Curing HIV Infection: Is it Possible?

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Disclosures:
Research support: Bionor, Gilead, GSK-ViiV, Janssen, Pfizer-ViiV, Vertex
Consultant/Advisor: Boehringer Ingelheim, CDC, Calimmune, Inc, Gilead, GSK-ViiV, Janssen,
DSMB: Janssen
Is It Possible to Cure HIV Infection?

- YES
- NO
Lecture Outline

• Why cure HIV infection? Isn’t ART enough?
• Definitions of HIV cure
  – Functional
  – Sterilizing
• HIV persistence and how to address it
• Shock and kill
• Genetically modified stem cell therapy
Life Expectancy of HIV-Infected Patients

- Life expectancy of Athena cohort (n=4,174) compared to general population
- Expected life years remaining at age 25
  - 53.1 (44.9-59.5) for general population
  - 52.7 for asymptomatic HIV+ patients

Cumulative Survival for HIV-infected Persons and the General Population from Age 25

Lohse et al, Ann Int Med 2007
Why Are We Now Talking about Cure?

• HAART does not fully restore health
• HAART require life-long therapy
  – Costs ($20-30k/person/year), toxicity, adherence
  – 75% of persons qualified for HAART in low- and middle-income countries are not being treated
  – For every 10 people treated in 2011, 16 others became infected
• Mechanisms of viral persistence are now better understood
• The “Berlin Patient”, the “Mississippi Baby” and the “Visconti Cohort” indicate a cure may be feasible
Sterilizing Cure

• Complete eradication of all replication competent virus ("sterilizing cure")
  • Is this possible?
  • Is this necessary?
  • How can this be proven?
• Approach #1: Destroy all HIV-producing or HIV-infected cells; replace with HIV-resistant cells
• Approach #2: Induce transcription of latent HIV genomes in resting CD4+ T cells during completely effective antiretroviral therapy

**A sterilizing cure may require potent host responses to clear residual virus-producing cells**
The First Case of Eradication of HIV Infection?
### Treatment Schema

**Assays for HIV**: (blood, BM, brain, colon)

**Procedures to obtain specimens:**
- Leukapheresis
- Sigmoidoscopy
- Colonoscopy
- LN biopsy
- LP

**Samples were sent to 6 different labs:**
1. BSRI (Michael Busch)
2. NIH (Tae-Wook Chun)
3. Solna, Sweden (Sarah Palmer)
4. UCSD/SDVAMC (Matt Strain, Doug Richman)
5. Johns Hopkins (Robert Siliciano)
6. UCSF/SFVAMC (Joseph Wong)
# Summary of Virology Results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Measure</th>
<th>Lab</th>
<th>Consensus</th>
<th>Avg levels in ART-suppressed pt</th>
<th>Fold difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>HIV RNA</td>
<td>BSRI NIH Sweden UCSD JH UCSF</td>
<td>Intermittent + &lt;1 c/ml</td>
<td>1-2 c/ml</td>
<td>2-20</td>
</tr>
<tr>
<td>PBMC</td>
<td>HIV DNA</td>
<td>BSRI NIH Sweden UCSD JH UCSF</td>
<td>ND ≤1 in 10⁶-⁷</td>
<td>750 in 10⁶</td>
<td>&gt;750-7500</td>
</tr>
<tr>
<td>HIV RNA</td>
<td>-</td>
<td>-</td>
<td>ND ≤1 in 10⁶-⁷</td>
<td>66 in 10⁶</td>
<td>&gt;66-660</td>
</tr>
<tr>
<td>IUPM</td>
<td>-</td>
<td>-</td>
<td>ND ≤1 IU in 10⁷-⁹</td>
<td>1 IU in 10⁶</td>
<td>&gt;10-1000</td>
</tr>
<tr>
<td>Rectum</td>
<td>HIV DNA</td>
<td>BSRI NIH Sweden UCSD JH UCSF</td>
<td>Positive 8 c/10⁶ cells</td>
<td>780-3300 in 10⁶</td>
<td>97-413</td>
</tr>
<tr>
<td>HIV RNA</td>
<td>-</td>
<td>-</td>
<td>ND &lt;1 in 10⁷</td>
<td>21-57 in 10⁶</td>
<td>210-570</td>
</tr>
<tr>
<td>CSF</td>
<td>HIV RNA</td>
<td>BSRI NIH Sweden UCSD JH UCSF</td>
<td>ND ≤0.1 c/ml</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>HIV DNA</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>
Functional Cure

• Long-term health in absence of antiretroviral therapy = “functional cure”
  – Occurs in ~1% of natural infections,
  – may be occurring in “post-treatment controllers” (e.g., Visconti Cohort)
  – “Mississippi Baby”

• Approach: Reduce or limit the size of the latently HIV-infected reservoir

• Will there be residual disease?
Visconti Cohort:
Post-treatment controllers had high viremia before HAART and low proviral DNA post-HAART

Saez-Cirion and colleagues, 2012
Primary strategy to eliminate latent HIV infection

Other Challenges:
- Clearance of infected cells
- Clearance of virions
- Complete block of new infection
Shock and Kill

Diagram showing the process of "Shock and Kill" in the context of HIV treatment. The diagram illustrates the interaction between Vorinostat, the immune system, and antiretroviral therapy to control HIV replication and reduce viral load.
Vorinostat:
Suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor with nanomolar potency licensed for the treatment of cutaneous T cell lymphoma

Archin ARHR 2009
Contreras JBC 2009
**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
CD8⁺ T Cells from Patients on ART DO NOT Kill Latently Infected CD4⁺ T cells after Virus Reactivation

Shan Immunity 2012
CD8⁺ T Cells from Patients on ART DO NOT Kill Latently Infected CD4⁺ T cells after Virus Reactivation

Shan Immunity 2012
Can Stem Cell Transplants Cure HIV Infection?
Patient A (4.3 years post-HSCT)

### Sample Input Assay Result / Detection Limit

<table>
<thead>
<tr>
<th>Sample</th>
<th>Input</th>
<th>Assay</th>
<th>Result / Detection Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMC DNA</td>
<td>25 x 10⁶ PBMC</td>
<td>qPCR for LTR/Gag</td>
<td>Not Detected &lt; 0.07 copies/10⁶ PBMC</td>
</tr>
<tr>
<td>Peripheral CD4+ T Cells</td>
<td>150 x 10⁶ CD4+ T cells</td>
<td>Co-culture</td>
<td>Not Detected &lt; 0.01 IU/10⁶ CD4+ Cells</td>
</tr>
</tbody>
</table>

Minimum 3.3 log₁₀ reduction of PBMC DNA after alloHSCT
Patient B (2.6 years post-HSCT)

Sample Input Assay Result / Detection Limit

<table>
<thead>
<tr>
<th>Sample</th>
<th>Input</th>
<th>Assay</th>
<th>Result / Detection Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMC DNA</td>
<td>50 x 10^6 PBMC</td>
<td>qPCR for LTR/gag</td>
<td>Not Detected* &lt; 0.04 copies/10^6 PBMC</td>
</tr>
<tr>
<td>Peripheral CD4+ T Cells</td>
<td>150 x 10^6 CD4+ T cells</td>
<td>Co-culture</td>
<td>Not Detected &lt; 0.01 IU/10^6 CD4+ cells</td>
</tr>
<tr>
<td>Rectal Biopsies</td>
<td>DNA from 1.3 x 10^6 cells</td>
<td>qPCR for LTR/gag</td>
<td>Not Detected &lt; 2 copies/10^6 cells</td>
</tr>
</tbody>
</table>

Minimum 3.5 - 4 log reduction of PBMC DNA after alloHSCT, CCR5 wildtype

Henrich T et al. WELBA05
Ongoing Studies / Preliminary Results

- Results reported July 2013:
  Patient A ≈ 8 weeks off ART
  Patient B ≈ 15 weeks off ART

- No detectable HIV-1 plasma RNA or PBMC DNA to date from either patient
Ongoing Studies / Preliminary Results

• Results reported July 2013:
  Patient A ≈ 8 weeks off ART
  Patient B ≈ 15 weeks off ART
• **No detectable HIV-1 plasma RNA or PBMC DNA** to date from either patient

• UPDATED REPORT December 2013
  Patient A ≈ 12 weeks off ART
  Patient B ≈ 32 weeks off ART
• **HIV-1 RNA detected in plasma in both patients.**
Can Gene-modified Cellular Treatments Be Used in HIV+ Patients without Cancer?
GENE-MODIFIED HEMATOPOIETIC STEM/PROGENITOR CELL BASED THERAPY FOR HIV DISEASE

Geoffrey Symonds, PhD
Louis Breton
Jeffrey Bartlett, PhD
Louis Evans, MD
W. David Hardy, MD

CIRM Disease Team Therapy Development Research Award
DR1-06893
Investigational product (IND 15258)

LVsh5/C46 (Cal-1) modified autologous CD34+ Hematopoietic Stem/Progenitor Cells and autologous CD4+ T Lymphocytes

active anti-HIV agents
Natural genetic mutation provides HIV protection

- ~1% Caucasians lack the CCR5 receptor (homozygous) and have complete protection against HIV
- ~10% Caucasians have half the CCR5 receptor (heterozygous), are susceptible to HIV but have ~3-5 year delay in disease progression

Cell-delivered gene therapy is a strong HIV therapeutic candidate

- Our therapy has shown 5-10 fold reduction of CCR5 and may confer the same protection to any HIV+ individual
- 100% down-regulation not required for clinical significance
- Modified HSPC can engraft and preferentially expand over time
- Can also introduce to CD4 T cells
- Support for one-time or infrequent delivery requirement
- Could solve issues of adherence
Therapeutic Rationale

☑ Combination Therapy – Cal-1 (combined sh5/C46)
   • Active against both R5- and X4- strains of HIV
   • May be more effective than either agent alone
   • Two points of inhibition for R5-tropic HIV-1
   • Mitigates against resistance of HIV

☑ Target Cell Population –
   • HSPC for long-term protection
   • CD4+ T Cells for short- to medium-term protection

☑ Engineering of Host Resistance –
   • Selective expansion mediated by ongoing HIV-1 infection

☑ Conditioning – Busulfan
   • Enhanced engraftment of transplanted cells
Scientific Rationale

- Delivery to Hematopoietic Stem/Progenitor Cells for long-term protection
- Delivery to CD4+ T Cells for short- to medium-term protection

Intracellular immunization:
Progressive population of immune system with cells protected against HIV (Baltimore)
Efficacy Studies (in vivo)

In vivo efficacy in hu-NSG (BLT) mice

Fetal Liver → CD34+ HSPC → Transduce with Cal-1

HSPC^TN (Cal-1 transduced CD34+)

Combine with fetal thymus and liver in matrigel

Irradiate

3 weeks

Analysis of engraftment

Ex vivo challenge analysis

12 weeks

Splenocytes

Infect with HIV-1

In vivo challenge analysis

14 weeks

Based on Shimizu et al. 2010 Blood 115:1534-1544
Preclinical - Efficacy Studies

*In vivo* efficacy in hu-NSG (BLT) mice

*Ex vivo* challenge experiments using splenocytes from BLT mice humanized with Cal-1 transduced CD34\(^+\) HSPC (Cal-1) or mock transduced CD34\(^+\) HSPC (Control)
**Preclinical - Efficacy Studies**

*In vivo* efficacy in hu-NSG (BLT) mice

**CD4⁺ T-cell protection**

[Graph showing the percentage of CD4⁺ T-cells over weeks post-infection for Control and Cal-1 groups.]

**Viral Load Reduction**

[Graph showing the normalized HIV-1 copies per μl plasma over weeks post-infection for Control and Cal-1 groups.]

Effective protection of CD4⁺ T-cells and reduction of HIV-1 viremia *in vivo*
Preclinical - Safety/Toxicity Studies

- Cell progenitor skewing assessment (methylcellulose colony progenitor assays on CD34\(^+\) cells)
- Apoptosis (caspase assays on primary human PBMC)
- Proliferation studies (in primary human PBMC)
- Inflammation studies (via IFN\(\gamma\), TNF\(\alpha\), and IL-6 ELISA)
- Recombination and replication competent lentivirus (RCL) formation
- Mutagenic potential of Cal-1 and insertional mutagenesis
- Safety and toxicology assessment of Cal-1 transduced CD34\(^+\) HSPC in NSG mice

No results from these studies have been indicative of any toxicity risk.
Key Considerations To Improve Clinical Outcomes

1. Use of conditioning to create bone marrow “space”
   • We will use two doses of busulfan (4mg/kg and 8mg/kg) in the second and third cohorts with the aim of making bone marrow “space”
   • These are doses successfully used in other gene therapy stem cell trials

2. Dual therapeutic targeting host rather than the virus
   • Sh5/C46 combination may protect against both CCR5 & CXCR4-tropic HIV
   • Dual therapeutic mitigates development of resistance
   • Engineering host resistance to HIV allows selection of modified cells
Key Considerations To Improve Clinical Outcomes

3. Subjects with active HIV, which may supply selective pressure
   • Subjects have ceased ART for at least 6 weeks coming into the study
   • HIV viral load provides selective pressure

4. Use of two cell populations
   • CD4+ T cells to provide short – medium term benefit
   • CD34+ HSPC to provide longer term benefit
Hypotheses – CAL-USA-11

Primary Hypothesis

Delivery of the Investigational Product will be a safe and feasible means to reduce HIV-1 RNA and increase CD4+ T cell counts (as compared to baseline) in the absence of ART

Secondary Hypotheses

• Transduced HSPC will home to the bone marrow, engraft and provide a population of peripheral blood hematopoietic cells protected from HIV-1
• Intravenous busulfan will facilitate engraftment of transduced HSPC, resulting in increased levels of Cal-1 marking compared to the non-busulfan cohort
• Transduced T cells will be protected from the pathogenic effects of HIV-1 and provide a short to medium term replenishment of CD4+ cells in the peripheral blood
Study Overview

1. Apheresis
   - Small volume apheresis
   - CD4⁺ cell isolation with CliniMacs
   - Standard volume apheresis
   - CD34⁺ cell isolation with CliniMacs

2. Cell isolation
   - G-CSF
   - HIV-Infected individual

3. Lentiviral Transduction
   - Isolated CD34⁺
   - Transduction with Cal-1
   - Isolated CD4⁺

4. Harvest of transduced CD4⁺ and CD34⁺ cells
   - HSPC<sup>tn</sup>
   - T<sup>tn</sup>
   - Infuse cells
   - Busulfan to create bone marrow “space”

5. Autologous transplant of genetically modified cells
Study Design CAL-USA-11

- Eligibility screening & enrolment
- CD4 Apheresis
- CD34 Apheresis
- G-CSF
- G-CSF 10 µg/kg daily SC
- Infusion
- Clinical Protocol
- DSMB reviews x2 interim analysis
- DSMB reviews x2 final analysis

-63 -39 -32 -28 Day 0 4w 12w 24w 48w

**Cohort 1:** No busulfan preconditioning

**Cohort 2:** Preconditioning with a single dose of 4mg/kg Busulfan

**Cohort 3:** Preconditioning with a total dose of 8mg/kg Busulfan
Primary Objectives and Outcome Measures

- The **safety and feasibility** of the introduction of Cal-1, gene-transduced, hematopoietic cell populations
- The **safety** of low and moderate dose intravenous busulfan non-myeloablative conditioning as a means to improve engraftment

### Safety Measures

<table>
<thead>
<tr>
<th>CBC</th>
<th>Vital signs</th>
<th>Pregnancy test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemistry</td>
<td>Weight</td>
<td>HIV-1 R5/X4 tropism</td>
</tr>
<tr>
<td>T cell count</td>
<td>Chest x-ray</td>
<td>Integration analysis</td>
</tr>
<tr>
<td>Physical Exam</td>
<td>RCL</td>
<td></td>
</tr>
</tbody>
</table>

### Feasibility Measures

- Number of successful manufacturing runs (i.e. complying with all release criteria)
- CD4+ and CD34+ purity
- CD4+ and CD34 + phenotype
- Number of target cells harvested/administered
Secondary Objectives and Outcome Measures

To assess the difference between the 3 treatment cohorts in:

• **The extent of HSPC contribution and T cell survival by;**
  - evaluation of Cal-1 marking and expression in peripheral blood and GALT

• **The benefit of busulfan conditioning as determined by evaluation of engraftment and differentiation of HSPC by;**
  - evaluation of Cal-1 marking and expression in peripheral blood subpopulations (monocytes, granulocytes, CD4+ and CD8+ lymphocytes), and bone marrow

• **The potential efficacy of Cal-1, as measured by;**
  - Plasma HIV-1 RNA relative to baseline, and over time
  - CD4+ T lymphocyte count, percentage and CD4+/CD8+ T lymphocyte ratio relative to baseline, and over time
  - Time to commencement of ART
Research Questions

• Can HIV eradication be proved using the best currently available virologic assays? Can they adequately detect ultra-low levels of residual virus which may re-activate without ART?

• Enhanced, acquired immune control of HIV is the objective. What correlates of this new immunity are we looking for to confirm that we are on the right track?

• Can activation of latently HIV-infected immune cells be specific enough to avoid deleterious immune activation?

• ART interruptions will be necessary to test for HIV control or eradication. How can they be done safely when prevailing data suggest that even brief exposure to viremia may be harmful?

• Are any of these novel therapies scalable for general use?
Is It Possible to Cure HIV Infection?

- YES
- NO