Towards an HIV cure: understanding the major barriers

Sharon R Lewin
Director, Doherty Institute for Infection and Immunity, The University of Melbourne and Royal Melbourne Hospital and Consultant physician, The Alfred Hospital, Melbourne, Australia

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Only one case of HIV cure:
Timothy Brown

Long-Term Control of HIV by CCR5 Delta32/Delta32 Stem-Cell Transplantation

Gero Hütter, M.D., Daniel Nowak, M.D., Maximilian Mossner, B.S.,
Susanne Ganepola, M.D., Arne Müßig, M.D., Kristina Allers, Ph.D.,
Thomas Schneider, M.D., Ph.D., Jörg Hofmann, Ph.D., Claudia Kücherer, M.D.,
Olga Blau, M.D., Igor W. Blau, M.D., Wolf K. Hofmann, M.D.,
and Eckhard Thiel, M.D.

The Emerging Race To Cure HIV Infections

Timothy Ray Brown’s startling fate has pushed to the front a daunting research challenge that long seemed a fool’s errand
Outline: major barriers to cure

- **HIV persistence**
  - HIV latency
  - HIV persistence in tissues

- Impaired **immune function**
  - HIV-specific T-cell function
  - Inflammation

- **Measuring** virus persistence and biomarkers of functional cure
HIV persistence on ART: many diverse forms

Chun TW, Nature Immunol 2015
HIV latency
Establishing HIV latency

Saleh et al., Blood 2007; Cameron et al., Proc Natl Acad Sci 2010;107(39):16934-9; Evans et al Plos Pathogens 2013; 9:12; e1003799; Shen A et al., J Virology 2013 Sep;87(17):9768-79
Multiple molecular factors maintain HIV latency

Reversing HIV latency: shock and kill

Latent infection

“shock”

HIV US RNA

HIV proteins

HIV virions

Cell death
Many drugs activate latency in vivo but none eliminate latently infected cells

<table>
<thead>
<tr>
<th>Latency reversing agent</th>
<th>Site of action</th>
<th>HIV latency</th>
<th>US HIV RNA</th>
<th>Plasma RNA</th>
<th>HIV DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vorinostat</td>
<td>HDACi</td>
<td>Single dose$^1$ Intermittent$^2$ Continuous$^3$</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Panobinostat</td>
<td>HDACi</td>
<td>Intermittent dose$^4$</td>
<td>↑</td>
<td>+/-</td>
<td>↔</td>
</tr>
<tr>
<td>Romidepsin</td>
<td>HDACi</td>
<td>Weekly dose$^5$</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↔</td>
</tr>
</tbody>
</table>

Enhancing potency of latency reversing agents: combination activation

Multiple synergistic combinations
HDACi + 5 aza-C (methylation inhibitor)$^1$
HDACi + DNA methyltransferase inh$^2, 3$
HDACi + bryostatin $^4$
HDACi + disulfiram $^4$
HDACi + screening drug library (Merck)$^5$
HDACi + IL-15$^6$

Enhance immune mediated killing
T-cell vaccines
Antibody – bNAB, bispecific, ADCC

Enhance apoptosis
Inhibitors of apoptosis (IAP) antagonists
BCL2 antagonists
PI3k antagonists
Inhibiting BCL2 to trigger apoptosis of latently infected cells

- HIV protease cleaves procaspase 8 to Casp8p41
- Casp8p41 binds BAK and induces apoptosis
- Also binds BCL2 enhances cell survival
- BCL2 antagonist (venetoclax) in clinical development for CLL (phase II/III)

Patient derived cells (on ART) treated ex vivo with PHA/IL2+ BCL2 antagonist (venetoclax)

Cummins et al., J Virol 2016
HIV persistence in tissue
Anatomical reservoirs

Lymph node: persistence of HIV in B cell follicles in T follicular helper cells

B cell follicle sanctuary permits persistent productive simian immunodeficiency virus infection in elite controllers

Yoshinori Fukazawa¹,², Richard Lum¹,², Afam A Okoye¹,², Haesun Park¹,², Kenta Matsuda³, Jin Young Bae¹,², Shoko I Hagen¹,², Rebecca Shoemaker⁴, Claire Deleage⁴, Carissa Lucero⁴, David Morcock⁴, Tonya Swanson¹,², Alfred W Legasse¹,², Michael K Axthelm¹,², Joseph Hesselgesser⁵, Romas Geleziunas⁵, Vanessa M Hirsch³, Paul T Edlefsen⁶, Michael Piatak, Jr⁴, Jacob D Estes⁴, Jeffrey D Lifson⁴ & Louis J Picker¹,²
T follicular helper cells are highly enriched for replication competent virus

n=9; HIV-infected individuals on ART

Banga et al., CROI 2016, Boston MA
Rectum: HIV is enriched in CCR6+CXCR3+ (Th1/Th17) T-cells

Blood

Integrated HIV DNA copies / 10^6 cells

P < 0.001
P < 0.001
P < 0.001

n=20

n=48

Rectum

Blood

Brain: Microglia are infected on ART but rare

In 5 patients on ART, HIV DNA detected in only one patient

|    |    |      |      |      |      |      |      |      |      |      |      |      |    |      |      |      |      |      |      |      |      |      |      |      |    |      |      |      |      |      |      |      |      |      |    |
|----|----|------|------|------|------|------|------|------|------|------|------|------|----|------|------|------|------|------|------|------|------|------|------|------|----|------|------|------|------|------|------|------|------|----|
| Con B | R5 | CT RPNNNTRK | S I H I | - | GPGR | AFYT TG EI IG | D | RQAHC | 35 |
| ADA  | R5 | ............ | - | - | . . | D | . . . . . . | 35 |
| YU2  | R5 | ............ | . N | - | - | L | . . . . . . | 35 |
| JRCSF| R5 | ............ | S | - | - | - | . . . . . . | 35 |
| T82S1| X4 | . R | - | . . | - | - | R | . . | R | . . | . . | . . | . . | 34 |
| T82S2| R5 | . R | - | - | - | - | - | D | . . | . . | . . | 34 |
| T82S3| X4 | . R | - | - | - | - | - | R | . . | R | . . | E | . . | 34 |
| T82M1| R5 | - | . | - | - | - | - | D | . . | . . | . . | 34 |
| T82P3| R5 | . I | - | . . | P | - | R | . . | E | . . | . . | 35 |

Melissa Churchill and Lachlan Gray, Burnet Institute
Persistent virus replication on ART in tissue: remains controversial!

Persistent HIV-1 replication maintains the tissue reservoir during therapy

Ramon Lorenzo-Redondo, Helen R. Fryer, Trevor Bedford, Eun-Young Kim, John Archer, Sergei L. Kosakovsky Pond, Yoon-Seok Chung, Sudhir Penugonda, Jeffrey G. Chipman, Courtney V. Fletcher, Timothy W. Schacker, Michael H. Malim, Andrew Rambaut, Ashley T. Haase, Angela R. McLean & Steven M. Wolinsky
## Interventions targeting tissue

<table>
<thead>
<tr>
<th>Tissue site</th>
<th>Persistent virus</th>
<th>Target</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph node</td>
<td>T follicular helper cell (B cell follicle)</td>
<td>Disrupt follicle</td>
<td>Anti CD20 (NHP)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Enhance migration of CTL</td>
<td>CXCL13</td>
</tr>
</tbody>
</table>

1. InMIND, clinicaltrials.gov NCT02519777;  
3. DIORR, clinical trials.gov NCT02500446
impaired immune function on ART
HIV persists during ART in part due to lack of effective T cell immunity

LETTER

Broad CTL response is required to clear latent HIV-1 due to dominance of escape mutations

Kai Deng\(^1\), Mihaela Pertea\(^1,2\), Anthony Rongvaux\(^3\), Leyao Wang\(^4\), Christine M. Durand\(^1\), Gabriel Ghiaur\(^5\), Jun Lai\(^6\), Holly L. McHugh\(^1\), Haiping Hao\(^9\), Hao Zhang\(^7\), Joseph B. Margolick\(^7\), Cagan Gurer\(^8\), Andrew J. Murphy\(^9\), David M. Valenzuela\(^8\), George D. Yancopoulos\(^6\), Steven G. Deeks\(^9\), Till Strowig\(^3\), Priti Kumar\(^10\), Janet D. Siliciano\(^1\), Steven L. Salzberg\(^2,11\), Richard A. Flavell\(^3,12\), Liang Shan\(^3\) & Robert F. Siliciano\(^1,13\)

Stimulation of HIV-1-Specific Cytolytic T Lymphocytes Facilitates Elimination of Latent Viral Reservoir after Virus Reactivation

Liang Shan\(^{1,2}\), Kai Deng\(^1\), Neeta S. Shroff\(^1\), Christine M. Durand\(^1\), S. Alireza. Rabi\(^1\), Hung-Chih Yang\(^3\), Hao Zhang\(^4\), Joseph B. Margolick\(^4\), Joel N. Blankson\(^1\) and Robert F. Siliciano\(^{1,5,*}\)
CD8+ T-cells contribute to viral control in SIV-infected macaques on ART

n=11; SIVmac239, ART=PMPA/FTC/DRV/RGV

PERIOD 1 = before CD8 depletion
PERIOD 2 = after CD8 depletion
PERIOD 3 = after CD8 reconstitution

Silvestri G et al., CROI 2016, Boston MA
Breakthrough of the Year

Cancer Immunotherapy

T cells on the attack
Blocking immune checkpoint markers to boost immune function

Ex vivo blockade of PD-1, PDL-1, CTLA-4, and TIGIT enhance HIV-specific T-cells

Latent HIV is enriched in T-cells expressing immune checkpoint markers

Chomont et al., Nature Med 2009

Fromentin, Chomont et al., submitted
ALT-803 (IL15 superagonist) reverses latency and boosts CTL activity ex vivo

ALT-803 is an IL-15 superagonist currently in phase I/II for solid organ malignancy.

Open label, single arm, dose escalation study in HIV infection planned (Schacker).

Jones B et al., Plos Path 2016; Jones et al., Keystone meeting on HIV Persistence, April 2015
HIV persistence on ART correlates with markers of activation and proliferation

Integrated HIV DNA copies/million CD4+ T-cells

%CD38+HLA-DR+ CD8+ T-cells

p=0.023

p<0.001

Similar findings for CCR5, Ki67, PD-1, TIM3 and others in blood and tissue

Khoury, Fromentin, Chomont et al
measuring the HIV reservoir
## Quantifying HIV persistence

**HIV genomes**
- Integrated genomes
  - Intact genomes
- Genomes producing viral transcripts
- Genomes producing viral proteins
- Genomes producing virions

### Detectable targets

<table>
<thead>
<tr>
<th>Detectable targets</th>
<th>Assay target</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gag DNA</td>
<td></td>
<td>1000</td>
</tr>
<tr>
<td>Alu-LTR</td>
<td></td>
<td>300</td>
</tr>
<tr>
<td>Intact genomes</td>
<td></td>
<td>60</td>
</tr>
<tr>
<td>usRNA, msRNA</td>
<td></td>
<td>30-70</td>
</tr>
<tr>
<td>HIV proteins</td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>Viral particles</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Infectious virus</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

Detection of HIV RNA producing cells by flow cytometry

CEM (uninfected) : Activated ACH2 (infected)

HIV gag

HIV env

HIV gag

HIV env

Brightfield

eenv RNA/

gag RNA /DAPI

Hao Lu, Doherty Institute
Detection of SIV env using CT/PET

Poly(ethylene glycol)-modified, $^{64}$Cu-labeled SIV Gp120–specific antibody

Santangelo et al., *Nature Methods* 2015
We need a biomarker that can predict “cure” or “remission”

- **HIV DNA**: SPARTAC, Swiss HIV Cohort Study, SALTO
- **CA-US HIV RNA**: ACTG ATI Cohort Study
- **PD1 expression** on CD4 and CD8 (prior to ART): SPARTAC

Conclusions

- Multiple barriers to eliminating HIV including both HIV persistence and impaired immunity.

- Agents that reverse latency need to be more potent, more specific and induce death of the infected cell. Alternative approaches may be needed to specifically target different tissue reservoirs.

- Long term immune control also required to both boost T-cell function and reduce inflammation. Future role of immune checkpoint markers to be determined.

- New modalities needed to quantify the frequency and location of infected cells and predict time of remission.
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