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

**Bruce Levine, Ph.D.**
**Division of Transfusion Medicine**
**Department of Pathology and Laboratory Medicine**
**University of Pennsylvania**

Penn Medicine
Center for Cellular Immunotherapies

# Conflict of Interest Statement

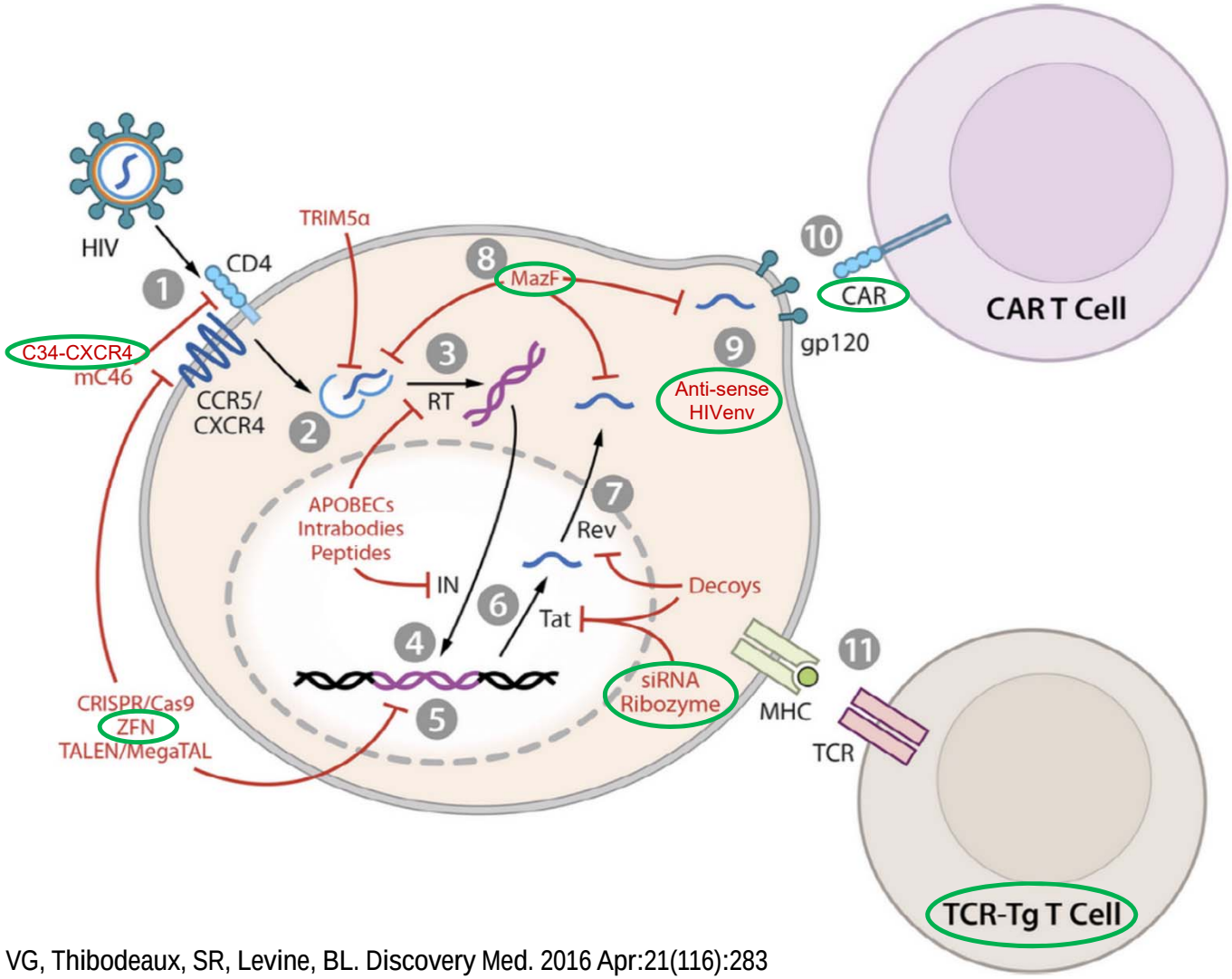
- Declaration of financial interest due to intellectual property and patents in the field of cell and gene therapy.
- Consultant for GE Healthcare and Intrexon; SAB for Brammer Bio and Incysus
- Conflict of interest is managed in accordance with University of Pennsylvania policy and oversight

# 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




Bhoj, VG, Thibodeaux, SR, Levine, BL. Discovery Med. 2016 Apr:21(116):283

**Table 1. Clinical Trials of Gene-modified Cells in HIV Infection.**

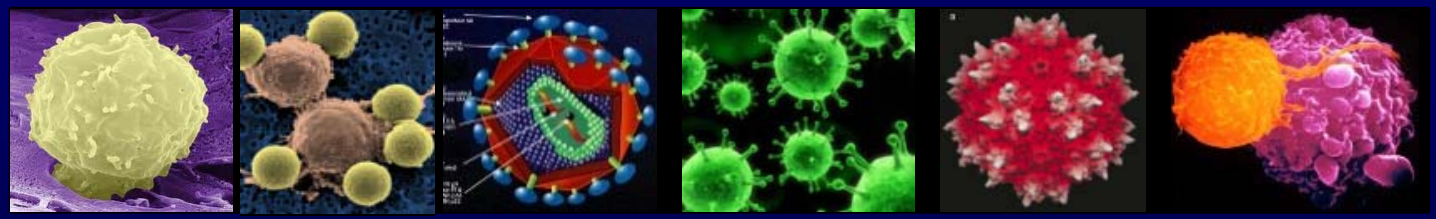
| Strategy* | Target | Mechanism | Cell Type | Gene Delivery Mechanism | Clinical Trial Identifier | Reference (if published) |
|---|---|---|---|---|---|---|
| *Viral Factors* | | | | | | |
| Viral entry (1) | HIV-1 env protein | Fusion inhibitor peptide (C46) | Autologous CD4+ T cells | Retroviral vector | | Van Lunzen *et al.*, 2007 |
| | HIV-1 env mRNA | Antisense RNA | Autologous CD4+ T cells | Conditionally replicating lentiviral vector | NCT00295477 | Levine *et al.*, 2006; Tebas *et al.*, 2013 |
| Provirus (4, 5) | HIV-1 U5 and pol mRNA | Ribozyme | Autologous CD4+ T cells | Retroviral vector | | Wong-Staal *et al.*, 1998 |
| RNA transcription (6) | HIV-1 tat-vpr mRNA | Ribozyme | Autologous CD34+ HSCs | Retroviral vector | NCT00074997 | Amado *et al.*, 1999; MacPherson *et al.*, 2005; Mitsuyasu *et al.*, 2009; Amado *et al.*, 2004; Cooper *et al.*, 1999 |
| RNA nuclear export (7) | HIV-1 Rev protein | RNA decoy | Autologous CD34+ HSCs | Retroviral vector | | Kohn *et al.*, 1999 |
| | | Transdominant mutant protein | Allogeneic CD34+ HSCs | Retroviral-based vector | | Kang *et al.*, 2002 |
| | | | Autologous T cells | Plasmid or retroviral-based vector | | Woffendin *et al.*, 1996; Ranga *et al.*, 1998 |
| | HIV-1 TAR and/or Rev | Antisense RNA | Lymphocytes from uninfected identical twin donor | Retroviral vector | | Morgan *et al.*, 2005 |
| RNA stability (8) | HIV-1 MazF | Endoribonuclease | Autologous CD4+ T cells | Retroviral vector | NCT01787994 | |

Discovery Med. 2016 Apr:21(116):283

| Table 1. Clinical Trials of Gene-modified Cells in HIV Infection. | | | | | | |
|---|---|---|---|---|---|---|
| **Strategy*** | **Target** | **Mechanism** | **Cell Type** | **Gene Delivery Mechanism** | **Clinical Trial Identifier** | **Reference (if published)** |
| ***Host Factors*** | | | | | | |
| Surface targets and viral entry (1) | Human CCR5 | ZFN | Autologous CD4+ T cells | Adenoviral vector | NCT02388594 NCT01252641 NCT01044654 NCT01543152 | Tebas *et al.*, 2014; Maier *et al.*, 2013 |
| | Human CCR5 | ZFN | Autologous CD34+ HSCs | mRNA electropo-ration | NCT02500849 | |
| ***Immune Response*** | | | | | | |
| TCR trans-genic T cells (10) | SL9 epitope (HIV-1 Gag) | Enhanced TCR | Autologous T cells | Lentiviral vector | NCT00991224 | |
| Chimeric anti-gen receptor (CAR) T cells (11) | CD4ζ chain | CAR-modified, HIV-specific T cells | Autologous CD4+ and CD8+ T cells | Retroviral vector | | Mitsuyasu *et al.*, 2000; Deeks *et al.*, 2002 |

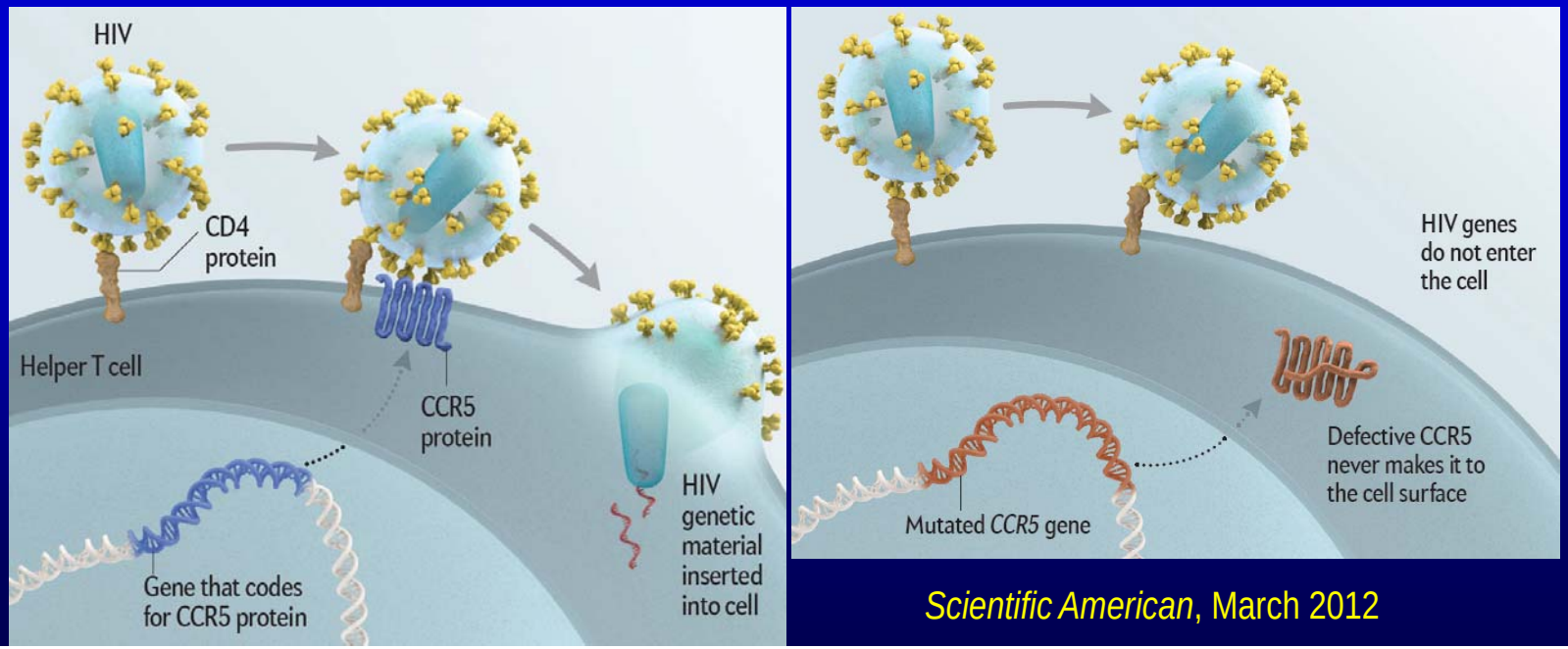
Discovery Med. 2016 Apr:21(116):283

# Editing the Genome to Confer HIV Resistance



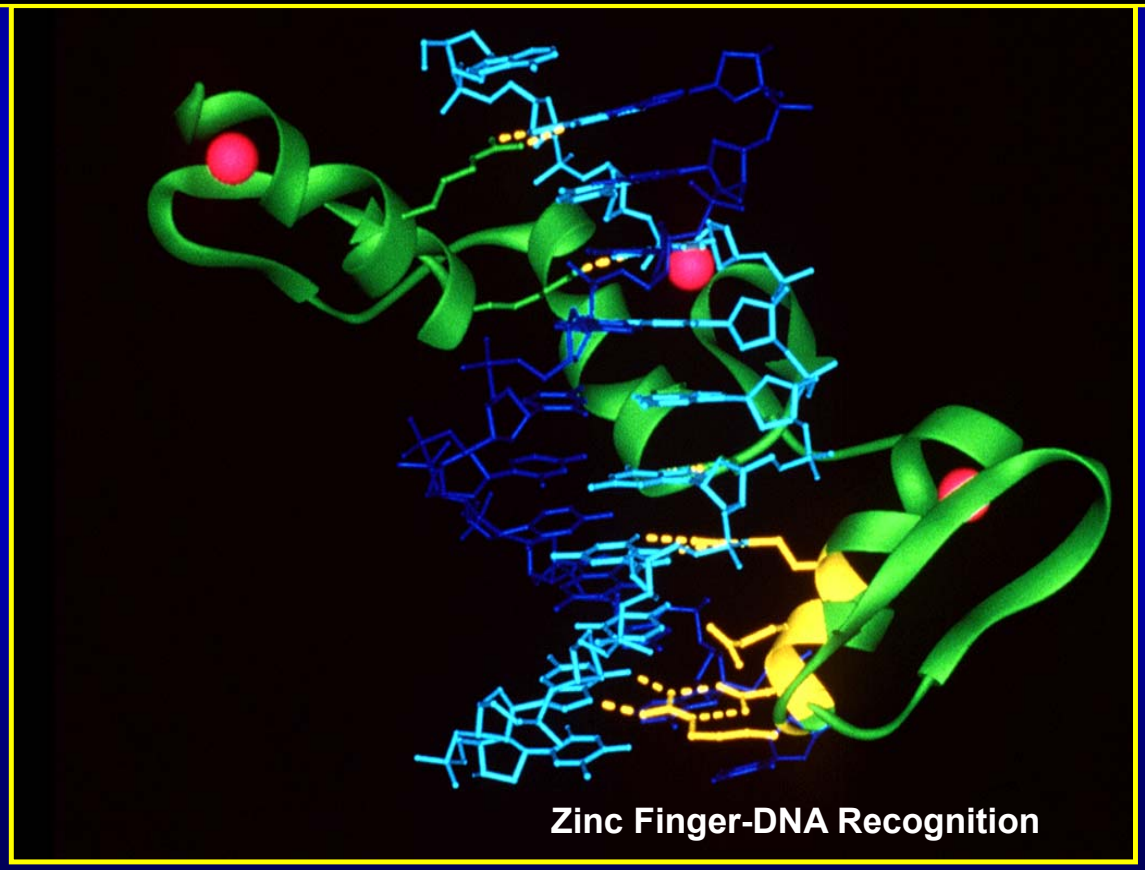
# Why Target CCR5 in HIV?

- 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

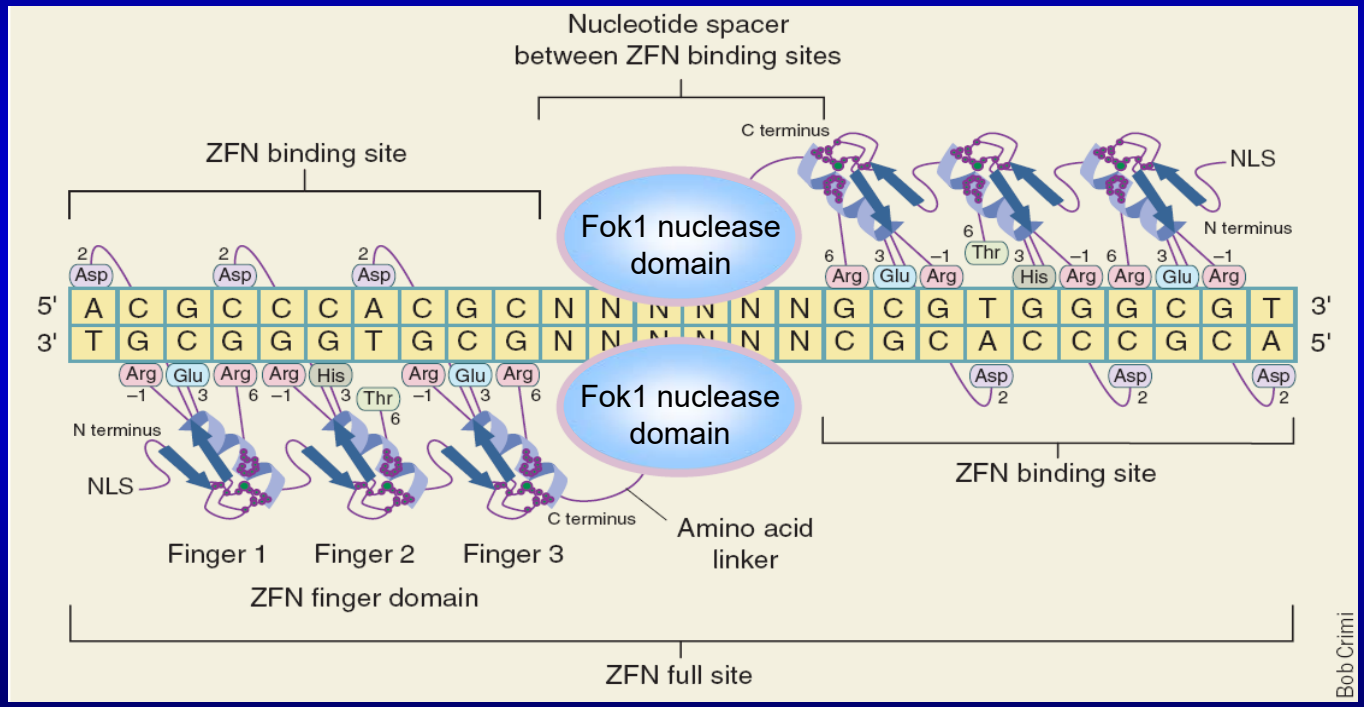
# What are Zinc Finger Proteins?
# -specific DNA binding proteins, e.g transcription factors and other regulatory proteins



**Zinc Finger-DNA Recognition**

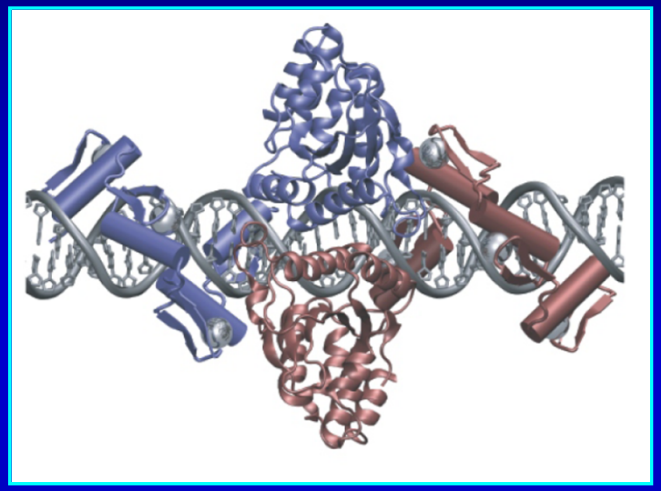
Paveltich and Pabo. *Science* **252:**809 (1991)

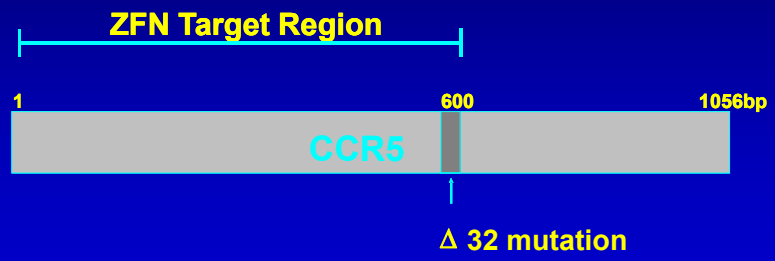
# Combinatorial Strategy with ZFNs To Achieve Genome Specific Targeting



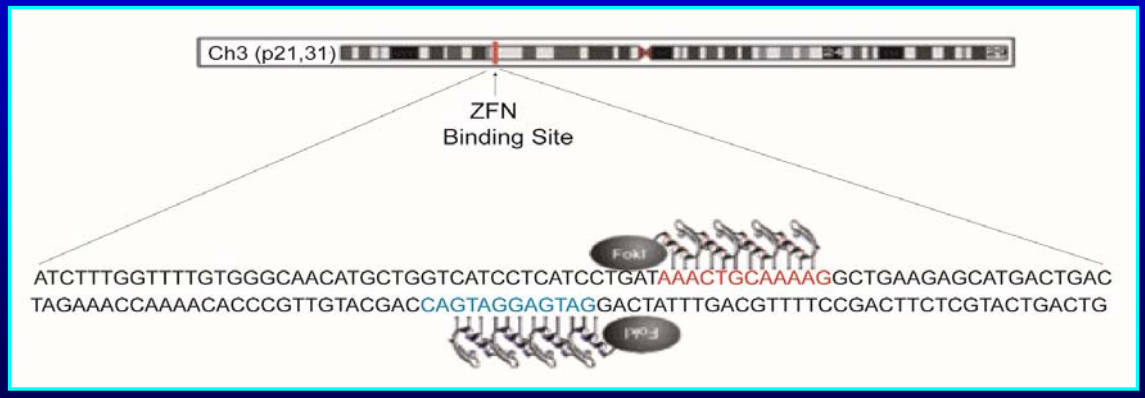

Porteus, Nat Biotech 2005

# Zinc-finger protein-targeted gene regulation: Genomewide single-gene specificity

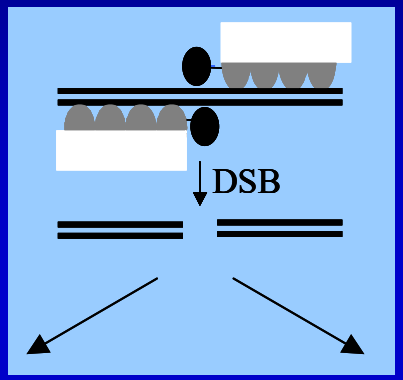




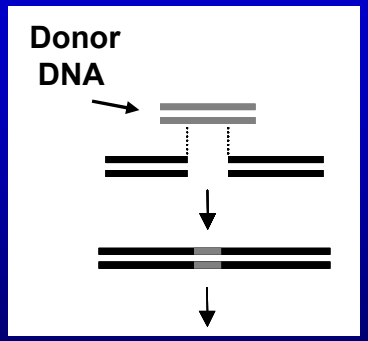

- To disrupt CCR5 and prevent HIV entry, ZFN pairs were targeted to the region upstream of where the Δ32 mutation occurs



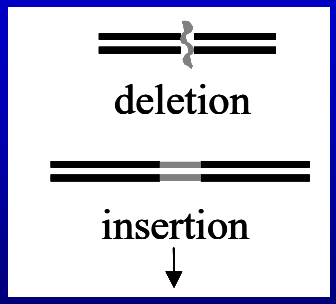
# ZFN-Mediated Targeted Gene Repair or Disruption




ZFN Mediated Cleavage




Gene Repair via
Homologous Recombination




Gene Disruption via
Non-Homologous End Joining

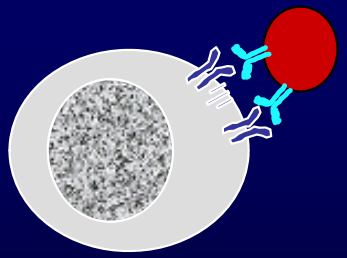
# Activated T Cell Therapy for Infection or Malignancy

## 10 Day Ex Vivo Process

1. Leukapheresis, enrich, deplete, or isolate cells of interest

2. Stimulate cells with aAPC

(insert genes)



Bead or LV K562

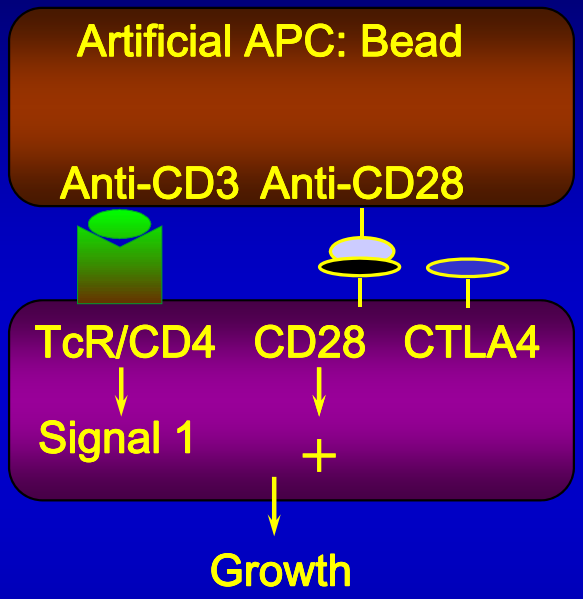
3. Reinfuse cells

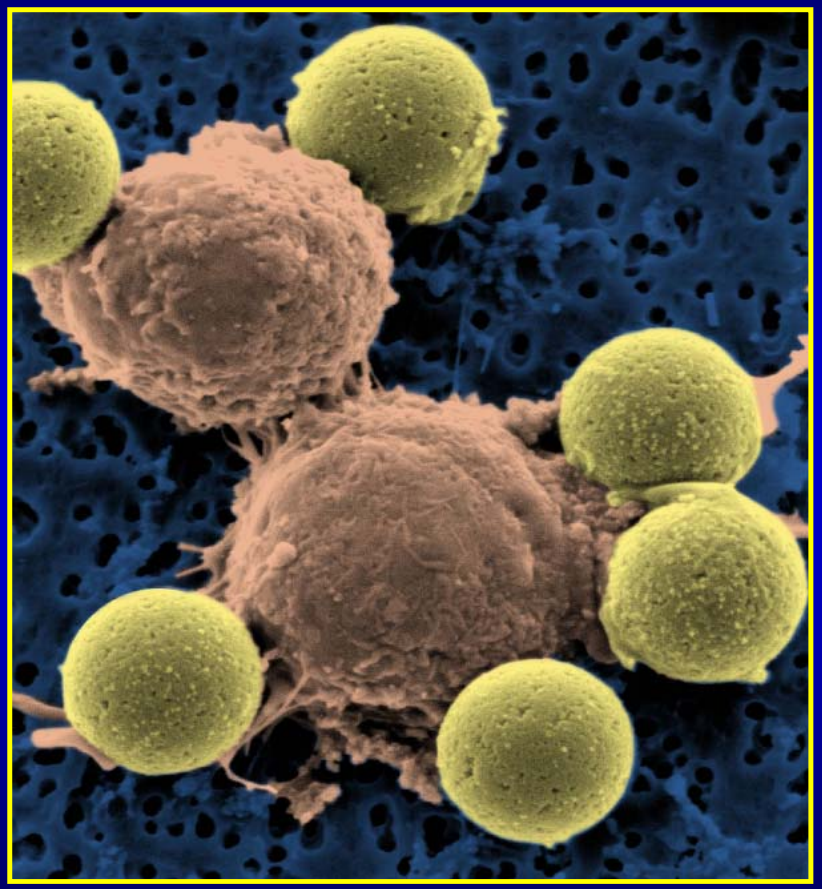
4. Large scale cell expansion

5. Remove beads, wash and concentrate cells

6. Quality Control

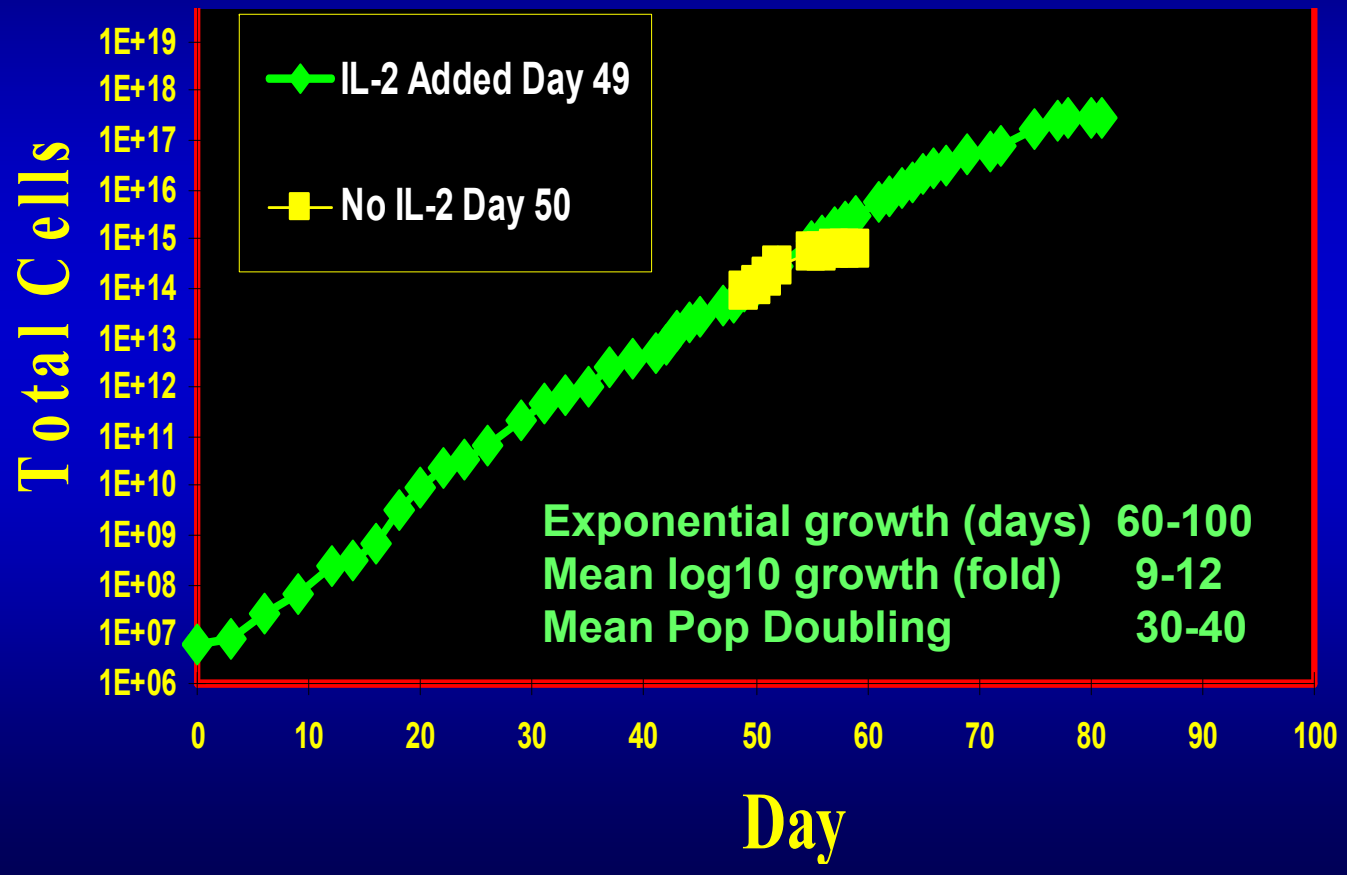
# Bead Based in vitro T Cell Culture System



*J Immunol* 1997; 159: 5921
*Science* 1997; 276: 273
*Immunol. Rev.* 1997; 160: 43
*Mol. Ther.* 2004; 9; 902
Exp. Opin. Biol. Ther. 2008; 8: 475

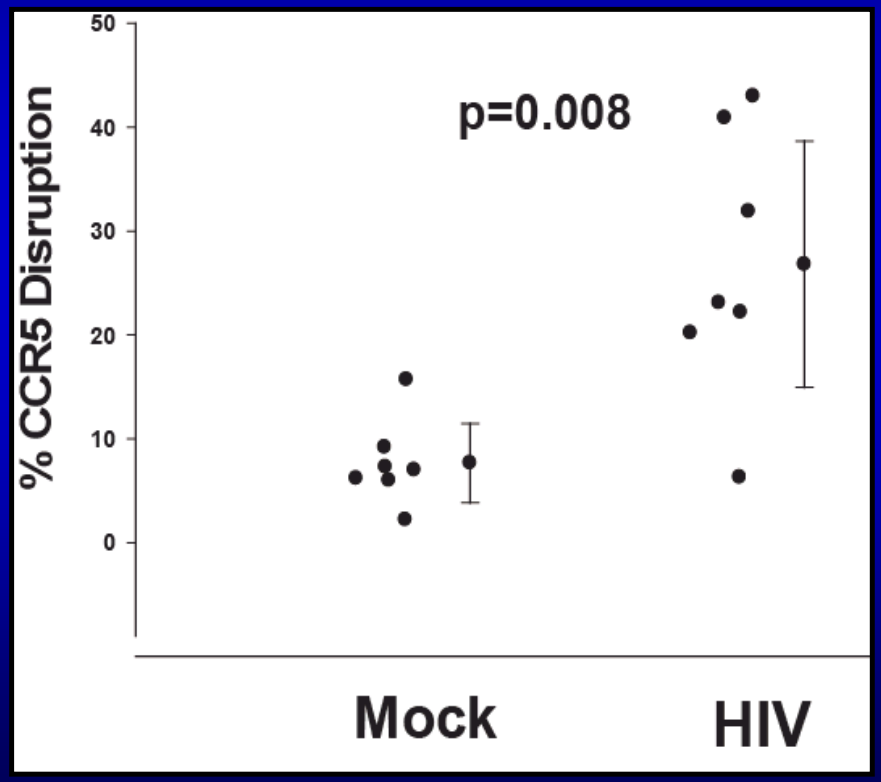
# Polyclonal Replicative Potential of Adult CD4 T Cells In Vitro



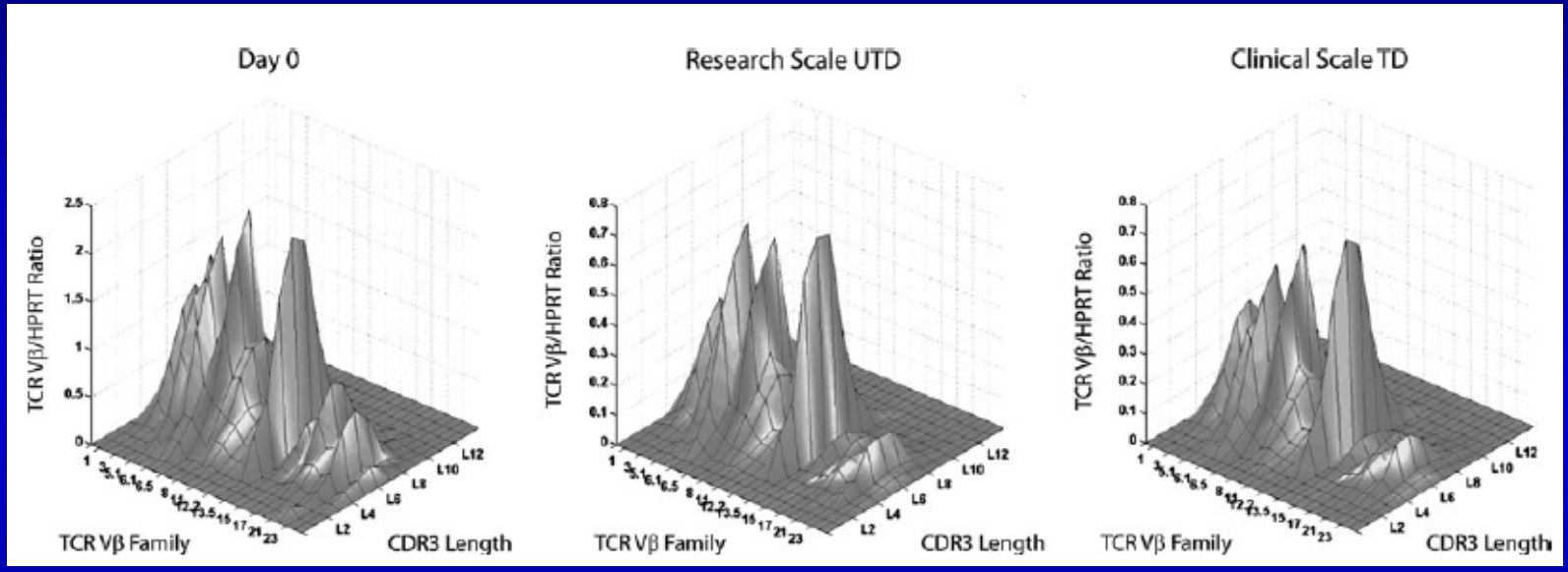

Nc020795.xls

# In Vivo Selection of CCR5-ZFN Modified Cells in NOD/SCID IL-2R$\gamma^{null}$ Mice

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



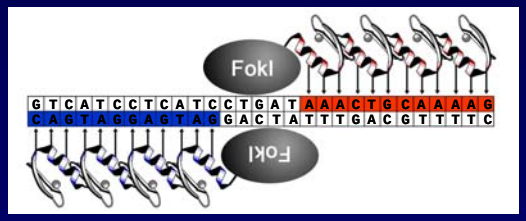
# High Definition T Cell receptor Repertoire Following CCR5 ZFN gene disruption



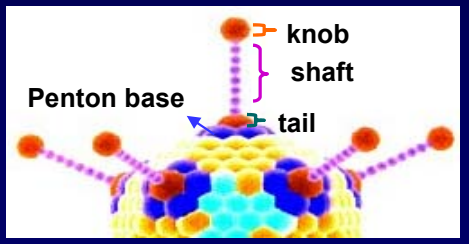

- No change compared to unmodified cultures
- Karyotyping also showed no differences
- Extensive animal studies show no increase in adverse events/deaths

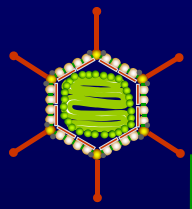
Human Gene Therapy 24:245–258 (March 2013)

# Ex Vivo CCR5 Genetic Modification of CD4+ T-cells Via Zinc Finger Nucleases in HIV+ Pts




Ad5/F35 Chimeric Vector

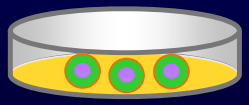


SB-728

Pheresis

Enrich CD4+



Expand, Cryopreserve, Test cell product

Infuse

Follow
Safety, CD4/HIV,
CCR5 selection

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 x $10^{10}$ CCR5- modified cells

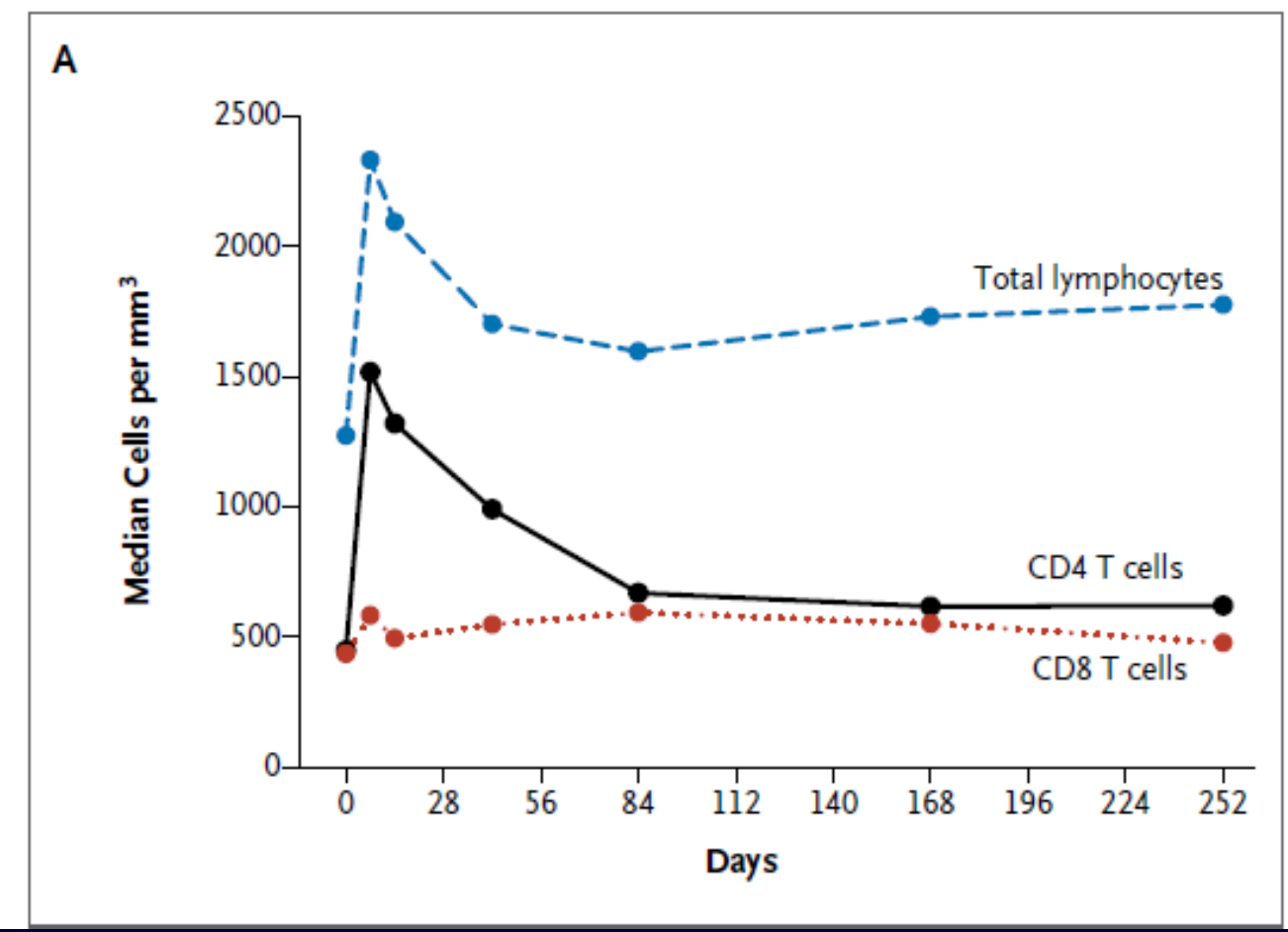
**Quest Clin. Res. / UCLA* (NCT01044654)**

- Open label, single dose study
- Study population – All subjects on HAART

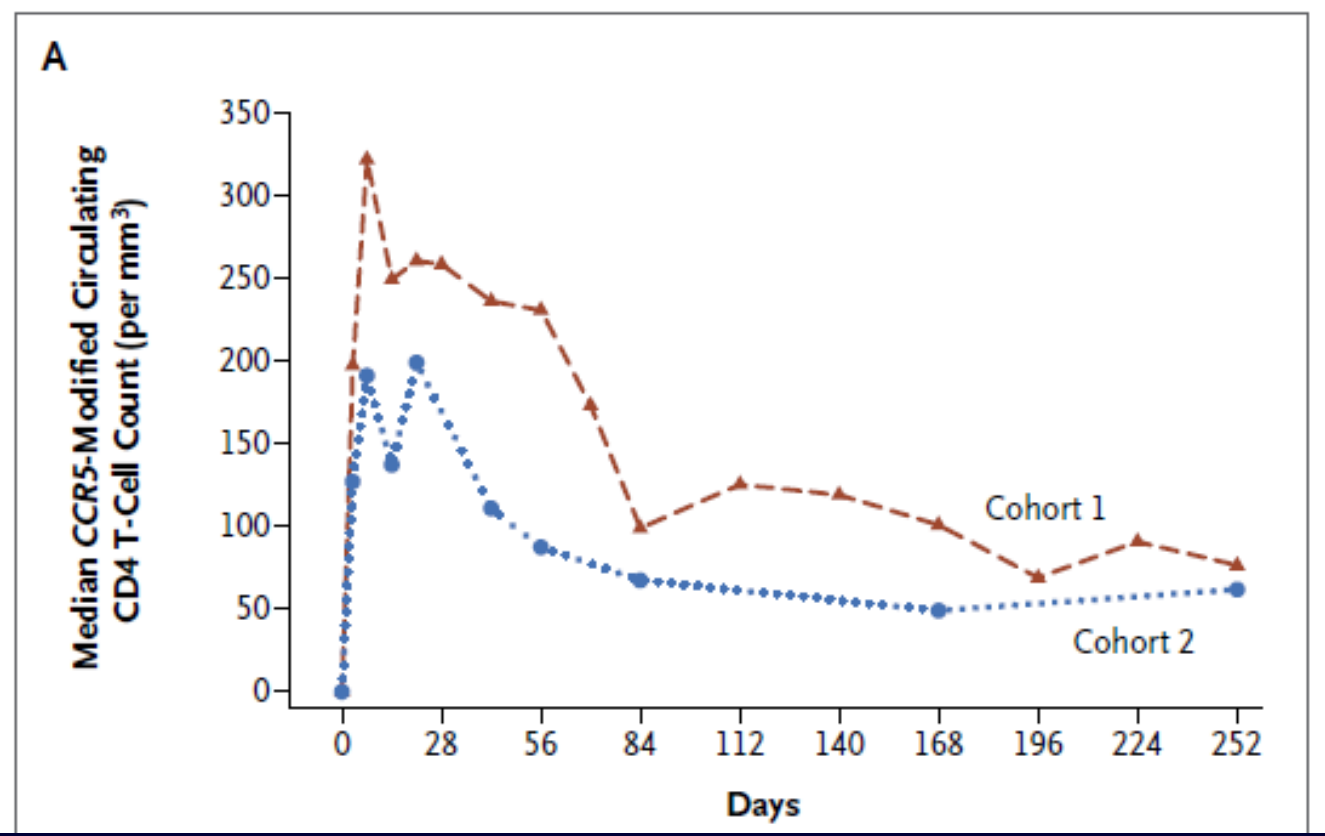
**Endpoints**

**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

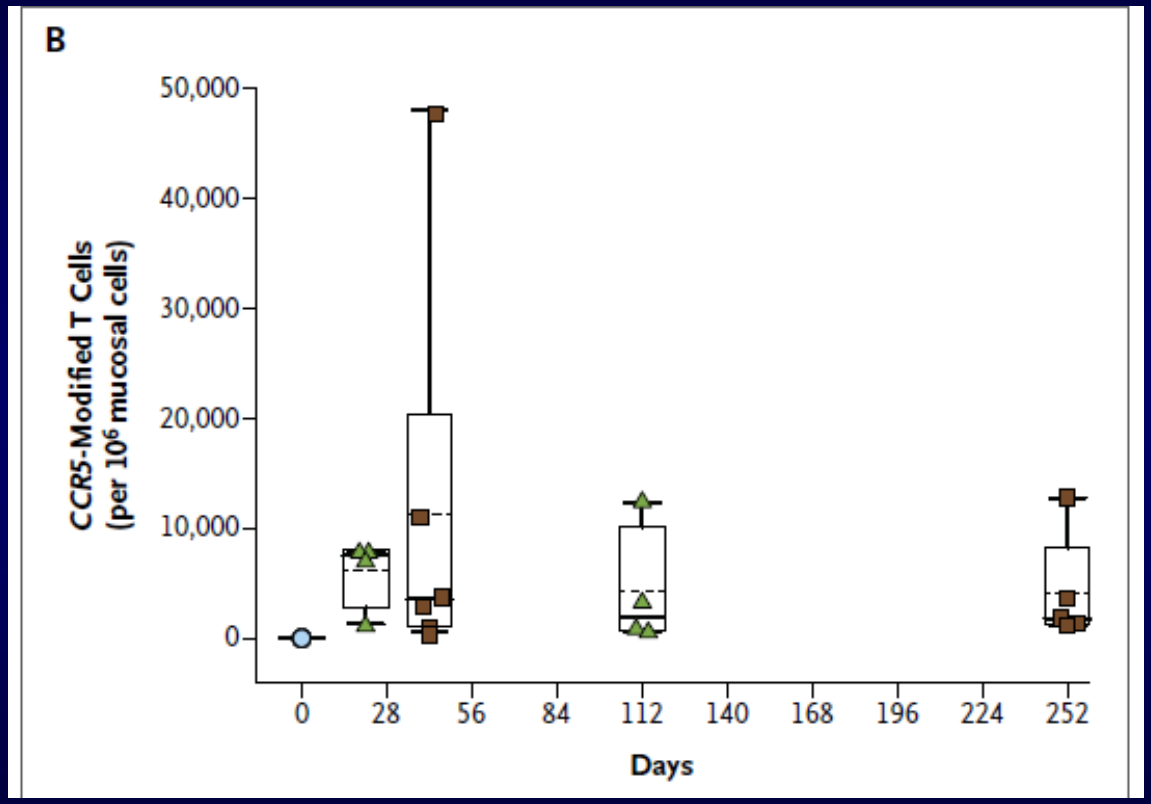


# CCR5-Modified T Cell Persistence in Peripheral Blood



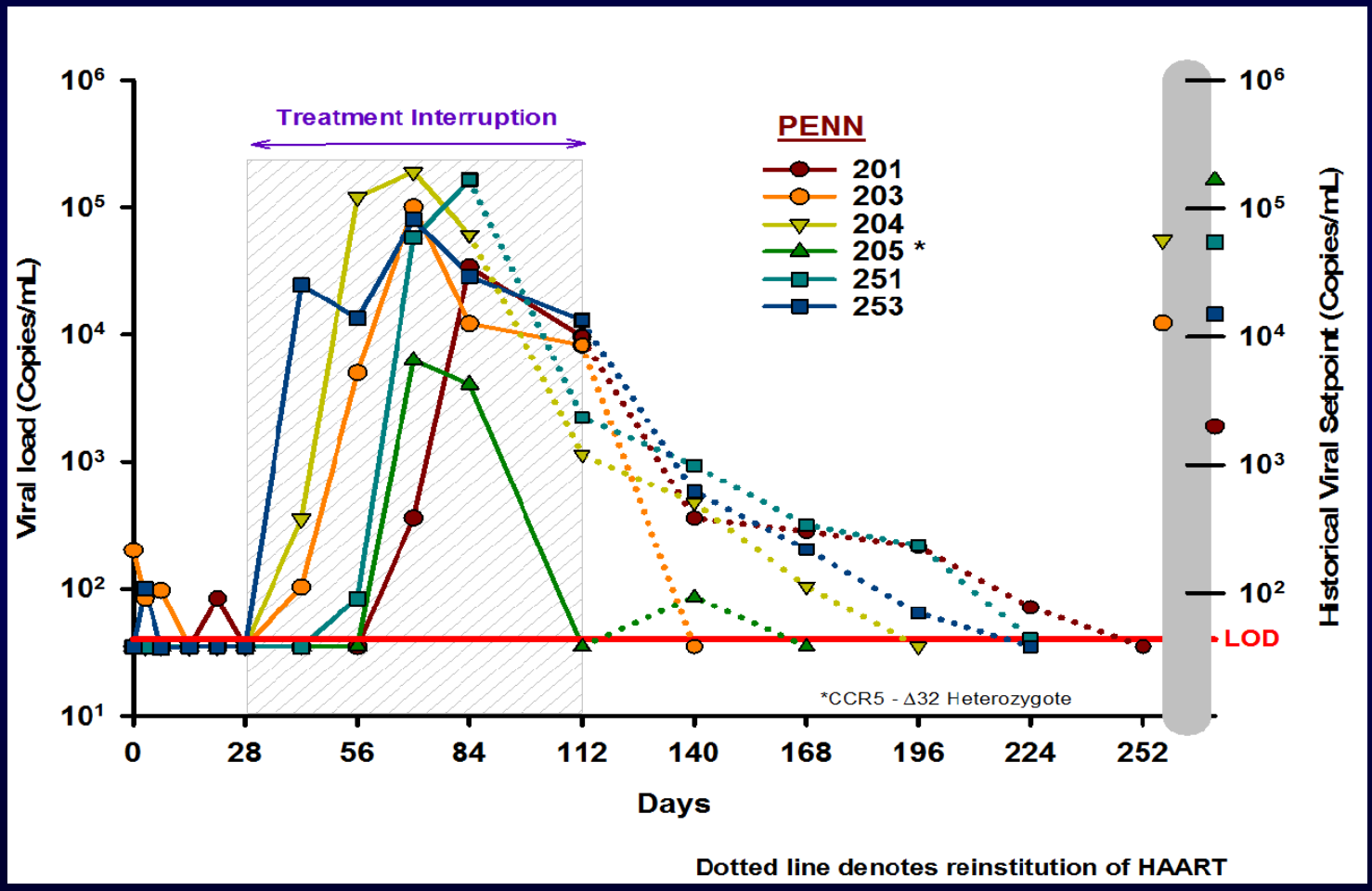

Interim analysis

# CCR5-Modified T Cells Traffic to Rectal Mucosa

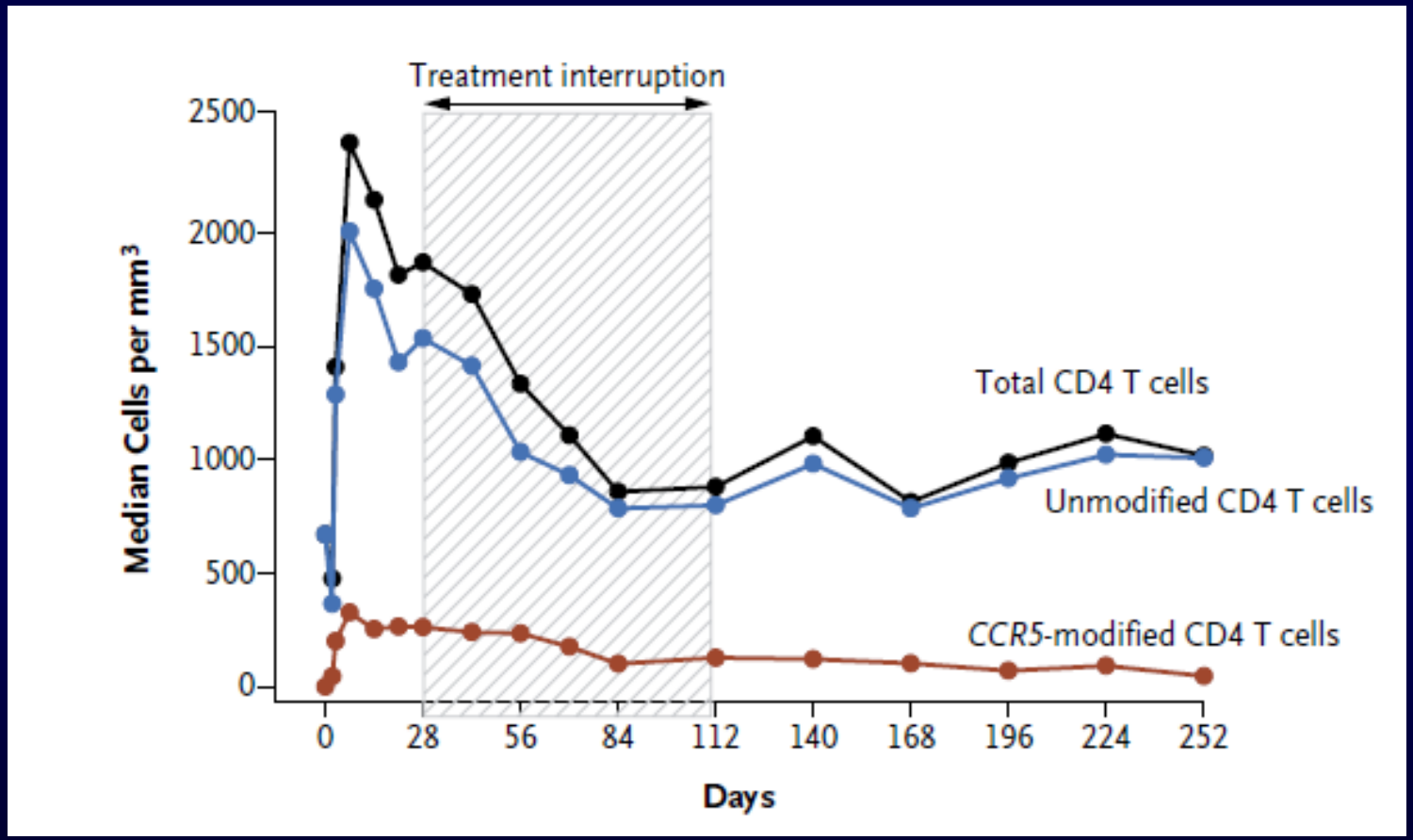



- **R5 gene disruption levels in rectal mucosal CD4s qualitatively tracks with the disruption levels in peripheral blood CD4s**

# Unexpected Finding: HIV-RNA Decreases During STI in Immune Responders



# CD4 Count Decay:
# Unmodified vs Gene-Edited T Cells

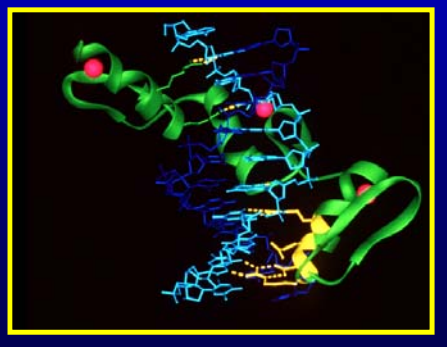


# CCR5 modified CD4 T cell Infusions in HIV

- 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

# CCR5 modified CD4 T cell Infusions in HIV
# Next Steps

- 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



Molecular Therapy
Methods & Clinical Development
Volume 3, 2016, Article 16007

Open Access

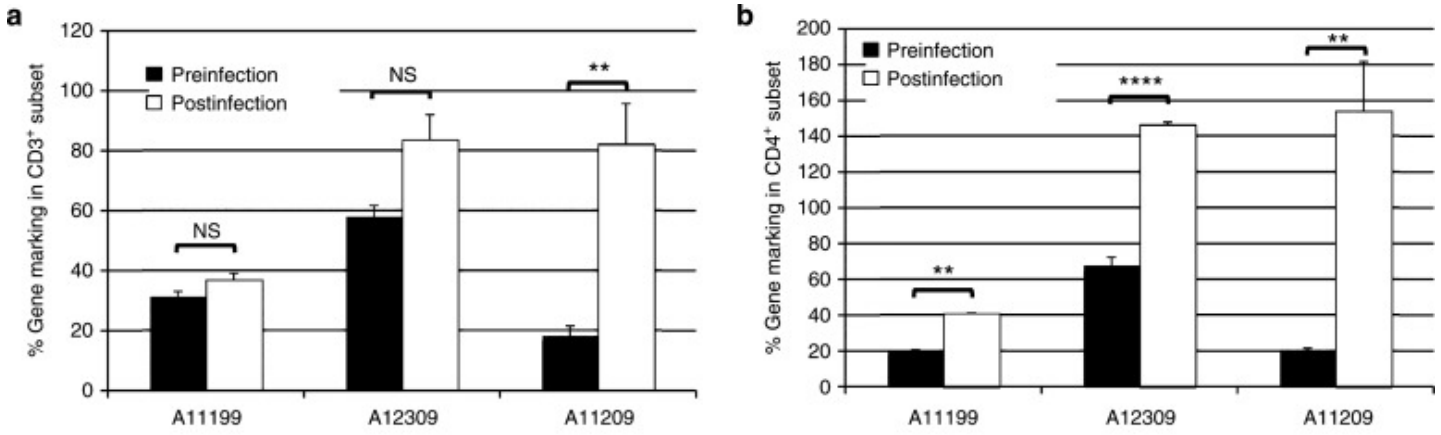
Article

# Multilineage polyclonal engraftment of Cal-1 gene-modified cells and in vivo selection after SHIV infection in a nonhuman primate model of AIDS

Christopher W. Peterson[a], Kevin G. Haworth[a], Bryan P. Burke[b], Patricia Polacino[c], Krystin K. Norman[a], Jennifer E. Adair[a], Shiu-Lok Hu[c, d], Jeffrey S. Bartlett[b], Geoff P. Symonds[b], Hans-Peter Kiem[a, e]

- 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

PLOS | PATHOGENS

Browse | Publish | Abo

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

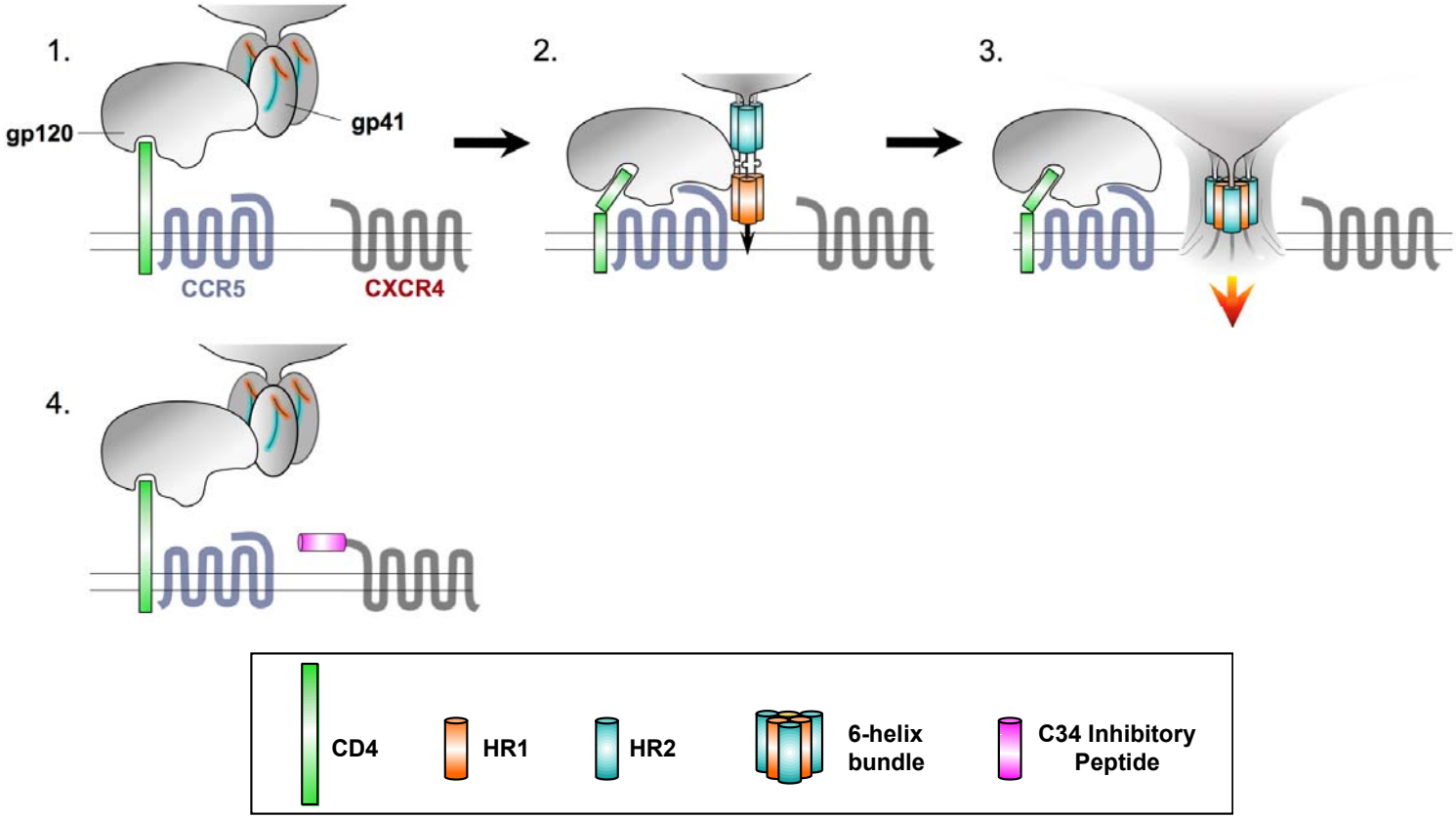
# Potent and Broad Inhibition of HIV-1 by a Peptide from the gp41 Heptad Repeat-2 Domain Conjugated to the CXCR4 Amino Terminus

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 ]

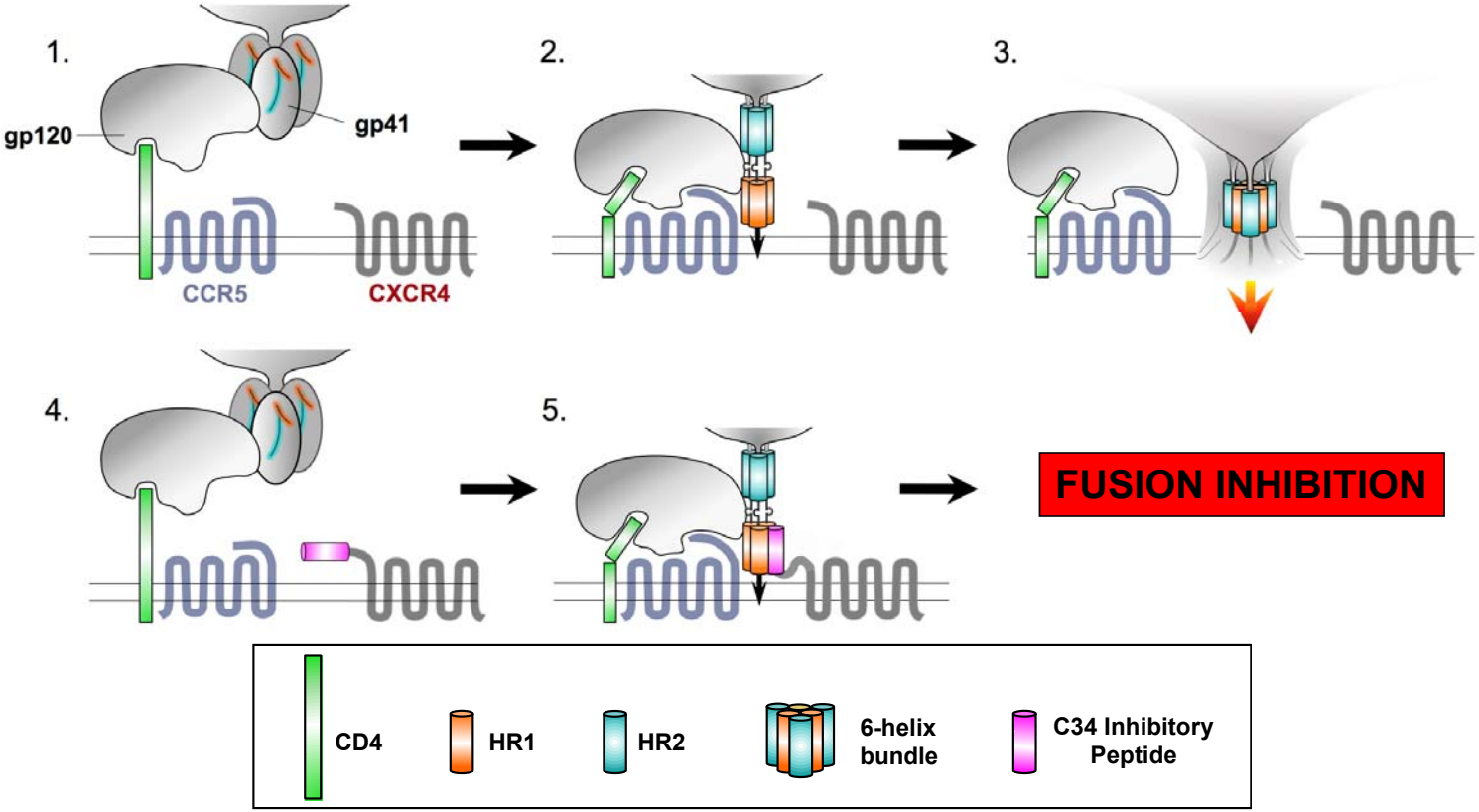
Published: November 17, 2016 • https://doi.org/10.1371/journal.ppat.1005983

- 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)



# 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. Leslie**[1], Beth Haggarty[1], Josephine Romano[1], Andrea P. O. Jordon[1], Jianbin Wang[2], Max W. Richardson[3], James L. Riley[3], Michael C. Holmes[2], Pablo Tebas[1], James Hoxie[1]

# 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?

*Nature Medicine* **15**, 285 - 292 (2009)
Published online: 15 February 2009 | doi:10.1038/nm.1932

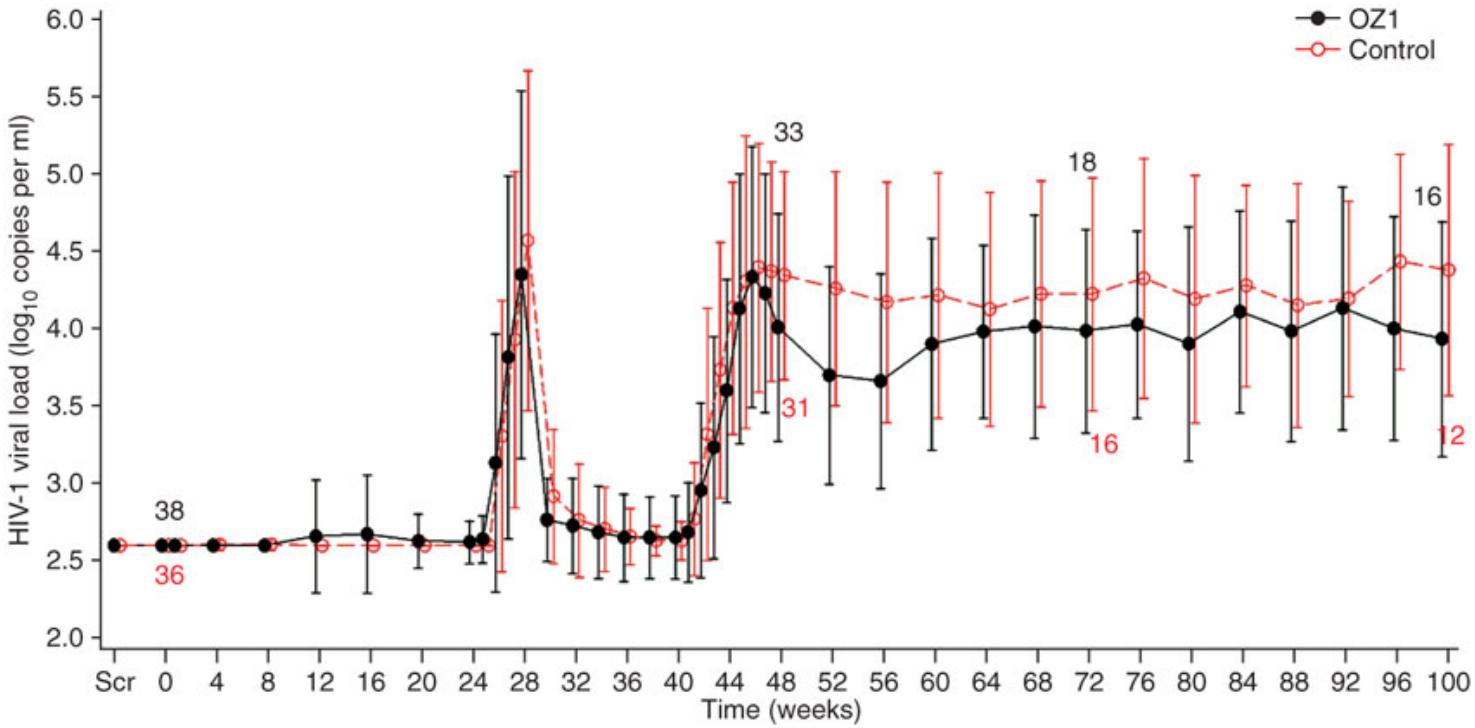
nature medicine

# Phase 2 gene therapy trial of an anti-HIV ribozyme in autologous CD34$^+$ cells

Ronald T Mitsuyasu[1], Thomas C Merigan[2], Andrew Carr[3], Jerome A Zack[4], Mark A Winters[2], Cassy Workman[5], Mark Bloch[6], Jacob Lalezari[7], Stephen Becker[8], Lorna Thornton[8], Bisher Akil[9], Homayoon Khanlou[10], Robert Finlayson[11], Robert McFarlane[12], Don E Smith[13], Roger Garsia[14], David Ma[3], Matthew Law[15], John M Murray[15,16], Christof von Kalle[17,18], Julie A Ely[19], Sharon M Patino[19], Alison E Knop[19], Philip Wong[19], Alison V Todd[19], Margaret Haughton[19], Caroline Fuery[19], Janet L Macpherson[19], Geoff P Symonds[19], Louise A Evans[19], Susan M Pond[19] & David A Cooper[3,15]

- 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



RESEARCH ARTICLE | GENE THERAPY

# RNA-Based Gene Therapy for HIV with Lentiviral Vector–Modified CD34+ Cells in Patients Undergoing Transplantation for AIDS-Related Lymphoma

David L. DiGiusto[1,*], Amrita Krishnan[1,*], Lijing Li[1], Haitang Li[2], Shirley Li[3], Anitha Rao[1], Shu Mi[4], Priscilla Yam[3], Sherri Stinson[5], Michael Kalos[6], Joseph Alvarnas[1], Simon F. Lacey[4], Jiing-Kuan Yee[3], Mingjie Li[7], Larry Couture[3,8], David Hsu[8], Stephen J. Forman[1], John J. Rossi[2,†] and John A. Zaia[3]

[1]*Department of Hematology and Hematopoietic Cell Transplantation, City of Hope, Duarte, CA 91010, USA.*

[2]*Department of Molecular and Cellular Biology, City of Hope, Duarte, CA 91010, USA.*

[3]*Department of Virology, City of Hope, Duarte, CA 91010, USA.*

[4]*Clinical Immunobiology Correlative Studies Laboratory, City of Hope, Duarte, CA 91010, USA.*

[5]*General Clinical Research Center, City of Hope, Duarte, CA 91010, USA.*

[6]*Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA.*

[7]*Department of Neurology, Washington University School of Medicine, St. Louis, MO 63110, USA.*

[8]*Center for Applied Technology Development, City of Hope, Duarte, CA 91010, USA.*

†To whom correspondence should be addressed. E-mail: jrossi@coh.org

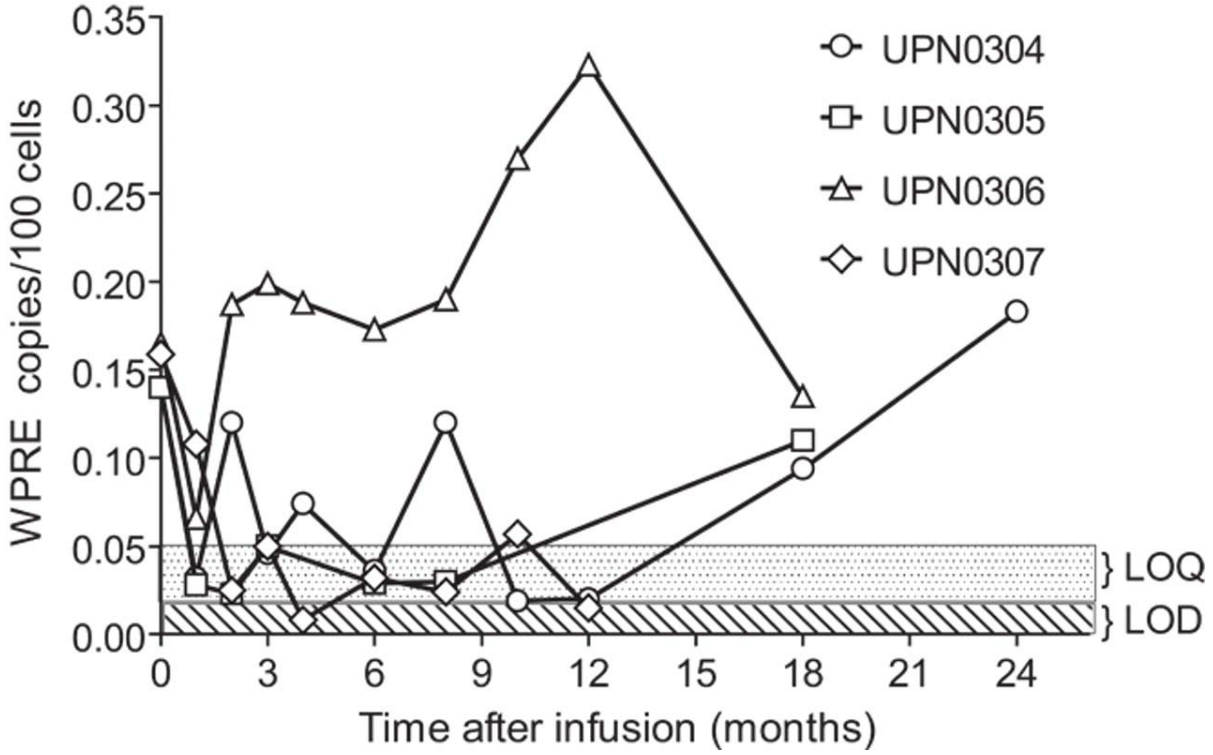
+ See all authors and affiliations

*Science Translational Medicine* 16 Jun 2010:
Vol. 2, Issue 36, pp. 36ra43
DOI: 10.1126/scitranslmed.3000931

Science Translational Medicine

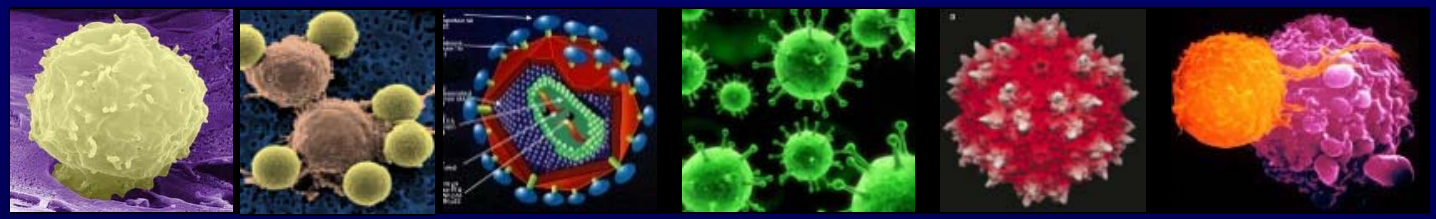
(CD34+) hematopoietic progenitor cells expressing three RNA-based anti-HIV moieties (tat/rev short hairpin RNA, TAR decoy, and CCR5 ribozyme). In vitro analysis of these gene-modified cells showed no differences in their hematopoietic potential compared with nontransduced cells. In vitro estimates of 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

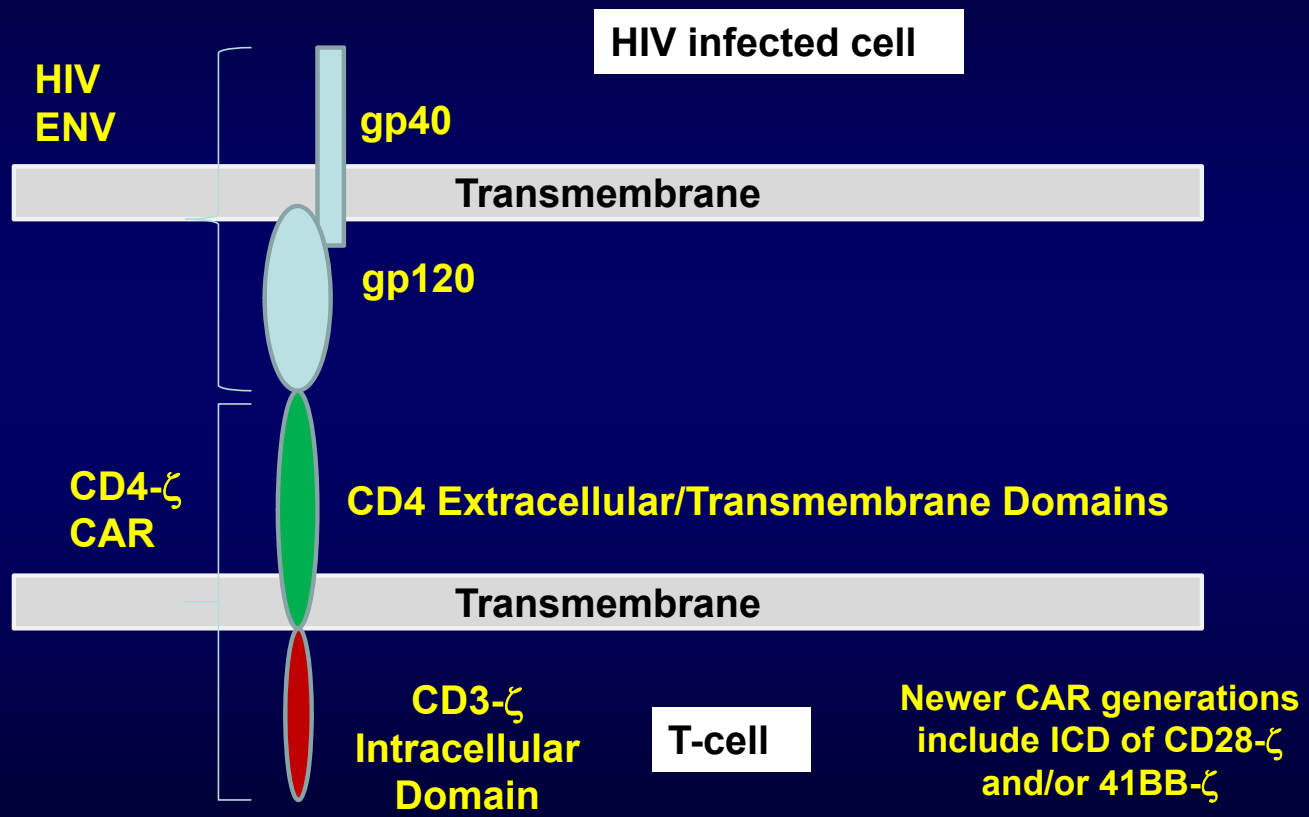



- Ethical requirement to provide standard of care- unmodified HSC infused 1 day after gene marked HSC
- Possibly/likely reduced engraftment of gene-modified HSC?

# HIV CAR T Cells and Gene Modified T Cell Longevity in vivo



# CD4-zeta Chimeric Antigen Receptor

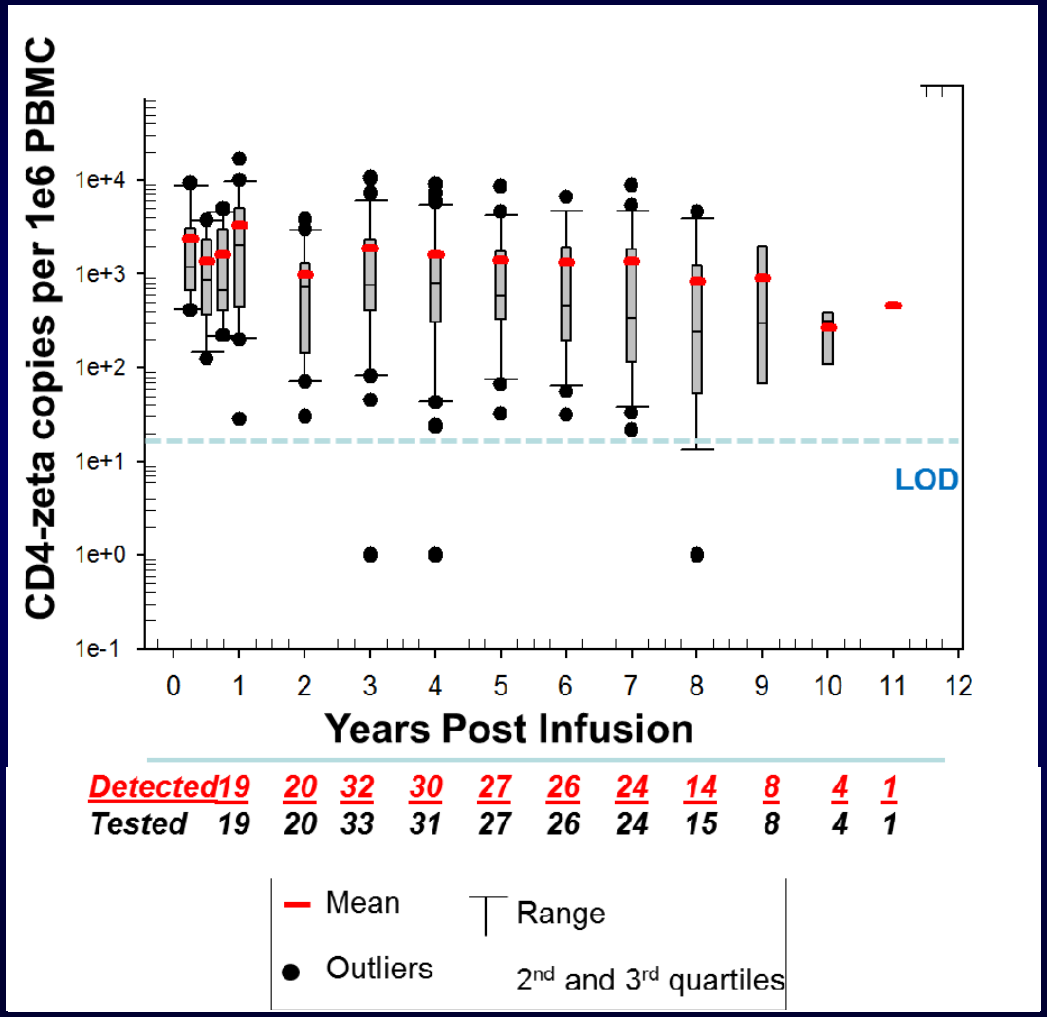


# Longitudinal Analysis of CD4ζ CAR

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**

Scholler et al.
Science Trans. Med.
May 2, 2012

# CD4-ζ RNA Present in Patient

| PBMCs<br>Study/Patient | Annual Follow up | % Copies CD4ζ in PBMCs | RNA Detection |
|---|---|---|---|
| Mitsuyasu / H | 2nd | 0.122 | +++ |
| Mitsuyasu / H | 5th | 0.148 | +++ |
| Mitsuyasu / H | 8th | 0.218 | +++ |
| Mitsuyasu / A | 9th | 0.021 | + |
| Mitsuyasu / D | 9th | 0.008 | + |
| Mitsuyasu / K | 8th | 0.014 | UD |
| Deeks / B | 6th | 0.103 | +++ |
| Deeks / B | 8th | 0.455 | +++ |
| Deeks / B | 9th | 0.301 | +++ |
| Deeks / L | 5th | 0.173 | +++ |
| Deeks / L | 9th | 0.037 | +++ |
| Deeks / L | 10th | 0.034 | +++ |
| Deeks / P | 5th | 0.145 | +++ |
| Deeks / P | 10th | 0.040 | +++ |
| Deeks / C | 3rd | 0.272 | +++ |
| Deeks / C | 9th | 0.226 | +++ |
| Deeks / N | 7th | 0.260 | +++ |
| Deeks / J | 7th | 0.189 | +++ |
| June/Levine / 27 | 8th | 0.046 | +++ |
| June/Levine / 35 | 5th | 0.141 | +++ |
| June/Levine / 36 | 5th | 0.199 | +++ |
| Control / 209 | N/A | UD | UD |
| Control / 207 | N/A | UD | UD |
| Control / 207 | N/A | UD | UD |

Engraftment Limit for RNA detection

**Levels of CD4-ζ RNA Detected**

N/A = not applicable

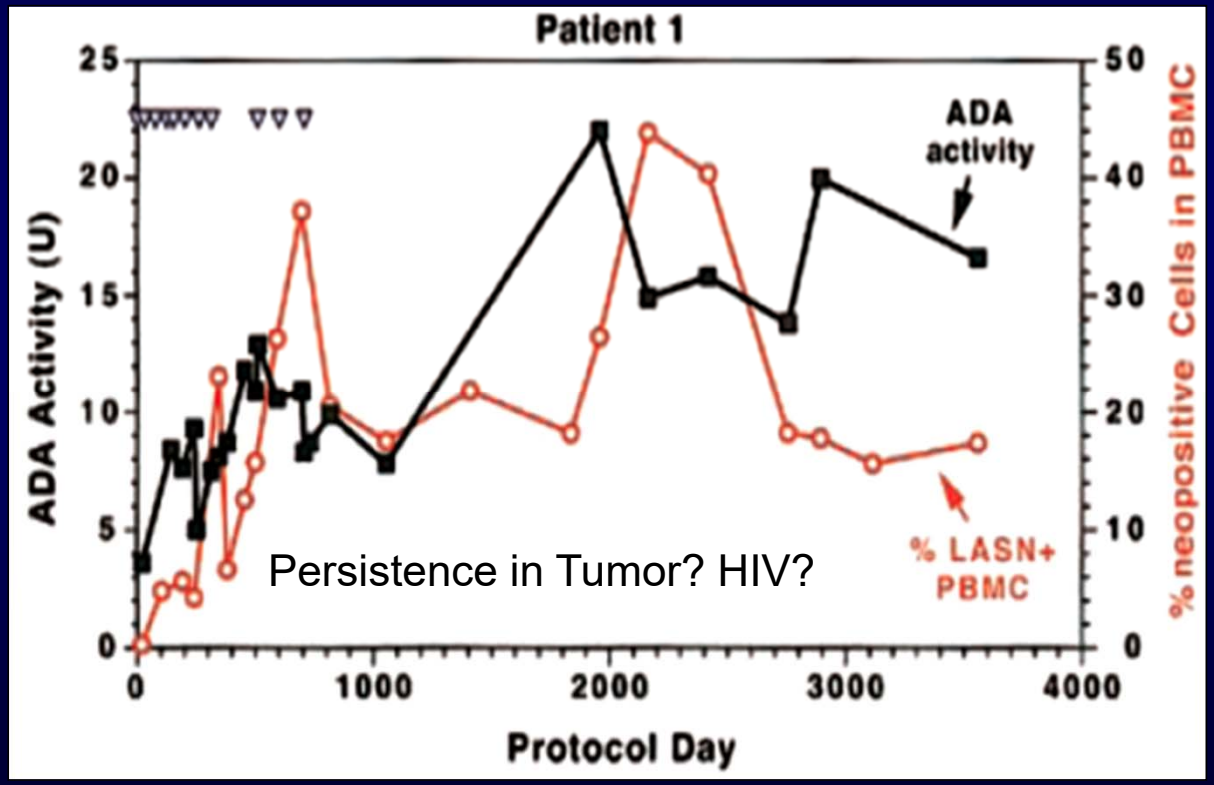
+++ = reliable levels

\+ = at limits of detection

UD = no RNA Detected

# SCID-ADA Transduced T cells

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



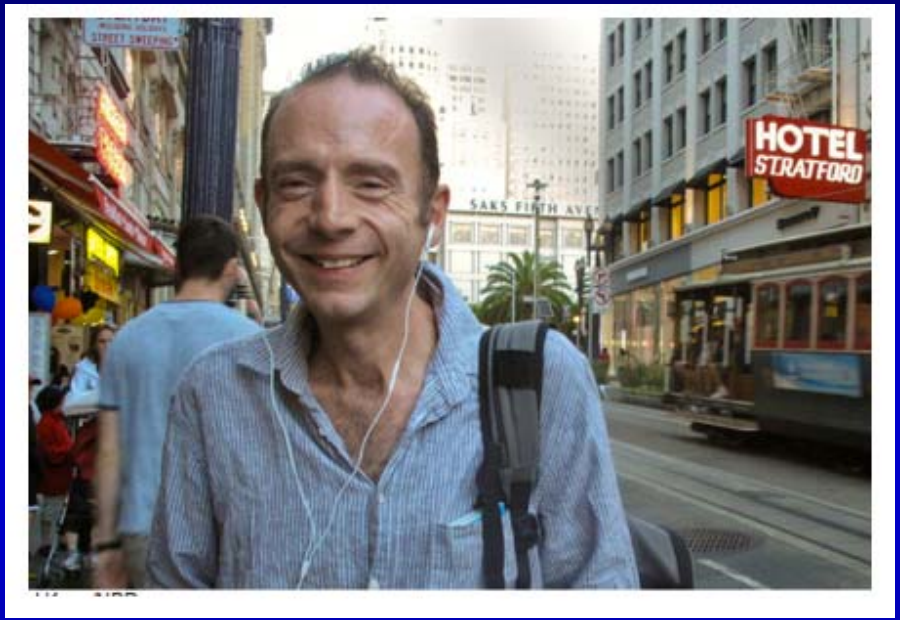

Muul et al. Blood. 2003 Apr 1;101(7):2563-9

# Patient Safety Years of Genetically Modified T cells

## University of Pennsylvania

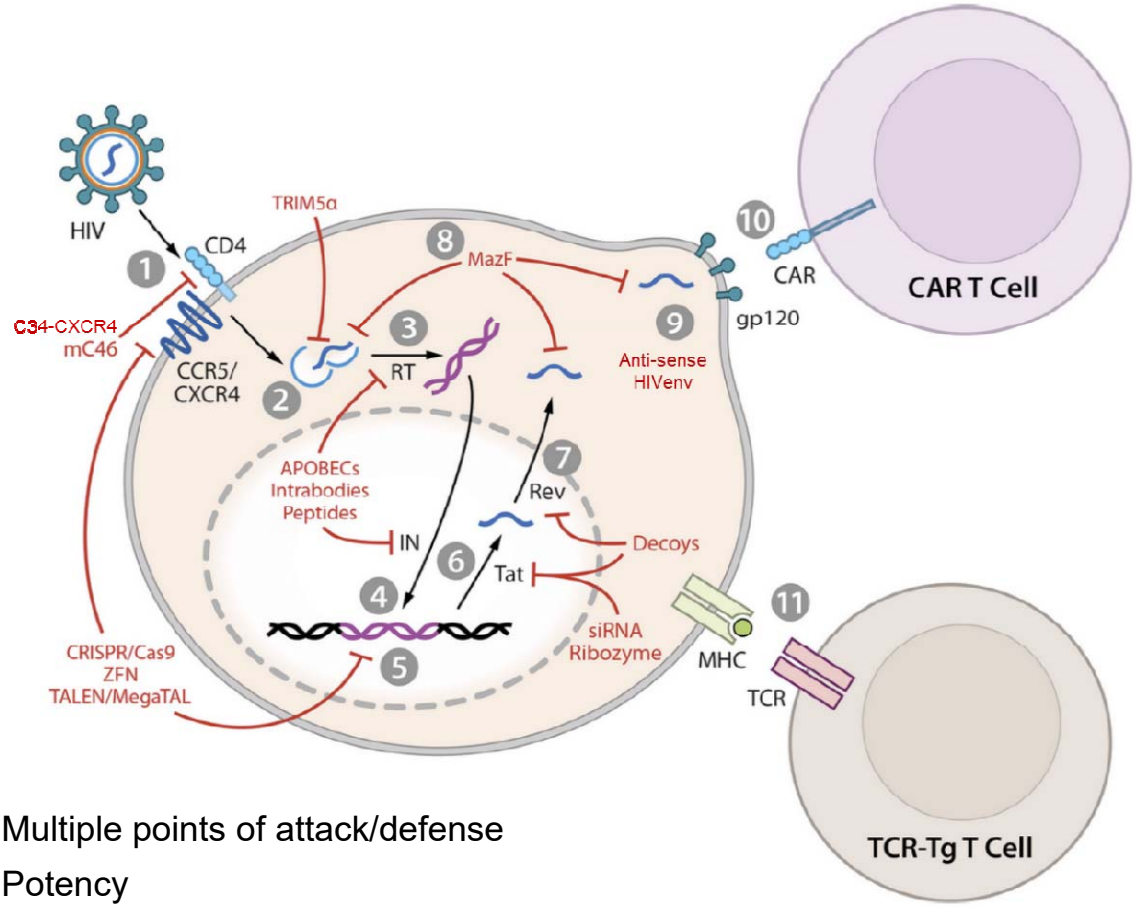
| Trial | Engineered T Cell | # Patients Infused | Safety (Patient-Years) | # Patients Alive (as of last date enrolled in study/LTFU) |
|---|---|---|---|---|
| Sangamo ZFN (HIV) | Ad5/35 zinc finger nuclease | 12 | 74.4 | 12 |
| CD4z CAR (HIV) includes CG trials | Retroviral CAR | 44 | 783.6 | 44 |
| SB-728mR CCR5 ZFN | CCR5 ZFN | 11 | 10.9 | 11 |
| MAZ-Takara (HIV) | Retroviral MazF | 10 | 22.7 | 10 |
| VirxSys VRX496 (HIV) | Lentiviral antisense HIVenv | 20 | 204.1 | 20 |
| Adaptimmune (HIV) | Lentiviral gag TCR | 2 | 10.8 | 2 |
| Adaptimmune Myeloma and Sarcoma | Lentiviral NY-ESO1 TCR | 21 | 100.8 | 21 |
| Penn/Novartis CART19/CTL019 | Lentiviral 19:BBz CAR | 311 | 467.3 | 214 |
| EGFR | Lentiviral CART-EGFR | 10 | 8.5 | 3 |
| UPCC19214 CART-MESO-19 | CART-MESO-19 | 3 | 3.2 | 2 |
| UPCC31213 | CART-MESO | 15 | 10.2 | 2 |
| UPCC31415 | CART22 | 3 | 1.5 | 1 |
| UPCC14415 | CART-BCMA | 14 | 10.9 | 10 |
| **Total** | | **476** | **1709** | **352** |

# 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