RAC Protocol #1110-1130 CAL-USA-11

"An Adaptive Phase I/II Study of the Safety of CD4+ T lymphocytes and CD34+ Hematopoietic Stem/Progenitor Cells Transduced with CAL-1, a Dual Anti-HIV Gene Transfer Construct, in Busulfan Conditioned HIV-Infected Adults Previously Exposed to ART"

Ron Mitsuyasu, University of California, LA;
Principal Investigator
Jay Lalezari, Quest Clinical Research;
Investigator
Geoff Symonds, Calimmune;
Chief Scientific Officer

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Background

HIV impairs immune function

- Primarily decreases CD4+/CCR5+ cells
- Can be controlled through HAART
  - Requires daily compliance and life-long adherence
  - Has short and long term toxicities (GI, liver, bone and metabolic)
  - Issues with resistance (15% of new infections are resistant HIV strains)
  - Expensive (lifetime pt. costs in US approaching $750K) -$20B/yr. worldwide
- No current cure

Global HIV Statistics

Population	U.S.	Rest of World
Living with HIV/AIDS	1,200,000	33,000,000
Diagnosed	840,000	10,000,000
Patients on therapy	672,000	4,500,000
* Patients well controlled & adherent on therapy	403,000	1,800,000
Newly infected/year	56,000	2,600,000

Cocohoba J, et al. 14th CROI 2007; Abstract 784
UNAIDS Report on the Global AIDS Epidemic 2010

HIV-1 entry into CD4$^+$ cells is mediated by the chemokine receptor CC-CKR-5

Tatjana Dragic*, Virginia Litwin†, Graham P. Allaway†, Scott R. Martin*, Yaoxing Huang*, Kirsten A. Nagashima†, Charmagne Cayanan*, Paul J. Maddon†, Richard A. Koup*, John P. Moore* & William A. Paxton*

NATURE · VOL 381 · 20 JUNE 1996

Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene

Michel Samson*, Frédérick Libert*, Benjamin J. Doranz†, Joseph Rucker†, Corinne Liesnard‡, Claire-Michèle Farber§, Sentob Saragosti||, Claudine Lapouméroulie¶, Jacqueline Cognaux#, Christine Forceille#, Gaetan Muyldermans#, Chris Verhofstede#, Guy Burtonboy#, Michel Georges☆, Tsuneo Imai**, Shalini Rana††, Yanji Yi††, Robert J. Smyth††, Ronald G. Collman††, Robert W. Doms†, Gilbert Vassart*‡‡ & Marc Parmentier*

NATURE · VOL 382 · 22 AUGUST 1996

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Related Proof of Concept in Man

- Treated HIV/Leukemia Patient in Berlin with CCR5- donor cells
- Patient has no detectable HIV or leukemia 3 years after treatment
- American Foundation for AIDS Research has declared patient as "functionally cured"

Kristina Allers,[1] Gero Hütter,[2] Jörg Hofmann,[1] Christoph Loddenkemper,[4] Kathrin Rieger,[1] Eckhard Thiel,[1] and Thomas Schneider[1]

[1]Department of Gastroenterology, Infectious Diseases, and Rheumatology, Medical Clinic I, Campus Benjamin Franklin, Charité-University Medicine Berlin, Berlin, Germany; [2]Department of Hematology, Oncology, and Transfusion Medicine, Medical Clinic III, Campus Benjamin Franklin, Charité-University Medicine Berlin, Berlin, Germany; [3]Institute of Medical Virology, Helmut Ruska-Haus, Campus Mitte, Charité-University Medicine Berlin, Berlin, Germany; and [4]Institute of Pathology/Research Center Immunodeficiencies (RCIS), Campus Benjamin Franklin, Charité-University Medicine Berlin, Berlin, Germany

HIV entry into CD4+ cells requires interaction with a cellular receptor, generally either CCR5 or CXCR4. We have previously reported the case of an HIV-infected patient in whom viral replication remained absent despite discontinuation of antiretroviral therapy after transplantation with CCR5Δ32/Δ32 stem cells. However, it was expected that the long-lived viral reservoir would lead to HIV rebound and disease progression during the process of immune reconstitution. In the present study, we demonstrate successful reconstitution of CD4+ T cells at the systemic level as well as in the gut mucosal immune system after CCR5Δ32/Δ32 stem cell transplantation, while the patient remains without any sign of HIV infection. This was observed although recovered CD4+ T cells contain a high proportion of activated memory CD4+ T cells, a, the preferential targets of HIV, and are susceptible to productive infection with CCR5-tropic HIV. Furthermore, during the process of immune reconstitution, we found evidence for the replacement of long-lived host tissue cells with donor-derived cells, indicating that the size of the viral reservoir has been reduced over time. In conclusion, our results strongly suggest that cure of HIV has been achieved in this patient. (Blood 2011;117(10):2791–2799)

Introduction

Destruction of the immune system by the HIV is driven by the loss of CD4+ T cells in the peripheral blood and lymphoid tissues. Viral entry into CD4+ cells is mediated by the interaction with a cellular chemokine receptor, the most common of which are CCR5 and CXCR4.[1] Because subsequent viral replication requires cellular gene expression processes, activated CD4+ cells are the primary targets of productive HIV infection. Consequently, HIV infection leads predominantly to the depletion of activated memory CD4+ cells, emphasizes the importance for continuing research in the field of CCR5-targeted treatment strategies, but uncertainty has remained over whether a cure for HIV infection has been achieved in this patient.

In the setting of HIV infection, the effects of pretransplantation conditioning do not allow the complete elimination of HIV, as demonstrated by previous studies in which researchers detected HIV-infected patients who underwent stem cell transplantation.

Delta32 Stem-Cell Transplantation

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

SUMMARY

Infection with the human immunodeficiency virus type 1 (HIV-1) requires the presence of a CD4 receptor and a chemokine receptor, principally chemokine receptor 5 (CCR5). Homozygosity for a 32-bp deletion in the CCR5 allele provides resistance against HIV-1 acquisition. We transplanted stem cells from a donor who was homozygous for CCR5 delta32 in a patient with acute myeloid leukemia and HIV-1 infection. The patient remained without viral rebound 20 months after transplantation and discontinuation of antiretroviral therapy. This outcome demonstrates the critical role CCR5 plays in maintaining HIV-1 infection.

HIV-1 ENTERS HOST CELLS BY BINDING TO A CD4 RECEPTOR AND THEN interacting with either CCR5 or the CXC chemokine receptor (CXCR4). Homozygosity for a 32-bp deletion (delta32/delta32) in the CCR5 allele results in an inactive CCR5 gene product and consequently confers high resistance against HIV-1 acquisition.[1]

A Bone Marrow Transplant to Treat a Leukemia Patient Also Gives Him Virus-Resistant Cells; Many Thanks, Sample 64

By MARK SCHOOFS

(See Corrections & Amplifications item below.)

The startling case of an AIDS patient who underwent a bone marrow transplant to treat leukemia is stirring new hope that gene therapy strategies on the far edges of AIDS research might someday cure the disease.

The patient, a 42-year-old American living in Berlin, is still recovering from his leukemia therapy, but he appears to have won his battle with AIDS. Doctors have not been able to detect the virus in his blood for more than 600 days, despite his having ceased all conventional AIDS medication. Normally when a patient stops taking AIDS drugs, the virus stampedes through the body within weeks, or days.

"I was very surprised," said the doctor, Gero Hütter.

The breakthrough appears to be that Dr. Hütter, a soft-spoken hematologist who isn't an AIDS specialist, deliberately replaced the patient's bone marrow cells with those from a donor who has a naturally occurring genetic mutation that renders his cells immune to almost all strains of HIV, the virus that causes AIDS.

The development suggests a potential new therapeutic avenue and curses as the search for a cure has adopted new urgency. Many fear that current AIDS drugs aren't sustainable. Known as

Proven efficacy of CCR5 knockdown in stem cell transplant patient (Blood, 2011)

First proof of long term control of HIV in patient (NEJM, 2008)

Early evidence of functionally cured HIV patient (WSJ, 2008)

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Therapeutic Approach

Natural genetic mutation provides HIV protection

- ~1% Caucasians lack the CCR5 receptor (homozygous) and have complete protection
- ~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

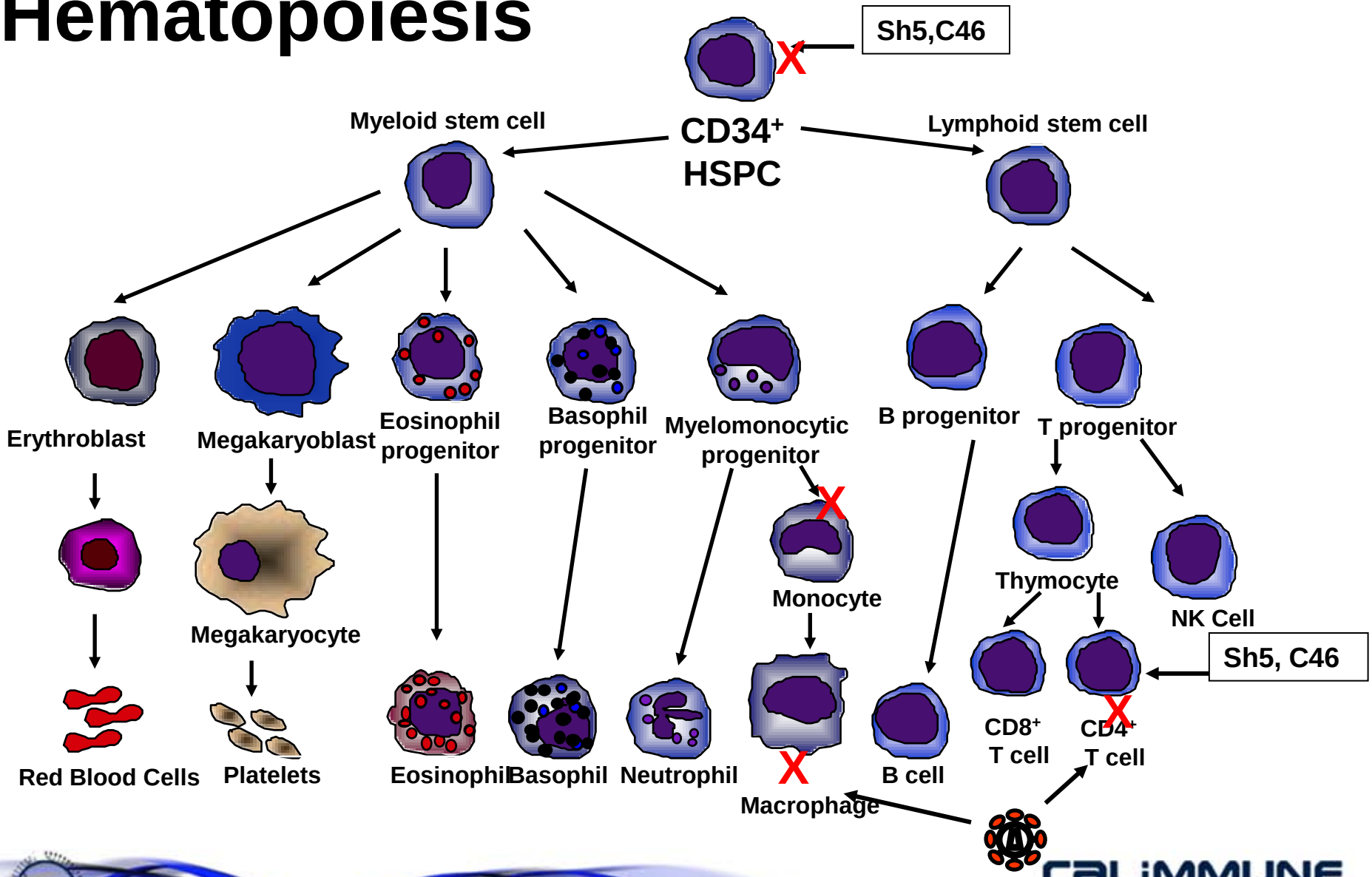
- Our therapy has shown 5-10 fold reduction of CCR5 and can confer the same type of rare advantage on any HIV+ individual
- 100% down-regulation not required for clinical significance
- Modified HSPC can engraft and preferentially expand over time
- Can also introduce to CD4 T cells
- One-time or infrequent delivery requirement
- Can solve issues of adherence
- The only FDA approved CCR5 inhibitor (Maraviroc) has known resistance

Engineering Protection

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

Intracellular immunization (David Baltimore): Progressive population of immune system with cells protected against HIV

Hematopoiesis



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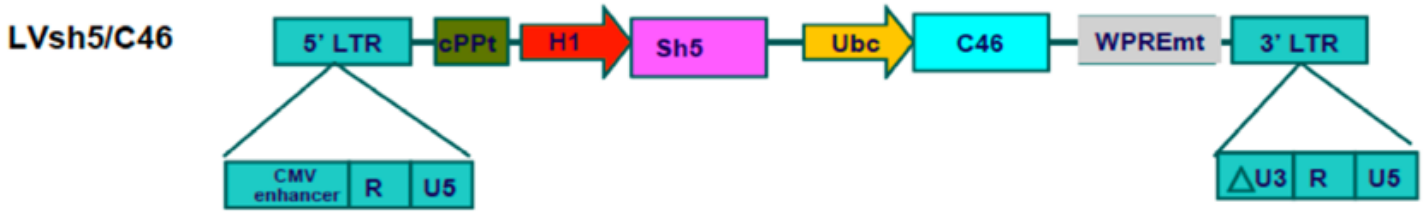
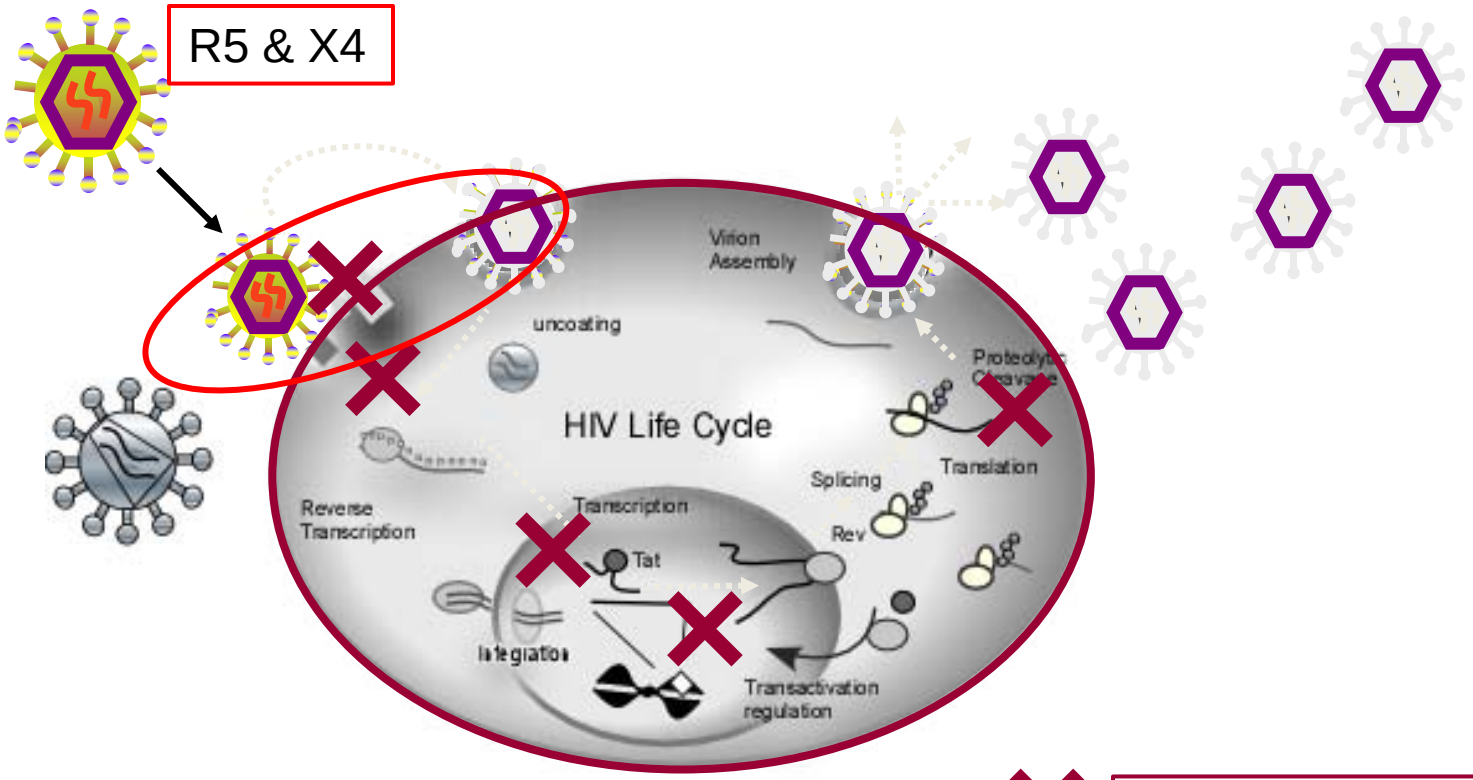
Self-Inactivating Lentiviral Vector (CAL-1)

Cal 1 Construct



LVsh5/C46 has a backbone that includes self-inactivating 5' and 3' long terminal repeats (LTRs); a central polypurine tract (cPPT); and a mutant Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPREmt). Inserts are Ubiquitin C promoter (Ubc); H1 promoter (H1); shRNA to CCR5 (sh5); gene for C46 (C46).

HIV Life Cycle



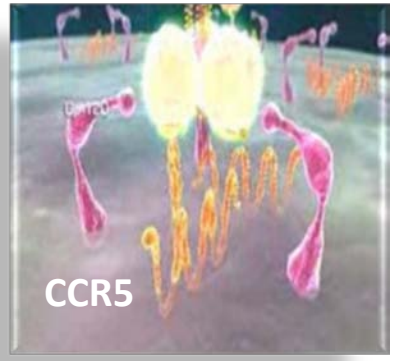
Potential points of inhibition

Rationale for Dual-Therapy

- Two active principles – one down-regulating the important co-receptor CCR5 and the other a potent fusion inhibitor
- Active against both R5 and X4 strains of HIV
- Two points of inhibition for R5
- Mitigates against resistance of HIV

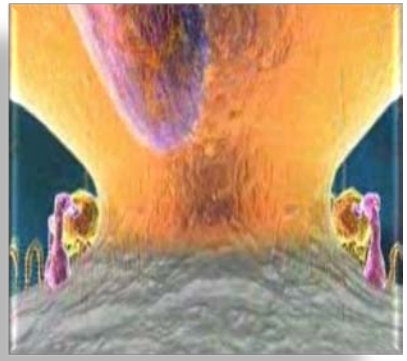
HIV Binding and Fusion

Contact

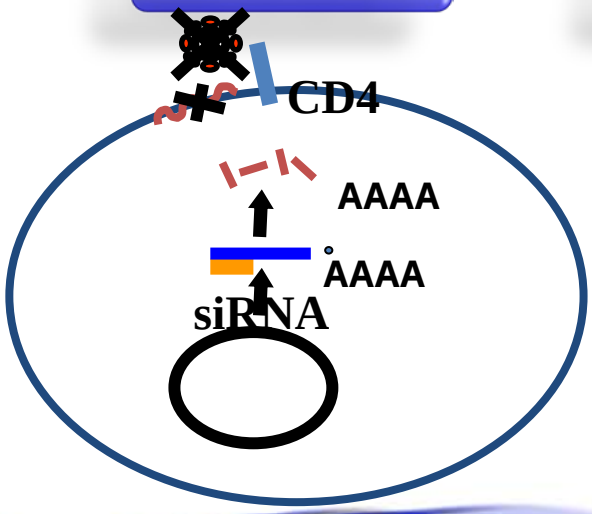


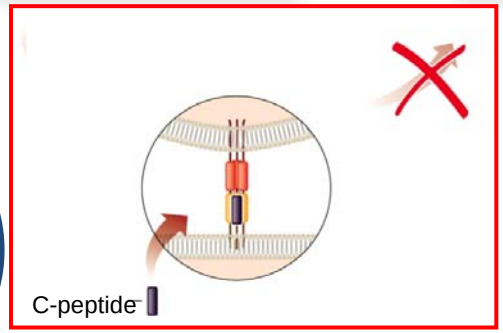
Attachment

Fusion

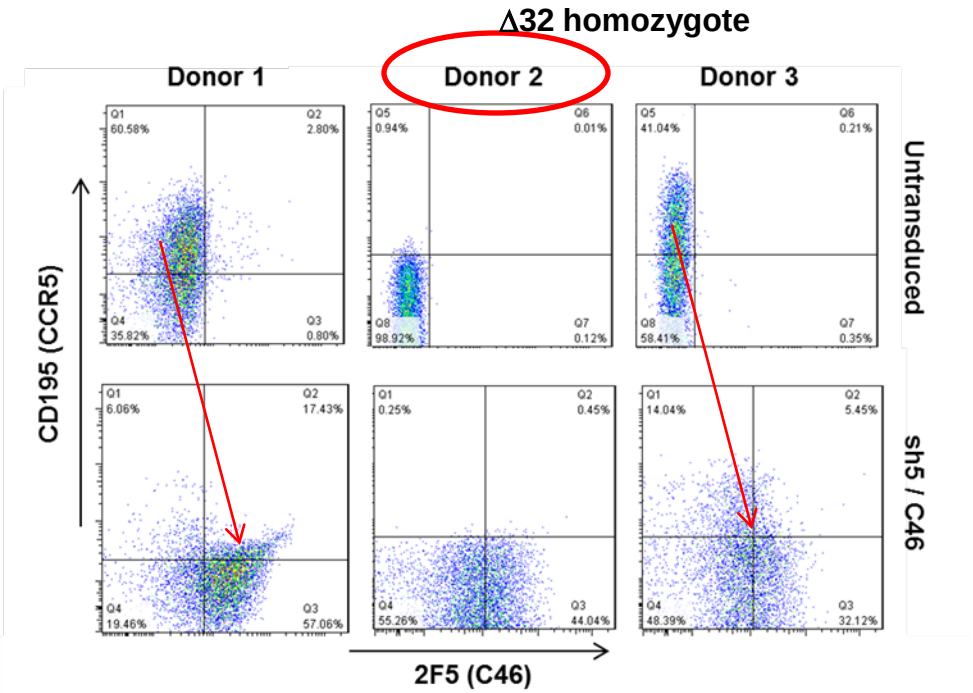


Entry





Simultaneous CCR5 Knockdown and C46 Expression in PBMC



Pre-Clinical Data

In Vitro

- No apparent toxicity – proliferation, colony formation, apoptosis, inflammatory response (IFNγ, IL6, TNFα)
- Assays – DNA PCR WPRE and C46; RNA PCR C46 and Sh5
- Copy number and expression
- Efficacy – T cell lines and PBMC (lab and clinical strains)

In Vivo

- Efficacy studies with Cal-1 underway in BLT mice
- GLP safety studies with Cal-1 underway in NSG mice
- Prior independent studies with CCR5 and C46 have shown efficacy and no toxicity

Overview of CAL-USA-11

Primary objective is to evaluate:

- The safety, feasibility & tolerability of Cal-1, gene-transduced, hematopoietic cell populations
- The safety & tolerability of low and moderate dose busulfan as a non-myeloablative conditioning agent as a means to improve engraftment of transduced HSPC

Clinical Trial – Not on ART

Cohorts

1. Transduced (4)
2. Transduced + low dose busulfan (4)
3. Transduced + moderate dose busulfan (4)

End-Points

Primary

- Safety and feasibility

Secondary

- Marking and expression
- Viral load
- T cell count
- TREC, CD31
- Development of resistance

Key Points

- Evaluation of a new dual lentiviral vector therapy for HIV
- Target of HSPC and T cells
- Use of a subject population with viral load to allow selective pressure on gene containing cells

Review Response – Dr Badley

1. Long-term follow up to be mandatory *clarified in Protocol and Informed Consent*
2. Increased studies on functionality of transduced cells *further studies of the type suggested will be conducted (mitogen stimulation PHA, IL2, T cell signalling CD3, CD28)*
3. Monitor immune response to C46 *yes, this is being done in the trial*
4. Relationship between MRC and DSMB and stopping rules *clarified in Protocol and will be in DSMB charter*
5. Insertional mutagenesis – cost of care if anything untoward *explanation further clarified*

Review Response – Dr Ross (1,2)

1. **Pre-Clinical Studies**
   a) Rationale for bi-functional vector *inhibits multi-tropic HIV /mitigates monotherapy resistance*
   b) Any off-target /toxic effects (sh5, C46) *none seen – either here or historically*
   c) Inhibition of virus in mixed percentage transduced cells *PBMC completed, Molt 4 underway*
   d) Donor 2 and Δ32 homozygosity *yes*
   e) GLP pharm/tox study endpoints *this is a GLP safety study measuring engraftment/toxicity*
2. **T-Cell and HSPC Transduction**
   a) Gene marking assay sensitivity *0.01%*
   b) Integration site analysis (CD34+ HSPC) *yes and clarified*
   c) Integration and expression in non-HIV target cells *yes, testing in humanized NSG mouse model (currently underway)*
   d) Transduction efficiency quantification *yes, use of C46 qPCR*
   e) MOI definition *ratio virus to cells and titer determined by 2F5 staining*
   f) Lower transduction limit *changed to ≥ 10%*

Review Response – Dr Ross (3-6)

3. **Pretreatment/pre-conditioning regimens**
   a) Busulfan and HIV virus load *no impact*
   b) Neupogen and HIV virus load *no impact*
4. **Clinical endpoints**
   a) Treatment options if withdraw *investigator's best judgement (last tolerable, most effective)*
   b) Integration site analysis and potential predominant clone *clarified in Protocol*
   c) Secondary long-term follow up *LTFU protocol will include integration analysis*
5. **Subject enrolment criteria**
   a) Time off ART and withdrawal therapy *6 weeks off ART and as above for 4a*
   b) Rationale for exclusion of non-R5 strain *as per FDA request to simplify study*
   c) Will Δ32 heterozygotes be allowed to participate? *yes, may provide important exploratory information*
6. **Informed Consent**
   a) Potential risk of insertional oncogenesis *explanation modified in Informed Consent*

Review Response – Dr Hammarskjold

Preclinical studies

1. Determination of RNA expression *method explained*
2. Potential off-target effects of shCCR5 *result explained & no off-target effects observed*
3. Rationale for use of dual vector *inhibits multi-tropic HIV and mitigates monotherapy resistance*
4. Cal-1 BLT experiments *experiments in progress*
5. Titering of vector stocks *titer on 293T with 2F5 read-out*
6. Use of resistant HIV strains *update given - strong inhibition in multiple strains*
7. Cal-1 resistant HIV strains; analysis of T20 resistant strain *$T20^r$ & $C46^r$ virus being analyzed*

Subject enrollment criteria

8. Will Δ32 heterozygotes be allowed to participate *yes, may provide important exploratory info*

Study endpoints

9. Analysis of potential development of resistance in clinical trial *yes, will be conducted*

Informed consent

10. Insertional mutagenesis and lentiviral vectors *explanation modified in Informed Consent*

Additional Slides

Modifications to Protocol

1.	Clarification of roles of and communications between Medical Review Committee (MRC) and Data Safety Monitoring Board (DSMB)	B4
2.	Clarification of cost of treating any Cal-1 related adverse events	B5
3.	Integration analysis of HSPC infusion product	R2b
4.	Rules for commencement of ART	R4a, R5a
5.	Clinical evaluation of integration site analysis	R4b

Modifications to Informed Consent

1. Clarification on requirement for long term follow-up B1
2. Clarification of cost of treatment B5
3. Description of risks for potential insertional mutagenesis R6, H10

"This study involves making a permanent change to the DNA of your blood cells with Cal-1. It is possible that this change can happen in an area of your cells' DNA that activates it in an unwanted way that may cause blood cancer. This is known as "insertional mutagenesis" and has happened for a very small number of people who have received gene therapy using other types of retroviral vectors, but has generally not been seen to date with lentiviral vectors. However, this could be due to the field's limited clinical use of lentiviral vectors. The risk of this happening is very low, and we will conduct regular tests called "Cal-1 integration site analysis" to look for this so that any possible blood cancer is found and treated early."

Sh5 – No Off-Target Effects

Potential off-target effects of sh5 have been extensively addressed and shown to be negative in tissue culture (TC), non-human primates (NHP) and BLT mice (M)

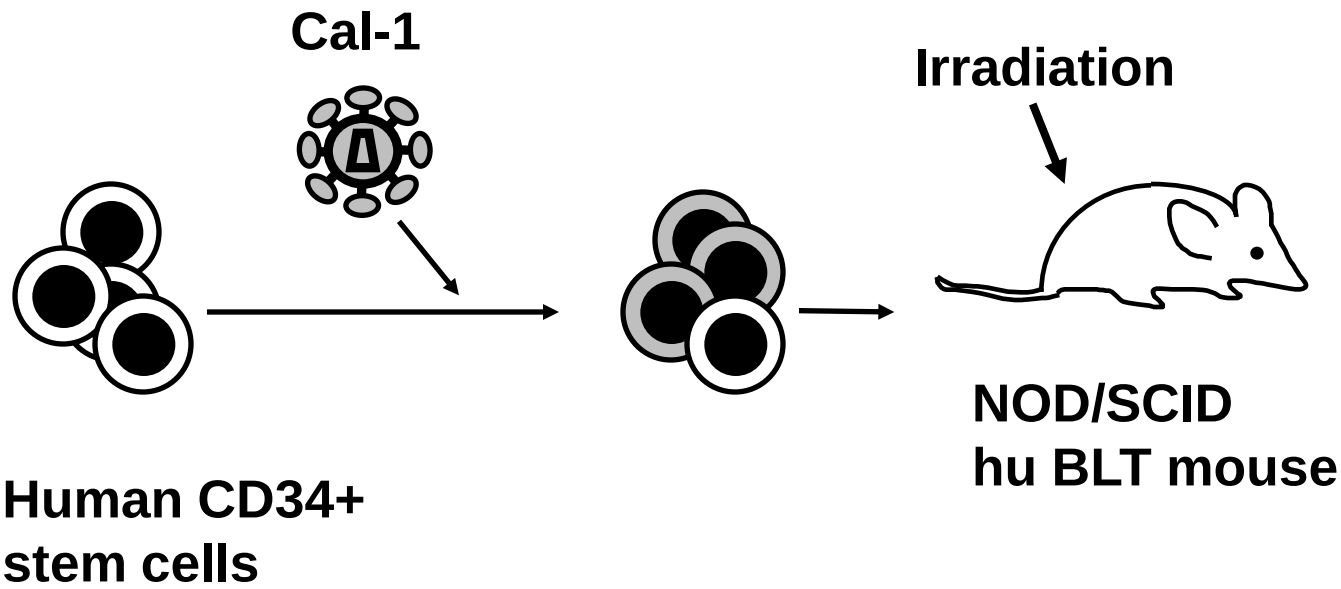
1. Qin et al 2003 Inhibiting HIV-1 infection in human T cells by lentiviral-mediated delivery of small interfering RNA against CCR5 PNAS 100: 183-188 (TC)
2. An et al 2006 Optimization and functional effects of stable short hairpin RNA expression in primary human lymphocytes via lentiviral vectors Mol Ther 14: 494-504 (TC)
3. An et al 2007 Stable reduction of CCR5 by RNAi through hematopoietic stem cell transplant in non-human primates PNAS 104: 13110-13115 (NHP)
4. Shimizu et al 2009 Characterization of a potent non-cytotoxic shRNA directed to the HIV-1 co-receptor CCR5. Genet Vaccines Ther 7: 8 (TC, NHP)
5. Shimizu et al 2010 A highly efficient short hairpin RNA potently down-regulates CCR5 expression in systemic lymphoid organs in the hu-BLT mouse model Blood 115: 1534-1544 (M)
6. Liang et al 2010 Inhibition of HIV-1 infection by a unique short hairpin RNA to chemokine receptor 5 delivered into macrophages through hematopoietic progenitor cell transduction. J Gene Med 12: 255-265 (TC)

C46 - No Toxicity/Immunogenicity

Potential toxicity and immunogenicity of C46 has been extensively addressed and shown to be negative in tissue culture, non-human primates and humans

1. Egelhofer et al 2004 Inhibition of human immunodeficiency virus type 1 entry in cells expressing gp41-derived peptides J Virol 78: 568-575..
2. Perez et al 2005 Suppression of HIV-1 infection in primary CD4 T cells transduced with a self-inactivating lentiviral vector encoding a membrane expressed gp41-derived fusion inhibitor Clin Immunol 115: 26-32.
3. Schambach et al 2006 Towards hematopoietic stem cell-mediated protection against infection with human immunodeficiency virus. Gene Ther 13: 1037-1047
4. van Lunzen et al 2007 Transfer of autologous gene-modified T cells in HIV-infected patients with advanced immunodeficiency and drug-resistant virus. Mol Ther.15:1024-33
5. Zahn et al 2008 Efficient entry inhibition of human and nonhuman primate immunodeficiency virus by cell surface-expressed gp41-derived peptides. Gene Ther 15: 1210-1222
6. Hermann et al 2009 Mutations in gp120 contribute to the resistance of human immunodeficiency virus type 1 to membrane-anchored C-peptide maC46. J Virol 83: 4844-4853
7. Trobridge et al 2009 Protection of stem cell-derived lymphocytes in a primate AIDS gene therapy model after in Vivo selection.PLoS One 4:e7693
8. Kimpel et al 2010 Survival of the fittest: positive selection of CD4+ T cells expressing a membrane-bound fusion inhibitor following HIV-1 infection. PLoS One 5: e12357.

Modeling Cal-1 in NOD/SCID hu BLT mouse



READ-OUT
- Reconstitution
- HIV challenge

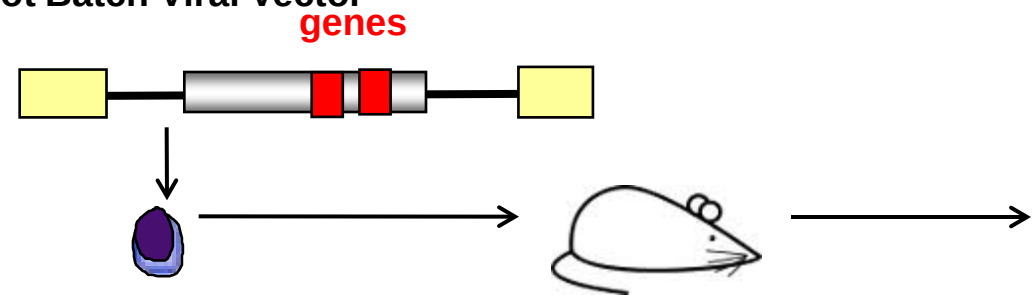
Shimizu et al 2010 Blood 115:1534-1544

Pre-Clinical Safety Studies

1.GLP Pharm/Tox Study

NOD-scid IL2r γ^{null}
(NSG) mice

Pilot Batch Viral Vector



Human CD34+ HSC

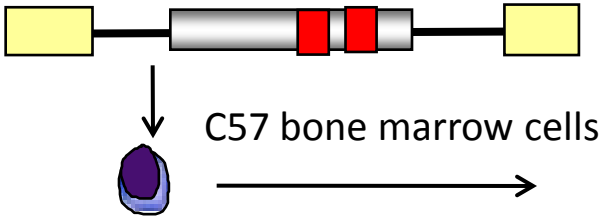
4 week old animals

Similar Lineage Reconstitution; no untoward toxicity +/- vector

2. IVIM Assay (C Baum)

Insertional transformation of hematopoietic cells by self-inactivating lentiviral and gammaretroviral vectors *Mol Ther 17; 1919-1928 (2009)*

Pilot Batch Viral Vector



C57 bone marrow cells

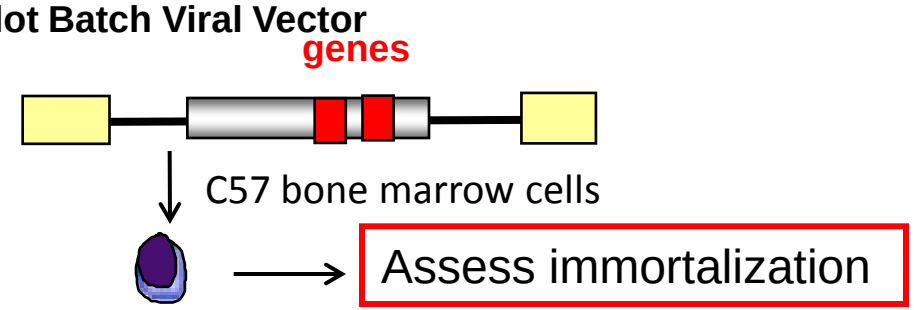
Assess immortalization

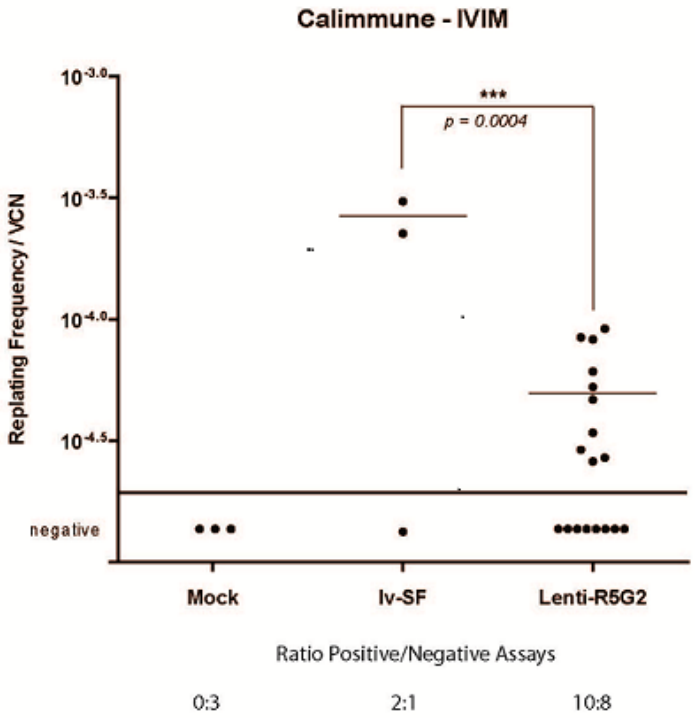
Pre-Clinical Safety Studies

2. IVIM Assay (C Baum)

Insertional transformation of hematopoietic cells by self-inactivating lentiviral and gammaretroviral vectors *Mol Ther 17; 1919-1928 (2009)*

Pilot Batch Viral Vector





Preliminary: "The IVIM assay shows a strongly reduced mutagenic potential of Lenti-R5G2 compared to the positive control lv-SF".

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