Targeting latent HIV infection: on the road towards an HIV Cure

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Towards an HIV Cure
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Last public appearance of the entire AIDS Quilt, 1996
HIV persists despite ART

- Latently infected cells
  - resting CD4+ T cells
  - other (potential) cell types
- Residual viral expression
  - Low-level viremia
  - HIV RNA detected in tissues
  - True residual replication?
- Potential pharmacologic reservoirs
- Failure of HIV-specific immunity and generalized immune dysfunction
Primary strategy to eliminate latent HIV infection

Other Challenges:
- Clearance of infected cells
- Clearance of virions
- Complete block of new infection
1. Integration site & host gene transcriptional interference
2. Restriction at mRNA transcript initiation:
   1. Resting state of memory T cells
   2. Transcription factors sequestered (eg. NF-kB, NFAT)
   3. Epigenetic chromatin modifications
3. Restriction at transcript elongation:
   1. Tat levels are restricted
   2. P-TEFb levels are restricted
   3. NELF impairs elongation that does occur
**Vorinostat:**
Suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor with nanomolar potency licensed for the treatment of cutaneous T cell lymphoma

Inhibits HDACs 1, 2, 3, and 8 (class I)
and HDAC 6 (class II)

Archin ARHR 2009
Contreras JBC 2009
Measures of persistent HIV in fully suppressed patients on ART

- Low-level Plasma HIV RNA
- Cell-associated HIV RNA in resting CD4+ T cells
- Resting CD4+ T cell
- HIV DNA
- HIV RNA
Resting CD4+ Cell Outgrowth Assay:
Gold standard method for quantification of replication-competent HIV (“latency”)

Leukapheresis:
4 billion lymphocytes from aviremic patient on ART

Antibodies Used:
- CD4 purification
- CD8, CD14, CD16
- CD19, CD56
- Glycophorin A
- Resting cell cocktail
- CD41, CD25, HLA-DR

Assay Variance:
- 1.2 log
- Range: 24850 to 5 infected cells per Billion

Antigen-magnetic bead cocktail

Mix cells with antibody-magnetic bead cocktail

Cells maintained 2 days
Integrase Inhibitor & N/NNRTI

24 hr Activation or Drug Test

Outgrowth assay

2.5 million cells/well
2.5 million cells/well
0.5 million cells/well
0.1 million cells/well

200 million resting CD4+ cells

Purified Resting cells
Resting CD4+ Cell Outgrowth Assay:
Gold standard method for quantification of replication-competent HIV ("latency")

Leukapheresis:
4 billion lymphocytes from CD4+ T cells; aviremic patent on ART

Antibodies Used:
- CD4 purification
- CD8, CD45, CD14
- CD19, CD56
- Glycoprotein A
- Resting cell cocktail
  - CD41, CD25, HLA-DR

HIV RNA per million Resting CD4+ T cells (detects 1 copy, LOQ 10 copies/million cells)

Mix cells with Antibody-magnetic bead cocktail

Magnet

200-1000 million resting CD4+ cells

6 hr drug exposure then RNA assay

Purified Resting cells

Immediate RNA assay
Single dose proof-of-concept pilot study with vorinostat

Stable cART >6 months; HIV RNA<50 c/ml; CD4>300 cells/µl

Hypothesis:
- HIV RNA expression in circulating resting CD4 T cells will be increased during the period of VOR intracellular effect
- VOR will disrupt latency in vivo
Harvest resting CD4+ T cells, establish baseline resting cell HIV RNA, and demonstrate that a change is detectable after a physiological exposure to VOR ex vivo.
Single 400 mg VOR dose: 12 hr VOR PK and cellular biomarkers of HDACi effect: total cell histone acetylation and histone acetylation at human p21 gene
Correlate VOR level with HDACi biomarkers

Repeat 400 mg dose and collect resting CD4+ cells: Compare change in intracellular HIV RNA from baseline pheresis
Single 400 mg VOR dose: Remeasure resting CD4+ T cell HIV RNA expression. Define potential for VOR to disrupt latency

- Mean 5.2-fold induction (range 1.5- to 10-fold)
- All increases significant (p < 0.01)
- No AE > Grade I
- No AE due to VOR

![Graph showing HIV-1 gag RNA copies per well with three bars for each patient after Baseline ART and VOR 400 mg treatment.]
What we have found so far:

• A single dose of VOR induces expression of full-length HIV RNA within latently infected resting CD4+ T cells.

• This is the first direct measurement of disruption of latent HIV infection in vivo.

• The optimal dosing schedule of VOR, and its ability to repeatedly and completely perturb latency in all relevant infected cells, must be established.

• Separately, the potential for VOR to deplete (some or all) latently infected cells must be established.
Assuming that VOR is not enough: screening to find new anti-latency agents

- ~ 1.5 million compounds (MRL Library)
- LTR-bGal HTS
- ~ Confirmed 104 compounds (not known HDACIs)
- NFAT-BLA Jurkat cell assay
- ~ 92 compounds that did not activate T-cells
- HDAC activity assay (novel HDACIs)
- ~ 83 compounds with potential novel mechanisms
- Toxicity
- Chemical attractiveness
- Further characterization

Hazuda IAS 2010
Screen anti-latency candidates for the ability to augment low-dose SAHA effect

But this synergy in cell model not seen in patient cell assays

Archin, Margolis, Hazuda
IAS 2010
High-throughput screens for new anti-latency compounds in T cell model systems

- Siliciano laboratory (Yang JCI 2009)
- Micheva-Viteva (JBC 2011)
- Planelles laboratory
- Gilead
- Janssen
- Merck and CARE collaboratory
- others
Comparison of primary cell and other model systems for response to anti-latency reagents

<table>
<thead>
<tr>
<th>Model</th>
<th>Name</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cultured Tcm cells</td>
<td>Planelles, V.</td>
</tr>
<tr>
<td>2</td>
<td>Bcl2-transduced primary T cells</td>
<td>Siliciano, R.</td>
</tr>
<tr>
<td>3</td>
<td>Quiescent CD4 T cells</td>
<td>Spina, C.</td>
</tr>
<tr>
<td>4</td>
<td>Quiescent CD4 T cells</td>
<td>Greene, W.</td>
</tr>
<tr>
<td>5</td>
<td>Jurkat cell line (J-Lat 6.3)</td>
<td>Verdin, E.</td>
</tr>
<tr>
<td>6</td>
<td>Patient CD4 T cells, ex vivo</td>
<td>Margolis, D.</td>
</tr>
<tr>
<td>7</td>
<td>Quiescent T cells + CCL19/21</td>
<td>Lewin, Sharon</td>
</tr>
<tr>
<td>8</td>
<td>Quiescent T cells + DC</td>
<td>Romerio, Fabio</td>
</tr>
<tr>
<td>9</td>
<td>HeLa cell line assay (Merck)</td>
<td>Hazuda, D.</td>
</tr>
<tr>
<td>10</td>
<td>Jurkat cell line assay (J-Lat 1.1)</td>
<td>Hazuda, D./Karn, J.</td>
</tr>
</tbody>
</table>

Led by Planelles & Spina
Where can HIV eradication approaches be studied?

Dinoso J Virol 2009
Suppression of plasma viremia in humanized mice

Daily injected Raltegravir FTC TDF

Choudhary J Virol 2009
Choudhary J Virol 2012
Denton J Virol 2012
Resting CD4\(^+\) cell outgrowth assay in human cells purified from humanized mice

Harvest lymphocytes from Blood, LN, Spleen, BM, FRT, Lung, Liver and Thymus

Rest cells for 2 days in media with HIV RT and Integrase Inhibitors

24 hr maximal activation with PHA, IL-2, allogeneic cells

HIV outgrowth assay in limiting dilution of activated CD4\(^+\) T feeder cells

Calculate frequency of resting cell infection by maximum likelihood method

97-99\% pure resting CD4\(^+\) T cells
0.5-1.0 million cells/mouse

Anti-mouse cocktail:
CD45, TER119, CD31, CD105

Anti-Human cocktail:
CD8, CD14, CD16, CD19, CD56, Glycophorin A, CD41, HLA-DR, CD25

Choudhary J Virol 2012
Denton J Virol 2012
Purification of Resting Human CD4+ T Cells from Humanized Mice

A. Before Column Purification

B. After Column Purification

Measured frequency of human resting CD4+ T cell infection: 1 to 7 per million

Resting human CD4+ T cells
Anti-latency Discovery

Discovery & Basic Studies
- High-Throughput Screening
- Mechanistic Studies

Cell Models
- Primary validation
- Secondary Validation

Human Assays

Animal Models
- Pre-clinical validation

Clinical Testing
HIV persists despite ART

- Latently infected cells
  - resting CD4+ T cells
  - other (potential) cell types
- Residual viral expression
  - Low-level viremia
  - HIV RNA detected in tissues
- Potential anatomic or pharmacologic reservoirs
- Failure of HIV-specific immunity and immune dysfunction
CD8$^+$ T cells from patients on ART do not reliably kill latently infected CD4$^+$ T cells after virus reactivation.

Shan Immunity 2012
CD8⁺ T cells from patients on ART do not reliably kill latently infected CD4⁺ T cells after virus reactivation.

Shan Immunity 2012
Testing interventions in vivo to reduce resting cell infection or low-level viremia

Anti-latency small molecules
Immunodulators
HIV vaccines
Novel approaches

Unproven Assays

DNA Integrants
Digital PCR
Microscopic flow cytometry
Immune response assay

Leukapheresis & SCA

ART

Baseline
After intervention

Leukapheresis & SCA
Ending AIDS

- Find patients earlier, bring treatment to them
- Develop ways to use ART as prevention
- Develop vaccines that substantially reduce the risk of transmission
- Build platforms to develop and test curative therapy
  - ✅ Perturb latency
  - ✅ Block all infection
  - ✅ Reach all relevant cells
  - ✅ Clear infected cells
Special thanks to the HIV+ volunteers

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Special thanks to the HIV+ volunteers

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The University of North Carolina at Chapel Hill

NIMH
National Institute of Mental Health

NIDA
National Institute on Drug Abuse
“Towards an HIV Cure”

The Global Scientific Strategy
“Towards an HIV Cure”
was launched on 19 July 2012!

Visit the IAS website for more information and sign the Rome Statement for HIV Cure research to be accelerated: www.iasociety.org.

“Towards an HIV Cure”