T-Cell Therapy for HIV: Genetic Engineering of Enhanced Function and HIV Resistance

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Conflict of Interest Statement

- Declaration of financial interest due to intellectual property and patents in the field of cell and gene therapy.
- Consultant for GE Healthcare and Intrexon; SAB for Brammer Bio and Incysus
- Conflict of interest is managed in accordance with University of Pennsylvania policy and oversight
Background and Hypothesis

- The development of more than two dozen antiretroviral therapies to combat HIV has resulted in a dramatic decrease in morbidity and mortality associated with AIDS. But...
- Significant costs of a lifetime of antiviral drug chemotherapy.
- Drug access and compliance issues have contributed to an increase in drug-resistant viral strains.
- Hypothesis: Select, re-engineer HSC or expand T cells ex vivo outside of HIV milieu and re-infuse to prevent disease progression and enhance immune function.
Multiple Points in HIV Lifecycle for Intervention by Gene Therapy
<table>
<thead>
<tr>
<th>Strategy*</th>
<th>Target</th>
<th>Mechanism</th>
<th>Cell Type</th>
<th>Gene Delivery Mechanism</th>
<th>Clinical Trial Identifier</th>
<th>Reference (if published)</th>
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<tbody>
<tr>
<td>Viral entry (1)</td>
<td>HIV-1 env protein</td>
<td>Fusion inhibitor peptide (C46)</td>
<td>Autologous CD4+ T cells</td>
<td>Retroviral vector</td>
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<td>Van Lunzen et al., 2007</td>
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<td>HIV-1 env mRNA</td>
<td>Antisense RNA</td>
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<td>Levine et al., 2006; Tebas et al., 2013</td>
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<td>Provirus (4, 5)</td>
<td>HIV-1 U5 and pol mRNA</td>
<td>Ribozyme</td>
<td>Autologous CD4+ T cells</td>
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<td>RNA transcription (6)</td>
<td>HIV-1 tat-vpr mRNA</td>
<td>Ribozyme</td>
<td>Autologous CD34+ HSCs</td>
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<td>Amado et al., 1999; MacPherson et al., 2005; Mitsuyasu et al., 2009; Amado et al., 2004; Cooper et al., 1999</td>
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<td>RNA nuclear export (7)</td>
<td>HIV-1 Rev protein</td>
<td>RNA decoy</td>
<td>Autologous CD34+ HSCs</td>
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<td>Transdominant mutant protein</td>
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<td>Autologous T cells</td>
<td>Plasmid or retroviral-based vector</td>
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<td>HIV-1 TAR and/or Rev</td>
<td>Antisense RNA</td>
<td>Lymphocytes from uninfected identical twin donor</td>
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<td>Morgan et al., 2005</td>
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<td>RNA stability (8)</td>
<td>HIV-1 MazF</td>
<td>Endoribonuclease</td>
<td>Autologous CD4+ T cells</td>
<td>Retroviral vector</td>
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</table>

* Discovery Med. 2016 Apr:21(116):283
### Table 1. Clinical Trials of Gene-modified Cells in HIV Infection.

<table>
<thead>
<tr>
<th>Strategy*</th>
<th>Target</th>
<th>Mechanism</th>
<th>Cell Type</th>
<th>Gene Delivery Mechanism</th>
<th>Clinical Trial Identifier</th>
<th>Reference (if published)</th>
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<td>Surface targets and viral entry (1)</td>
<td>Human CCR5</td>
<td>ZFN</td>
<td>Autologous CD4+ T cells</td>
<td>Adenoviral vector</td>
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<td>Tebas et al., 2014; Maier et al., 2013</td>
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<td><strong>Immune Response</strong></td>
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<td>TCR transgenic T cells (10)</td>
<td>SL9 epitope (HIV-1 Gag)</td>
<td>Enhanced TCR</td>
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<td>Lentiviral vector</td>
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<tr>
<td>Chimeric antigen receptor (CAR) T cells (11)</td>
<td>CD4ζ chain</td>
<td>CAR-modified, HIV-specific T cells</td>
<td>Autologous CD4+ and CD8+ T cells</td>
<td>Retroviral vector</td>
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<td>Mitsuyasu et al., 2000; Deeks et al., 2002</td>
</tr>
</tbody>
</table>
Editing the Genome to Confer HIV Resistance
Why Target CCR5 in HIV?

- HIV (R5 virus) targets CD4 T-cells by binding to CCR5, one of the major co-receptors for HIV entry
- CCR5 delta-32 mutation produces a nonfunctional protein
  - Homozygotes are resistant to HIV infection
  - Heterozygotes have slower disease progression

*Scientific American, March 2012*
What are Zinc Finger Proteins?
-specific DNA binding proteins, e.g. transcription factors and other regulatory proteins

Combinatorial Strategy with ZFNs To Achieve Genome Specific Targeting

Porteus, Nat Biotech 2005
Zinc-finger protein-targeted gene regulation: Genomewide single-gene specificity

To disrupt CCR5 and prevent HIV entry, ZFN pairs were targeted to the region upstream of where the Δ32 mutation occurs.
ZFN-Mediated Targeted Gene Repair or Disruption

Gene Repair via Homologous Recombination

Gene Disruption via Non-Homologous End Joining

Donor DNA

DSB

ZFN Mediated Cleavage

deletion

insertion
Activated T Cell Therapy for Infection or Malignancy
10 Day Ex Vivo Process

1. Leukapheresis, enrich, deplete, or isolate cells of interest
2. Stimulate cells with aAPC (insert genes)
3. Large scale cell expansion
4. Remove beads, wash and concentrate cells
5. Reinfuse cells
6. Quality Control

Bead or LV K562
Bead Based in vitro T Cell Culture System

Artificial APC: Bead

Anti-CD3 Anti-CD28

TcR/CD4 CD28 CTLA4

Signal 1 Growth

J Immunol 1997; 159: 5921
Science 1997; 276: 273
Mol. Ther. 2004; 9: 902
Exp. Opin. Biol. Ther. 2008; 8: 475
Polyclonal Replicative Potential of Adult CD4 T Cells In Vitro

- **IL-2 Added Day 49**
- **No IL-2 Day 50**

Total Cells vs. Day

- **Exponential growth (days)** 60-100
- **Mean log10 growth (fold)** 9-12
- **Mean Pop Doubling** 30-40
In Vivo Selection of CCR5-ZFN Modified Cells in NOD/SCID IL-2Rγnull Mice

Primary CD4+ T Cells Isolated from Spleen Day 40 after HIV Challenge

p=0.008
High Definition T Cell receptor Repertoire Following CCR5 ZFN gene disruption

- No change compared to unmodified cultures
- Karyotyping also showed no differences
- Extensive animal studies show no increase in adverse events/deaths

Human Gene Therapy 24:245–258 (March 2013)
Ex Vivo CCR5 Genetic Modification of CD4\(^{+}\) T-cells
Via Zinc Finger Nucleases in HIV+ Pts

- Pheresis
- Enrich CD4+
- SB-728
- Expand, Cryopreserve, Test cell product
- Infuse
- Follow Safety, CD4/HIV, CCR5 selection

Ad5/F35 Chimeric Vector

Clinicaltrials.gov NCT00842634
Phase I Study Designs:
SB728T = CCR5-ZFN Modified T Cells (Enriched CD4+)

Penn / Jacobi (NCT00842634)
• Open label, single dose study
• Study population: 3 cohorts
  1. MDR, virologic failure
  2. Aviremic, CD4> 450 (N=6)
  3. Aviremic, CD4< 500 (N=6)
• Optional STI beginning 1 month post infusion (cohort 2)
• Single infusion of 0.5 - 1.0 \times 10^{10}
  CCR5- modified cells

Quest Clin. Res. / UCLA* (NCT01044654)
• Open label, single dose study
• Study population – All subjects on HAART

Endpoints
Feasibility, Safety and Tolerability
Change in CD4 count, CD4/CD8 ratio, proviral DNA
Persistence of ZFN CCR5 modified T-cells
Increased CD4 Counts in Peripheral Blood after CCR5-ZFN CD4+ T Cell Infusion
CCR5-Modified T Cell Persistence in Peripheral Blood

Interim analysis

[Graph showing the median CCR5-modified CD4 T cell count per mm$^3$ over days for Cohort 1 and Cohort 2.]
• R5 gene disruption levels in rectal mucosal CD4s qualitatively tracks with the disruption levels in peripheral blood CD4s
Unexpected Finding: HIV-RNA Decreases During STI in Immune Responders

Viral load (Copies/mL)

Treatment Interruption

Days

Viral Setpoint (Copies/mL)

*CCR5 - Δ32 Heterozygote

Dotted line denotes reinstitution of HAART
CD4 Count Decay: Unmodified vs Gene-Edited T Cells
CCR5 modified CD4 T cell Infusions in HIV

• Safe and well-tolerated
• Durable increases in total CD4 T cells, normalization of CD4:CD8 ratio.
• Engraft, expand, and persist (>1 yr) in circulation
• CCR5-modified CD4 T cells detected in gut mucosa, demonstrating homing and persistence
• Delay/decrease in viral setpoint during drug treatment interruption in a subset of study subjects
• Increase CCR5 ZFN-modified T cell engraftment with Cytoxan

• Demonstrate dose effect by enrolling Δ32 heterozygotes

• CXCR4 ZFN-modified T cells

• Delivery of ZFN by electroporation of RNA
Investigational agent mC46 (derived from C-terminal hydrophobic alpha helix region of gp41) inhibits conformational changes required for virus fusion
mC46 and a shRNA specific to human CCR5
Cal-1 transduction and autologous transplantation of hematopoietic stem cells
long-term, multilineage engraftment in blood and GI tract
Positive selection for gene-marked cells is observed in blood and tissues following SHIV challenge
Enrichment of mC46 and CCR5 shRNA modified cells following SHIV Challenge

Peterson et al. Mol Ther Methods Clin Dev. 2016 Feb 24;3:16007
Potent and Broad Inhibition of HIV-1 by a Peptide from the gp41 Heptad Repeat-2 Domain Conjugated to the CXCR4 Amino Terminus

George J. Leslie, Jianbin Wang, Max W. Richardson, Beth S. Haggarty, Kevin L. Hua, Jennifer Duong, Anthony J. Secreto, Andrea P. O. Jordon, Josephine Romano, Kritika E. Kumar, Joshua J. DeClercq, Philip D. Gregory, Carl H. June, James A. Hoxie [view all]

Published: November 17, 2016 • https://doi.org/10.1371/journal.ppat.1005983

- HIV-1 entry can be inhibited by soluble peptides from gp41 heptad repeat-2 (HR2) domain
- These peptides can be conjugated to anchoring molecules and over-expressed on the cell surface
- C34-conjugated coreceptors CCR5 or CXCR4 each exhibited potent and broad inhibition of HIV-1 isolates from diverse clades irrespective of tropism (i.e., each could inhibit R5, X4 and dual-tropic isolates)
Inhibiting HIV Entry with **C34 Peptide** Conjugated to a Chemokine Receptor (CXCR4)

1. gp120 → gp41
2. CCR5 → CXCR4
3. 6-helix bundle
4. CD4 HR1 HR2 C34 Inhibitory Peptide
Inhibiting HIV Entry with C34 Peptide Conjugated to a Chemokine Receptor

657 - Evolution of HIV-1 Resistance to the Fusion Inhibitor C34-CXCR4 and Potential Fitness Costs in Consideration of a Phase 1 Clinical Trial

George J. Leslie¹, Beth Haggarty¹, Josephine Romano¹, Andrea P. O. Jordon¹, Jianbin Wang², Max W. Richardson³, James L. Riley³, Michael C. Holmes², Pablo Tebas¹, James Hoxie¹
Autologous CD4 T-Cells Modified with Lentiviral Vector Expressing an HR2, C34-peptide conjugated to the CXCR4 N-terminus in HIV-infected Subjects

- **Study Design**
  - Single cohort
  - Open-label pilot study
  - Safety and tolerability
  - Single infusion of autologous CD4+ T-cells genetically modified with an HR2, C34-peptide conjugated to the CXCR4 N-terminus using a lentiviral vector in HIV-infected subjects

- **Investigational Objectives**
  - Safety and feasibility
  - Cell engraftment and persistence
  - Cell trafficking from blood to other sites
  - Expansion/extended persistence when ARV drugs are discontinued
  - HIV-1 rebound level when drugs are stopped
  - Will resistant virus emerge?
  - Will new anti-viral immune responses be generated?
• First randomized, double-blind, placebo-controlled, phase 2 cell-delivered gene transfer clinical trial
• 74 HIV-1–infected adults received a tat-vpr–specific anti-HIV ribozyme (OZ1) or placebo in autologous CD34+ hematopoietic progenitor cells
• No statistically significant difference in viral load between OZ1 and placebo group at primary end point
• But time-weighted areas under the curve significantly lower, CD4+ lymphocyte counts higher in OZ1 group
Quantification of the mean viral load from the analytic treatment interruption beginning at week 40
(CD34+) hematopoietic progenitor cells expressing three RNA-based anti-HIV moieties (tat/rev short hairpin RNA, TAR decoy, and CCR5 ribozyme). In vitro analysis of these gene-modified cells showed no differences in their hematopoietic potential compared with nontransduced cells. In vitro estimates of successful expression of the anti-HIV moieties were initially as high as 22% but declined to ~1% over 4 weeks of culture. Ethical study design required that patients be transplanted with both gene-modified and unmanipulated hematopoietic progenitor cells obtained from the patient by apheresis. Transfected cells were successfully engrafted in all four infused patients by day 11, and there were no unexpected infusion-related toxicities. Persistent vector expression in multiple cell lineages was observed at low levels for up to 24 months, as was expression of the introduced small interfering RNA and ribozyme.
Gene marking in peripheral blood after HSC transplantation

- Ethical requirement to provide standard of care: unmodified HSC infused 1 day after gene marked HSC
- Possibly/likely reduced engraftment of gene-modified HSC?
HIV CAR T Cells and Gene Modified T Cell Longevity in vivo
CD4-zeta Chimeric Antigen Receptor

HIV infected cell

Transmembrane

gp120

gp40

CD4 Extracellular/Transmembrane Domains

Newer CAR generations include ICD of CD28-\(\zeta\) and/or 41BB-\(\zeta\)

Transmembrane

CD3-\(\zeta\) Intracellular Domain

T-cell
15 year monitoring for delayed adverse events required by FDA (2006 Guidance).

Patients from 3 studies in HIV gene therapy utilizing a retroviral vector expressing the CD4-ζ CAR in CD4/8 T cells were evaluated annually for persistence of genetically modified T cells.
Combined Data of all 3 Studies

Annual Follow ups
Half-life
1-9 13.9yr

CD4-zeta copies per 1e6 PBMC

Detected: 19 20 32 30 27 26 24 14 8 4 1
Tested: 19 20 33 31 27 26 24 15 8 4 1

Scholler et al.
May 2, 2012
### CD4-ζ RNA Present in Patient

<table>
<thead>
<tr>
<th>Study/Patient</th>
<th>Annual Follow up</th>
<th>% Copies CD4ζ in PBMCs</th>
<th>RNA Detection</th>
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</table>

**Levels of CD4-ζ RNA Detected**
- N/A = not applicable
- +++ = reliable levels
- ++ = at limits of detection
- + = no RNA Detected

**Engraftment Limit for RNA detection**
SCID-ADA Transduced T cells

T Cells or their progeny, especially when conferred with a survival advantage, can persist in vivo for years


Persistence in Tumor? HIV?
# Patient Safety Years of Genetically Modified T cells

## University of Pennsylvania

<table>
<thead>
<tr>
<th>Trial</th>
<th>Engineered T Cell</th>
<th># Patients Infused</th>
<th>Safety (Patient-Years)</th>
<th># Patients Alive (as of last date enrolled in study/LTFU)</th>
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<td>Sangamo ZFN (HIV)</td>
<td>Ad5/35 zinc finger nuclease</td>
<td>12</td>
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<td>CART-BCMA</td>
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<td>10.9</td>
<td>10</td>
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<td><strong>Total</strong></td>
<td></td>
<td><strong>476</strong></td>
<td><strong>1709</strong></td>
<td><strong>352</strong></td>
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</table>
Why Is This Man Smiling?

- The HIV+ “Berlin Patient” Dx w/AML received HSC transplant from an allogeneic, HLA matched, CCR5 delta-32 homozygous donor. He is HIV-free w/o HAART (Hutter G., *NEJM*, 2009)
Keys to Intervention by Gene Therapy in HIV

- Multiple points of attack/defense
- Potency
- Persistence
- Sufficient number of gene modified cells