Gene Therapy and HSCT for HIV Cure: Science or Fiction?

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Why can’t we cure HIV with ARV Drugs
Where is the virus and how is it maintained in the face of “suppressive” therapy?

A game of hide and sleep?

These are not mutually exclusive mechanisms; will multiple approaches be required?
Approaches to Cure HIV by Gene Therapy

**CCR5 modification, T cells, stem cells**

**Other modifications of hematopoietic cells**

**Entry inhibitors, siRNA**

**Direct targeting of integrated virus**

Tilton and Doms, 2010
The main reason for failure of gene therapy: 
The number of gene-modified cells is too low.

Success with a low number of transduced cells is possible if

• the gene product has a therapeutic effect on non-modified cells also (e.g. secreted, immunizing gene product).
  -> bystander effect

• the transferred gene confers a selective advantage to the gene-modified cell.
  -> in vivo selection
In vivo selection of transduced cells

Autologous or syngeneic T cells

Selection

- Cells replicate HIV
- Cells are resistant to HIV
1. Modified CCR5, membrane-anchored peptides
2. scFv to RT
3. scFv to IN
4. Td Tat, TAR decoy
5. Td Rev, RRE, mEIF-5A
6. Anti-sense RNA, Ribozymes
7. td Gag, pbs decoy
8. Gag-Nuclease
Accumulation of gene-modified cells?

Selection?

Class I

Class II

Selection?

Class III

- Replicate HIV
- Contain antiviral gene
- HIV-infected
Retrovirus life cycle

Entry inhibition:
- fusion (gp41)
- CCR5d32
Integrated DNA:
- Tre recombinase
The membrane-anchored fusion inhibitory peptide M87: Mode of action

Membrane anchor

C-heptad repeat

N-heptad repeat

Fusion peptide

M87/C46

C-peptide (T20)

Linker

Membrane anchor

gp120

Cell
CD4 cells increase significantly after transfer of ex vivo expanded M87o-modified T cells

Relative changes in CD4+ absolute counts from baseline, means +/- confidence intervals

* p < 0.05

Van Lunzen et al, Mol Ther 2008
Make cells resistant to HIV: modify CCR5

Gene therapy to eliminate CCR5: Sangamo Biosciences Inc

SB728-902

Aviremic HIV subjects on HAART

SB728-T

n=6
Aviremic patients on cART
CD4>450
Treatment interruption

Lalezari et al., 18th CROI, Boston, Feb 2011

Ando et al., ICAAC, Chicago, Sept 2011

June et al., 19th CROI, Seattle 2012, #155
<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Sex</th>
<th>Ethnicity</th>
<th>Age</th>
<th>CD4 Count</th>
<th>% CD4</th>
<th>CD4:CD8</th>
<th>Viral Load</th>
<th>Years of HIV</th>
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<tbody>
<tr>
<td>Cohort 1</td>
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<td>01-102</td>
<td>M</td>
<td>Hispanic</td>
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<td>&lt;48</td>
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<tr>
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<tr>
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<td>53</td>
<td>281</td>
<td>16.5</td>
<td>0.30</td>
<td>&lt;48</td>
<td>21</td>
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<tr>
<td>02-302</td>
<td>M</td>
<td>Hispanic</td>
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<td>384</td>
<td>25.6</td>
<td>0.78</td>
<td>&lt;48</td>
<td>19</td>
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</tbody>
</table>
Increased CD4 T-cell Counts from Baseline after Single SB-728-T Infusion

Sustained increase from baseline observed in 5 of 6 subjects at most time points
Normalization of CD4:CD8 T-cell Ratio after Single SB-728-T Infusion

CD4:CD8 reversal (from <1 to >1) in 3 of 5 subjects
Expansion of CCR5-Disrupted CD4 T-cells in the Peripheral Blood

Median 2.9-fold increase of ZFN-modified cells at D14 post-infusion, suggests in vivo expansion
Viral load during treatment interruption

[Sangamo trial SB-728-T]
TALENs: Highly specific inhibition

- High efficiency
- Minimal impact on cells (vitality, function)
- Transient

Efficient!

 transient!
Specific TALENs cut CCR5 and decrease infectivity of modified cells

Very good correlation between molecular data (NGS) and FC-analysis
Summary (TALENs)

• Identification and cloning of novel CCR5-specific TAL effector nucleases with high on-target and low off-target activity

• Efficient transport of TALEN into T lymphocytes by the means of mRNA-electroporation for transient expression

• High-rate protection of edited T cells from HIV infection

→ Promising approach to protect autologous or allogeneic T cells from HIV infection

→ Early application seems to be preferable (protection of HIV-specific T-cell clones!)
Shift from CCR5 to CXCR4 tropism after allo transplant of CCR5-d32 homozygous graft

Kordelas et al. 2014 NEJM
Calimmune double strike

Membrane-anchored C peptides, maC46 (C46)

short hairpin RNA against CCR5

C46 peptide

Fusion peptide insertion

Six-helix bundle formation
Excision of HIV proviral DNA using Tre-recombinase
LTR-specific Tre-Recombinase effectively excise HIV-DNA in vitro

Real time qPCR from total genomic DNA

Gag expressing cells by indirect IF

Sarkar et al. 2007 Science
Humanized mouse models

- Transplantation with Tre-transduced human CD4⁺ T cells or CD34⁺ HSC
- Engraftment of the animals with human cells
- HIV-1 *de novo* infection
- Analysis of potential antiviral effects over time

Long-term Tre effect – The „Hamburg“ mouse?

- Tre-mediated decline of viral load below the detection limit
  (<20 HIV-1 RNA copies/ml)
2007 – first „natural“ HIV gene therapy
Transplant may lead to functional cure (1)

Timeline for the Berlin patient

- **AML diagnosis**
- **First bone marrow transplantation** (CCR5 Δ32 homozygous donor)
- **Second bone marrow transplantation** (same donor)

Search for residual HIV:
- Plasma RNA negative or equivocal
- Gastrointestinal tract RNA negative or equivocal
- HIV antibody decreasing

Donor search and genotyping

232 HLA identical donors

therefrom

80 registered at the DKMS

Request for blood samples

CCR5-Δ32 screening

Donor 61 CCR5-Δ32/Δ32
Probability in finding allo CCR5-d32/d32 donors

unrelated donors  
20% for 9/10 or 10/10 match  
(own experiences)

related donor  
1-(3/4)^n-1 x 0.01 (n= siblings)

cord blood  
up to 85% (4/6 match)

Projected HLA Match Rates with a 300-Unit Inventory of CCR5-Δ32/Δ32 Cord Blood Units

<table>
<thead>
<tr>
<th>Ethnic Group</th>
<th>Adult Patients</th>
<th>Pediatric Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 of 6 matches: .01%</td>
<td>6 of 6 matches: .01%</td>
<td></td>
</tr>
<tr>
<td>5 of 6 matches: 4.5%</td>
<td>5 of 6 matches: 10.6%</td>
<td></td>
</tr>
<tr>
<td>4 of 6 matches: 27.9%</td>
<td>4 of 6 matches: 73.6%</td>
<td></td>
</tr>
<tr>
<td>Includes need for TNCs of 1 x 10^7 cells/kg</td>
<td>Includes need for TNCs of 1 x 10^7 cells/kg</td>
<td></td>
</tr>
<tr>
<td>6 of 6 matches: .09%</td>
<td>6 of 6 matches: 1.01%</td>
<td></td>
</tr>
<tr>
<td>5 of 6 matches: 10.7%</td>
<td>5 of 6 matches: 10.8%</td>
<td></td>
</tr>
<tr>
<td>4 of 6 matches: 82.1%</td>
<td>4 of 6 matches: 85.6%</td>
<td></td>
</tr>
<tr>
<td>Minority</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>4 of 6 matches: 9.9%</td>
<td>4 of 6 matches: 28.6%</td>
</tr>
<tr>
<td>Mexican American</td>
<td>4 of 6 matches: 14%</td>
<td>4 of 6 matches: 44.1%</td>
</tr>
<tr>
<td>Chinese American</td>
<td>4 of 6 matches: 2.7%</td>
<td>4 of 6 matches: 12.3%</td>
</tr>
<tr>
<td>Includes need for TNCs of 2.5 x 10^7 cells/kg</td>
<td>Includes need for TNCs of 2.5 x 10^7 cells/kg</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>4 of 6 matches: 31.6%</td>
<td>4 of 6 matches: 34.1%</td>
</tr>
<tr>
<td>Mexican American</td>
<td>4 of 6 matches: 48.9%</td>
<td>4 of 6 matches: 52.5%</td>
</tr>
<tr>
<td>Chinese American</td>
<td>4 of 6 matches: 13.9%</td>
<td>4 of 6 matches: 15.7%</td>
</tr>
</tbody>
</table>

TNC indicates total nucleated cell.

[Petz, Biol Blood Marrow Transplant 2013]
Frequency of the CCR5-delta32 allele

- Highest frequency in Northern Europe
- Unknown selective advantage for d32 deletion
- Absent in Africans, Asians and Indians
<table>
<thead>
<tr>
<th>side</th>
<th>patient</th>
<th>cancer</th>
<th>graft</th>
<th>outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berlin, Germany</td>
<td>Male, 40y</td>
<td>AML</td>
<td>Matched unrelated</td>
<td>7 years alive</td>
</tr>
<tr>
<td>Utrecht, NL</td>
<td>Male, 53y</td>
<td>MDS</td>
<td>UCB &amp; haplo</td>
<td>Died 2 months (relapse, pneumonia)</td>
</tr>
<tr>
<td>Münster, Germany</td>
<td>Male, 51y</td>
<td>NHL</td>
<td>Missmatch unrelated</td>
<td>Died 3–4 months (infection)</td>
</tr>
<tr>
<td>Essen, Germany</td>
<td>Male, 30y</td>
<td>HIV–TNHL</td>
<td>Matched unrelated</td>
<td>Died 12 months (relapse)</td>
</tr>
<tr>
<td>Minnesota, US</td>
<td>Male, 12y</td>
<td>ALL</td>
<td>UCB</td>
<td>Died after 3 months (GvHD)</td>
</tr>
<tr>
<td>Santiago, Chile</td>
<td>Male, 46y</td>
<td>NHL</td>
<td>Matched related</td>
<td>Died 2–3 months (pneumonia)</td>
</tr>
<tr>
<td>Barcelona, Spain</td>
<td>Male, 37y</td>
<td>DLCBNHL</td>
<td>UCB &amp; haplo</td>
<td>Died 3 months (relapse)</td>
</tr>
</tbody>
</table>
Transplant may lead to functional cure

Timelines for the Boston patients

Patient A
Perinatal HIV infection, HLA-C MUD for recurrent B-NHL

- No HIV-1 DNA detected from 26x10^6 PBMCs (<0.12 cp /10^6 cells)
- Viral co-cultur of 150x10^6 CD4+ T cell negative (<0.007 IUMPs)
- No HIV-1 DNA detected from rectal tissues (<2.4 cp /10^6 cells)
- residual, post-HSCT host cells constituted ≈ 0.00041% - 0.00081% of PBMCs
- anti HIV-AB serodeconversion

[Henrich, 2012]
Patient B

HIV-1 infection >25 years, ART since 2003, MRD for MDS (prior lymphoma)

- No HIV-1 DNA detected from 24x10^6 PBMCs (<0.13 cp /10^6 cells)
- Viral co-cultur of 160x10^6 CD4+ T cell negative (<0.006 IUMPs)
- No HIV-1 DNA detected from rectal tissues (<2.4 cp /10^6 cells)
- residual, post-HSCT host cells constituted ≈ 0.00035% - 0.00096% of PBMCs
- anti HIV-AB serodeconversion

[Henrich, 2012]
A. Fevers, headache, nausea

- CSF VL detected
- Symptoms resolve

HIV-1 RNA (copies/ml)

HIV-1 DNA

TDF FTC EFV

TDF FTC DRV/r RAL

B. Fevers, malaise

- CSF VL 269 copies/ml
- Symptoms resolve

Plasma HIV-1 RNA

- <0.4 copies/ml

HIV-1 DNA

- <0.5 copies/10^6 PBMC

- <0.2 copies/10^6 PBMC

- TDF/FTC/DTG

HIV-1 RNA (copies/ml)

HIV-1 DNA

- + 1100 318 copies/10^6 PBMC

Henrich, 2014
What we’ve learned from the Boston patients

- Single copy assay negative
- No replication competent virus
- Tissues clear
- Anti HIV ab decline

Means nothing as long patient is on HAART
Immunological theory of HIV cure by stem cell Tx

HIV clearance by alloreactive NK cells?

The „Hamburg Experience“

- 4 patients treated with gene modified PBSC
- All relapsing ARL, myeloablative Tx. + Rdx.
- 3 autologous transplants (low cell number)
- 1 allogeneic Tx., CCR5-WT (25% transduction)
- No ART until engraftment (tox., DDI, selection)
- Engraftment okay, massive HIV replication
- Low number of transduced cells detected
- VL controlled when CTL reappeared
- 3 pts. died (1 sepsis, 2 recurrent disease)
- 1 patient well and alive (allo-Tx.) -> cured?
- Program stopped due to potential vector toxicity
Questions

- How much CTx. +/- irradiation is needed?
- Do we have to stop HAART until engraftment?
- How much conditioning and immunosuppression is needed?
- Is GvHD (graft vs. virus needed to eliminate latently infected cells?)
- Do we have to protect transplanted cells from reinfection?
- How good is the functional quality of Tx. cells?
- Sterilizing vs. functional cure?
Ethical considerations

- What are **acceptable risks and toxicities** of interventions in a population doing well on stable cART?

- What surrogate marker(s) of viral persistence will justify **treatment interruption** as a clinical endpoint in subsequent clinical trials?

- **Expectations** of study participants in early “proof of concept” studies

- **Community engagement** critical in all stages of study design and implementation
Conclusions

- Multiple barriers to eradication means a **combination approach** will be likely.

- Multiple strategies being tested in **early proof of concept studies** including activation strategies, gene therapy, vaccination Ø intensification.

- Early trials designed to demonstrate activity in vivo **may be negative** while we identify the best assays, dosing, testing strategies.

- Significant additional challenge to find a strategy that ultimately is **cheap, scalable** and widely available.
Project to guide and investigate the potential for HIV
cure in HIV-infected patients requiring allogeneic stem
cell transplantation for hematological disorders

Supported by AmfAR Research Consortium on HIV
eradication (ARCHE) Research Grant # 108930-56-RGRL
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1974

uses computer with 64 kb Rom
To get to the moon

2014

uses 64 gb iphone
To upload duckface photos