
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<tr>
<td>Antisense</td>
<td>Lexgenleucel-T (VX496)</td>
<td>Viral (env mRNA)</td>
<td>Lentiviral vector (LTR HIV)</td>
<td>Autologous CD34+ HPC</td>
<td>VIRxSYS Corporation</td>
<td>I–II</td>
<td>Ongoing</td>
<td>[47]</td>
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<tr>
<td>Antisense</td>
<td>HGTV43</td>
<td>Viral (TAR, tat/rev)</td>
<td>Retroviral (MMLV)</td>
<td>Autologous CD34+ HPC</td>
<td>Enzo Biochem</td>
<td>I–II</td>
<td>Ongoing</td>
<td>[68]</td>
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<tr>
<td>RNA decoy</td>
<td>L-RRE-neo</td>
<td>Viral (rev protein)</td>
<td>Retroviral (MMLV)</td>
<td>Autologous CD34+ HPC</td>
<td>Children’s Hospital Los Angeles, City of Hope</td>
<td>Pilot</td>
<td>Completed</td>
<td>[50]</td>
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<tr>
<td>shRNA RNA decoy (TAR) ribozyme</td>
<td>Tat/Rev shRNA</td>
<td>Viral (tat/rev mRNA)</td>
<td>Lentiviral vector (SIN HIV)</td>
<td>Autologous CD34+ HPC</td>
<td>Benitec</td>
<td>Pilot</td>
<td>Ongoing</td>
<td>[51**]</td>
</tr>
</tbody>
</table>
THE CLINICAL SPECTRUM OF HIV INFECTION

"Iceberg"

CDC-Reportable AIDS Opportunistic Diseases and Related Conditions

Nonspecific signs and symptoms of illness secondary to immunodeficiency (including "AIDS related complex")

Immune complex disease (e.g. thrombocytopenia)

Asymptomatic infections
What we can measure in the blood.

What exists in the rest of the body.
Caveats to all assays

- The measurable analytes represent only the “tip of the iceberg” of the reservoir that must be eradicated.
- The extra-circulatory reservoir is not homogeneous and is difficult to access.
- Most published reports have inadequate validation of performance characteristics.
What are the analytes that we can measure to assess a reduction in the latent reservoir

- Integrated HIV (proviral) DNA
- 2-LTR circular HIV DNA
- Cell associated HIV RNAs
- Plasma RNA
- Inducible infectivity by limiting dilution co-culture (Current Gold Standard)
- No detectable virus after withdrawal of ART (The Ultimate Standard)
Real-Time PCR

Template copies are estimated based on time (cycles).
→ Absolute numbers require a reference standard.

Exponential amplification provides high dynamic range.
→ Noise is also amplified.
HIV DNA Assay: Real-Time PCR

- At low copy numbers, errors explode:

Copies per million cells estimated in replicate wells.
Droplet Digital PCR

1. Partition
2. Amplify
3. Count

30 – 40 cycles
HIV DNA Assay: Droplet Digital PCR (ddPCR)

- PCR reactions were partitioned into 1 nL drops.
  - Bio-Rad QX-100
  - ≈ 10 human genomes / droplet
  - ≈ 500,000 cells assayed/ sample

- **2-LTR** and **pol** were multiplexed and normalized to RPP30.
  - RPP30 was assayed separately to compute cellular DNA input.
Digital PCR is more precise than real-time PCR.

- For rare HIV DNA targets, precision is still much better than real-time PCR.

Precision of ddPCR Assays

For 2-LTR, ddPCR is > 20-fold more precise.

Limit of detection (LOD)

- LOD can be decreased to < 1 copy / 10^6 cells by assaying additional replicates.

Digital PCR is less sensitive to sequence variation

- Even with sequence mismatches in both primers & probe, \( pol \) copy number varied by \(< \frac{1}{2} \log_{10} \)

The gold standard assay (limiting dilution co-culture assay) to measure replicative competent virus (infectious units per million, IUPM) is long (3 weeks), highly variable, donor dependent and not very sensitive.
What are the analytes that we can measure to assess a reduction in the latent reservoir

- Inducible infectivity by limiting dilution co-culture (Current Gold Standard)

Pro:
- True measure of replication competent infectivity. (The operational definition of latency for all viruses, like λ phage and HSV, is inducible infectivity in a cell not replicating virus.)

Con:
- Require large blood volumes (~100mL), expensive, imprecise, slow, cumbersome, labor intensive, insensitive, at the threshold of detectability
How to measure HIV reservoirs?

- The most sensitive detection of latently-infected cells is based on assays for HIV DNA, this does not distinguish between replication-competent and defective genomes.

Non-induced proviral clones (n=179)

- Internal deletion: 96 (53.6%)
- Hypermutated: 47 (26.3%)
- Nonsense/frameshift: 3 (1.75%)
- Truncated packaging signal: 3 (1.7%)
- Intact ORFs: 30 (16.8%), (range 6-36%)

Cell 155, 540–551, October 24, 2013
What are the analytes that we can measure to assess a reduction in the latent reservoir

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# Measuring the Latent Reservoir

<table>
<thead>
<tr>
<th></th>
<th>Proviral copies</th>
<th>Infectious Units</th>
<th>Plasma viremia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Average range</strong></td>
<td>10-1000</td>
<td>1-10</td>
<td>&lt;1-10</td>
</tr>
<tr>
<td>(per million cells or per ml plasma)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dynamic range of current assays (if you assay 10⁷ cells or 10 ml plasma)</strong></td>
<td>10^1-10^3</td>
<td>&lt;10^0-10^2</td>
<td>&lt;10^0-10^1</td>
</tr>
<tr>
<td><strong>Dynamic range needed to document eradication from that assayed reservoir</strong></td>
<td>&gt;10⁷-10⁹*</td>
<td>&gt;10⁶</td>
<td>&gt;10⁴*</td>
</tr>
</tbody>
</table>

*What proportion is replication competent?
The Berlin Patient

CCR5 ΔΔ32
No AML
No HIV

Graft-versus-host disease (GVHD)

SCT x 2
Chemo and Rad

CCR5 WT
AML
HIV

CCR5 ΔΔ32

The Berlin Patient


Now 7 years off antiretrovirals defines a sustained remission, if not a cure.

To what can we attribute this first (and only) case?

• Delta 32 CCR5 homozygous replacement of the bone marrow with no X4 virus in the host
• Graft vs. host disease (It improves survival with leukemia)
• Ablative chemotherapy
• Other?
The 2 Boston patients (Henrich et al, CROI, 2014)

Then we'll discuss, what we can measure is the tip of an iceberg.

The absence of detection is not the detection of absence.

- As we’ll discuss, what we can measure is the tip of an iceberg.
- The absence of detection is not the detection of absence.
The Mississippi Baby

Immediate ART
AZT-3TC-NVP

Maintained ART
AZT-3TC-LPV-r
For 18 months

ARVs stopped, HIV rebounds

Only one person has been “cured” of HIV.

Source: Adapted from Diana Finzi, U.S. National Institute of Allergy and Infectious Diseases
Clearance probabilities and rebound times following LRA therapy predicted from the model

Hill A L et al. PNAS 2014;111:13475-13480
The search for a cure will be prolonged and challenging. In fact, it may not be possible; nevertheless, like the search for a vaccine, the challenge should not preclude the effort.

- Achieving a cure would have a dramatic impact on morbidity, mortality, health care costs and transmission.
- The effort, regardless of the ultimate outcome, will yield significant insights into latency and reservoirs.
- Success should it be possible will take more than a decade.
  - acute lymphocytic leukemia in children
    - *Farber: aminopterin*
    - *Hitchings and Elion: 6-MP*
    - *Frei and Freireich*: combination therapy with RCTs
  - combination antiviral therapy for HIV (and HCV)
Acknowledgments

UCSD
Sara Gianella
Steven Lada
Marta Massanella Luna
Celsa Spina
Davey Smith
Matt Strain
Christina Yek

Johns Hopkins
Bob Siliciano
Janet Siliciano
Debbie Persaud
Jeff Lifson
Mike Piatak

NCI Frederick

UNC
Nancie Archin
Dave Margolis

UCSF / DARE
Leslie Cockerham
Steve Deeks
Hiroyu Hatano
Satish Pillai
Joe Wong
Steve Yukl

Barcelona
Julia Blanco
Ventura Clotet

NIAID