Gene Therapy for Primary Immune Deficiencies

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Human Gene and Cell Therapy Program

Broad Stem Cell Research Center

Jonsson Comprehensive Cancer Center

The Wooden Center
Hypothesis:

Gene therapy using autologous HSC that are corrected with the normal gene will have beneficial effects on blood cell production or function, without the immunologic complications of allogeneic HSCT.

May also use same techniques to genetically engineer HSC to produce blood cells with:

- a) resistance to HIV infection (shRNA, CCR5 k/o)
- b) specific anti-cancer reactivity (TCR, CAR)
- c) reduced suppression by chemotherapy (MGMT, DHFR)
Gene Therapy = Autologous Transplantation

- Collect Bone Marrow, PBSC or UCB
- Condition Bone Marrow with Chemotherapy
- Enrich Stem Cells (CD34-select)
- Culture with Hematopoietic Growth Factors
- Add or Correct Gene
- Formulate/Certify Stem Cells for Intravenous Infusion
- Autologous Transplantation
Gene Therapy Using Hematopoietic Stem Cells

Normal gene

Add it
Fix it

CD34+ - The 1%
Adenosine Deaminase (ADA)-Deficient Severe Combined Immune Deficiency (SCID)

Genetic deficiency of ADA enzyme activity is the underlying cause of 10-15% of human SCID.

In the absence of ADA, lymphocytes accumulate high levels of adenosine and deoxyadenosine metabolites (esp. dATP) which are toxic, leading to pan-lymphopenia (T-/B-/NK-)

Frequency of ADA-SCID is ~1.7-2.5/million births or approx. 7-10/yr in US + Canada.

Treatments options include allogeneic HSCT (MSD, MUD, haplo), PEG-ADA enzyme replacement therapy (ERT) or autologous transplant of gene-corrected HSC (gene therapy).
**MND-ADA γRV and EFS-ADA LV**

**MND-ADA γRV** from PG13 clone (GALV). MPSV γRV LTR drives hADA cDNA. Titer 3-5 x10^6 TU/ml (unprocessed).

**EFS-ADA LV** from 293T transient (VSV-G). Hu EF1α gene short (EFS) promoter drives codon-optimized hADA cDNA with WPRE, SIN LTR. Titer 3-5 x10^9 TU/ml (TFF concentrated).
Outcome after HSCT with Full, Partial or No Myeloablative Conditioning

Donor or Auto/Gtx HSC

Marrow

Patient’s Bone Marrow HSC

None

Full Myeloablation

Reduced Intensity Conditioning

Donor Chimerism

Donor Chimerism

Minimal

Full

Mixed
Clinical Trial Experimental Time-Line

- Consent
- Screen
- Admit
- BM Harvest (PICC)
- Busulfan (pK)
- γRV Transduction
- Transplant
- Immune Reconstitution
- D/C ERT

Day: -4 -3 -2 -1 0

MND-ADA γRV Trial
Clinical Trial Experimental Time-Line

- **Day: -4**: BM Harvest (PICC)
- **Day: -3**: Busulfan (pK)
- **Day: -2**: BM Harvest (PICC)
- **Day: -1**: Busulfan (pK)
- **Day: 0**: γRV Transduction
- **Day: +30**: Immune Reconstitution

**MND-ADA γRV Trial**
- Consent
- Screen
- Admit
- BM Harvest (PICC)
- Busulfan (pK)
- γRV Transduction
- Transplant

**EFS-ADA LV Trial**
- BM Harvest (PICC)
- LV Transduction
- Busulfan (pK)
Comparison of Grafts Between MND-ADA γRV and EFS-ADA LV

- Age At Gene Therapy
  - p=n.s.

- Busulfan AUC
  - p=n.s.
  - p=<0.0001

- CD34 Dose
  - p=0.01

- CD34 VCN
  - p=n.s.

- CD34 ADA Enzyme Activity
  - p=<0.0001

- CD34 ADA (U) / VCN
  - p=<0.0001
Comparison of Deoxyadenosine Nucleotides (dAXP) in RBC After Gene Therapy and ERT Withdrawal

ERT Withdrawal

MND-ADA \( γRV \)

EFS-ADA LV

\[ p = \text{<0.0001} \]

MND-ADA

EFS-ADA

ERT Withdrawal

Months After Gene Therapy
Vector Copy Number (VCN)

**PBMC**

- MND-ADA γRV
- EFS-ADA LV

- $p = n.s.$

**Gran VCN**

- MND-ADA γRV
- EFS-ADA LV

- $p = n.s.$

**PBMC ADA**

- MND-ADA γRV
- EFS-ADA LV

- $p <= 0.0001$

**PBMC ADA (U) / VCN**

- MND-ADA γRV
- EFS-ADA LV

- $p <= 0.0001$
Absolute Lymphocyte Counts (ALC)

- **MND-ADA γRV**
- **EFS-ADA LV**

![](chart.png)

- * p=<0.05
- ** p=<0.0001

ALC (cells/mm³)

Month After Transplant
Lymphocyte Recovery After Gene Therapy

Absolute CD3+ T Cells

- MND-ADA γRV
- EFS-ADA LV

* p=<0.05
** p=<0.0001

Absolute CD4+ T Cells

* p=<0.05
** p=<0.0001

Absolute CD19+ Cells

* p=<0.05

Absolute CD56+ NK Cells

* p=<0.05
Application to FDA for Orphan Drug Designation

Submitted: 072414
Date Designated: 102114
EFS-ADA Phase II/III Clinical Trial(s)

Based on guidance from FDA and EMA, a Phase II/III clinical trial is being planned to be done at UCL/UK and UCLA/US.

Major end-point will be survival compared to historical controls with unrelated or haploidentical allogeneic HSCT.

Will use BM for smaller or G-CSF mPBSC for older.

EFS-ADA LV transduced CD34+ final cell product will be cryopreserved and fully characterized prior to transplant.

Busulfan dose will be split into two, to allow subject-specific adjustments based on first dose pK to reach target net AUC = 4,900 uM*min = 20 ug/L*hr.
XSCID Gene Transfer Schema
Self-inactivating gammaretroviral vector

No gag, pol or env residues

eligibility criteria met

autologous BM harvest

CD34+ selection

d-4

3 rounds of transduction in retronectin coated bags

SCF IL3 TPO Flt3L

24h 24h 6h

Infuse

No CONDITIONING
Immune Reconstitution and Gene Marking after Gene Therapy

H

I

Average Vector Copies/Cell (VCN) by qPCR

XSCID 00007 VCN at Day +120

- Whole Blood
- MNC
- CD15 (My)
- CD19 (B)
- CD56 (NK)
- CD3 (T)
XSCID 3

Thrasher - UCL/GOSH. London
Cavazzana - Hopital Necker Enfants Malade, Paris
Pai – Boston Children’s Hospital
Filipovich - Cincinnati Children’s Hospital
Kohn - Mattel Children’s Hospital, UCLA

Lentiviral vector (EFS-gammaC)
  – developed by Genethon, France.
  David Williams – US IND Sponsor

Two arms:
  a) Good health: busulfan 6 mg/kg (target 30 mg*hr/L)
  b) Severe infection: no conditioning

U01 To submit - Sept 2015
Genetic Defects in CGD

- **CYBB** (~65% (X-linked))
- **CYBA** (~5%)
- **NCF1** (~25%)
- **NCF2** (~5%)
- **NCF4** 2 patients

**Rac2**

- **RAC2** (NCF3) 2 patients

**gp91phox**

**p22phox**

**p67phox**

**p40phox**

**p47phox**
A PHASE I/II, NON RANDOMIZED, MULTICENTER, OPEN-LABEL STUDY OF G1XCGD (LENTIVIRAL VECTOR TRANSDUCED CD34+ CELLS) IN PATIENTS WITH X-LINKED CHRONIC GRANULOMATOUS DISEASE

IND Sponsor: Donald B. Kohn, M.D.
University of California, Los Angeles (UCLA)

Primary Institution: Mattel Children’s Hospital, UCLA
Site PI: Donald B. Kohn, M.D.

Collaborating Institutions:
Children’s Hospital Boston
Site PI: David A. Williams, MD

National Institute of Allergy and Infectious Disease
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Thomas Coates - Children’s Los Angeles
Joseph Church - Children’s Los Angeles

Vector Production / Scientific Coord.
GENETHON – EVRY - FRANCE
Anne Galy, PhD

Cell Processing Facilities
UCLA HGMP Laboratories
Dana Farber Cancer Institute Cell Manipulation Core
NIH Dept. of Transfusion Medicine

NIH RAC OBA # 1304-1223
UCLA IRB # 13-001875; CHB IRB # P00012407; NIH IRB # 2014-564
FDA IND # 16141 (approved 10/11/15); NCT02234934
Funding - pending
Diagnosis and Inclusion Criteria


-Molecular diagnosis confirmed by DNA sequencing and supported by laboratory evidence for absent or reduction > 70% of the biochemical activity of the NAHPD-oxidase.

-At least one prior, ongoing or refractory severe infection and/or inflammatory complications requiring hospitalization despite conventional therapy.

-No HLA-matched donor available after registry search.

-No co-infection with HIV or hepatitis B or hepatitis C virus), CMV, adenovirus, parvovirus B 19 or toxoplasmosis.

-Written informed consent for adult patient or by parent/guardian and where appropriate child’s signed consent/assent.
A novel chimeric promoter targets transgene expression to myeloid cells and efficiently corrects a murine model of X-CGD

G Santilli et al. London, UK; Madrid, Spain; Frankfurt, Germany. Mol Ther 2011

Increased expression with myeloid maturation in murine X-CGD model.
Site-Specific Gene Modification

Genomic Sequence with Base-Pair Mutation (M)

Site-specific Endonuclease Creates DS DNA Break

Homologous Donor with Normal Base-Pair (N)

Edited Genome with Normal Base-Pair N
Conclusions

In Phase I/II trials, both γRV and LV were effective and safe to transduce HSC and express ADA.

---100% survival. 0% GVHD. No vector-related AE.

---Fair to good immune reconstitution –
  18/19 off ERT x 0.2-6 years.

---Better outcomes in infants than older children.

EFS-ADA LV expresses more ADA/VC than MND-ADA γRV.

Initial immune reconstitution may be more robust with EFS-ADA LV

Autologous HSCT with gene therapy provides a beneficial treatment option.
The Future of Gene Therapy for PID

Unlike allo HSCT, where one approach treats all SCID (or CGD, etc) genotypes, autologous gene therapy requires a genotype-specific therapeutic

Cost/time to develop for increasingly rare subset of disease is a challenge

Randomly integrating vectors, constitutively expressing transgene may be problematic, esp, for genes involved in signaling, etc

Risks from vectors must outweigh risks from GVHD, immune suppression with allo HSCT
The Future of Gene Therapy for PID

Site-specific gene correction under development using designer endonucleases to augment homologous recombination.

Would lead to physiologic expression of corrected endogenous gene.

Efficiency currently low, but improving.

Apply to less severe but more common disorders, e.g. X-HIM, XLA, as benefit/risk ratio improves.
U.C.L.A. Stem Cell Gene Therapy Group

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