Bone Marrow Transplant and Gene Therapy For Sickle Cell Disease

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Conflict of Interest Statement

Donald Kohn is a member of the Scientific Advisory Board of Orchard Therapeutics.

**UCLA** has licensed intellectual property, on which Dr. Kohn is an inventor, to Orchard Therapeutics (ADA SCID) and to BioMarin (Sickle Cell Disease).

Dr. Kohn serves on the SAB for Kite Pharma.
The 405, Friday 4 PM
Bone Marrow is Where New Blood Cells are Made

- Red Blood Cells
- White Blood Cells
  - Lymphocyte (T, B, NK cells)
  - Monocyte
  - Eosinophil
  - Basophil
  - Neutrophil
- Platelets
Hematopoietic Stem Cells (HSC) Produce All of the Blood Cells

**HSC come from:**
- Bone Marrow
- Umbilical Cord Blood
- or mobilized PBSC

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<th>Adults</th>
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Erythropoiesis

Epo
IL-3

Fetal Liver - Bone Marrow
Circulation

Bone Marrow
Blood stream

Stem cell
BFU-E
CFU-E
Colony formation (in vitro)
EPO

Pro-erythroblast
Basophilic erythroblast
Polychromatic erythroblast
Orthochromatic erythroblast

reticulocyte
eythocyte

nuclear extrusion
BMT Procedure for
Genetic Blood Cell Diseases

High dose chemotherapy is given to eliminate the patient’s own bone marrow and suppress their immune system.

Then, give stem cells from a healthy donor.

These donor stem cells engraft in the patient’s bone marrow and will make normal blood cells for the life of the patient.

This corrects the genetic disease of blood cells.
Bone Marrow Transplant - an example of Stem Cell Therapy

Harvesting bone marrow from the Donor

Processing Stem Cells

Patient is “conditioned” with high dose chemotherapy

Stem cells infused

Intensive medical support until stem cells grow
Collection of PBSC by Leukapheresis

Daily G-CSF

±Plerixafor

Infuse
Freeze
CD34-select
Gene Modify
Other
Use of Umbilical Cord Blood for Stem Cell Transplants

~50-100 ml of newborn’s blood in blood vessels of the umbilical cord and placenta.

Umbilical cord blood (UCB) has the same HSC as bone marrow and can be used for clinical stem cell transplants.

UCB normally wasted. Can be saved privately for a single child or family or can be saved in community banks to be publicly available.
Potential Benefits from BMT for Genetic Blood Cell Diseases

If successful, leads to cure from disease.

→ for SCD = no more pain crises, transfusions, ongoing organ damage, restrictions of activity.

→ Will not correct pre-existing problems, like stroke, kidney damage, etc.
Outcome after HSCT with Full, Partial or No Myeloablative Conditioning

Patient’s Bone Marrow HSC

None

Full Myeloablation

Reduced Intensity

Donor or Auto/Gtx HSC

Donor Chimerism

Minimal

Full

Mixed
Myeloablative Allogeneic HSCT is Curative for Children with SCD

- 22 children <16 years old transplanted
- HLA-Identical Sibling Donors
- Myeloablative Conditioning with Bu/Cy/ATG
Is Nonmyeloablative Conditioning Regimen with ptCY Applicable to SCD when using HLA-identical donors?

- **Hypothesis**
  - Lower-intensity conditioning combined with long-term administration of sirolimus can promote tolerance and suppress GVHD thus allowing for HSCT in SCD adults.

- **Results**
  - 287 patients screened (50 eligible for HSCT)
    - 30 transplanted (~10% of screened)
  - 26/30 (87%) achieved long-term stable engraftment with no GVHD
  - 15/26 (57%) of engrafted patients discontinued sirolimus with continued donor chimerism and no GVHD
  - 25/26 (96%) achieved full donor chimerism

- **Conclusion**
  - low-dose TBI, alemtuzumab, and sirolimus theoretically provide the necessary environment for stable mixed chimerism and minimal GVHD thus expanding this treatment option to adults with SCD.
Graft Versus Host Disease (GVHD)

A major limitation to the use of BMT to treat these genetic diseases of blood cells is the unwanted immune responses between a patient and the transplanted cells.

Patient can reject the transplanted HSC.

Donor T cells within the marrow can react against the recipient (GVHD).

Immune suppression is needed, yet significant clinical complications may still occur.
GVHD remains a major cause of morbidity and mortality after allogeneic HSCT, even with HLA-matched donor.
Gene Therapy
Gene Therapy / Autologous Stem Cell Transplant for Genetic Blood Cell Diseases

γ-Retroviral
Lentiviral
Foamy Viral
α-Retroviral

Package as Pseudotyped Vector

Gene Addition to Stem Cells with Vector

Collect, Isolate Stem Cells

Transplant Gene-Modified Stem Cells

Gene Correction of Stem Cells with Site-Specific Nuclease and Homologous Donor

Administer Marrow Conditioning

Patient
Sickle Cell Disease, Bone Marrow Transplant and Gene Therapy

For blood cell diseases, fixing the gene in the patient’s own bone marrow stem cells which are then transplanted back to the patient could correct the problem.

For gene therapy of sickle cell disease, the goal is to gene modify the bone marrow stem cells to prevent sickling of the red blood cells that are made.

How?
Gene Therapy Using Hematopoietic Stem Cells

Normal gene

Add it
Fix it

CD34+ - The 1%
Map of Retrovirus and Retroviral Vector

A. Retrovirus

B. Retroviral Vector

Exogenous gene(s)

5’ LTR | gag | pol | env | 3’ LTR

D

ψ⁺

5’ LTR | Exogenous gene(s) | 3’ LTR

D

E/P

ψ⁺

ATG

Vector RNA

Stpp

AAAA

Therapeutic Protein
Lentiviral Vector

Entry via Nuclear Pore

Entry, Uncoating

Reverse Transcription

SS RNA

DS DNA

Integration into Chromosomal DNA

Expression of Therapeutic mRNA and Protein

Stable Transmission to Progeny Cells

Hematopoietic Stem Cell
Severe Combined Immune Deficiency (SCID)
Severe Combined Immune Deficiency (SCID)

SCID is the most severe primary immune deficiency, with absent T and B (and sometimes NK) cell function (incidence ~1/58,000).

High early mortality from infections without treatment.

Bone marrow transplantation can be curative:
~95% success with HLA-matched sib donor (MSD)
~70-80% success with matched unrelated donor (MUD) or haplo-identical (parent) donor.
The EFS-ADA Lentiviral Vector

SIN Lentiviral Vector (pCCL)
Human Elongation Factor1-α Promoter (EFS)
Human ADA cDNA (codon-optimized)
WPRE (ORF removed)
VSV-G pseudotype.
Titer: 0.5-1.0x $10^{10}$ TU/ml (after ~1000X concentration)
Clinical Trials of Gene Therapy for ADA SCID

# ADA SCID Gene Therapy Subjects/Year


Subjects/Year: 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10
EFS-ADA Vector Copy Number (VCN)

Months After Gene Therapy

VCN

PBMC

Granulocytes

Mean +/- s.e.m.; n=20
EFS-ADA LV "500s"

RBC ADA

RBC %dAXP

CD3+ T Cells

CD19+ B Cells

Months After Gene Therapy

Months After Gene Therapy

Months After Gene Therapy

Months After Gene Therapy
Survival after EFS-ADA Gene Therapy

- EFS-ADA USA (n=21)
- EFS-ADA UK (n=15)

February 2017
Application to FDA for Orphan Drug Designation

Breakthrough Therapy Designation: Aug. 2015
Rare Pediatric Disease Designation: July 2017
Sickle Cell Disease, Bone Marrow Transplant and Gene Therapy

How could gene therapy be applied to SCD?
Single Amino Acid Change In β-Globin of SCD Leads to Hb Polymerization and RBC Sickling

Val 6 → Glu 6

Aggregated HbS molecules polymerize in RBC, making them stiff and deformed (sickled)
Fetal HbF Prevents Sickling

RBC’s have mostly adult HbA ($\beta_2\alpha_2$ >90%), but variable amounts of fetal HbF ($\gamma_2\alpha_2$ 1-10%). Patients with SCD who express >8.6% HbF (i.e. <90% sickle HbS $\beta^s_2\alpha_2$) have milder disease and improved survival.

HbF ($\gamma_2\alpha_2$) repels HbS

How could gene therapy be applied to SCD?

a) Add a normal $\beta$-globin gene (like trait).

b) Add a $\gamma$-globin (fetal) gene to act like hydroxyurea

c) Add a “$\gamma$-like” $\beta$ –globin to act like hydroxyurea

d) Directly correct sickle mutation in the $\beta$-globin gene
Gene Therapy for Hemoglobinopathies
Thresholds and Approaches

Need to express sufficient γ-globin to prevent RBC sickling (approx 10% of total or ≥1.5 gm/dl).

Express γ-globin - Malik

Express amino acid substituted β-globin:
  T87Q - Leboulche, bluebird bio
  AS3 - Townes, Kohn

Induce endogenous fetal (γ)-globin expression by suppressing/knocking-out Bcl11a or its binding sites in the γ-globin gene promoter

Edit to correct the sickle mutation in beta-globin gene
Clinical Trial of Stem Cell Gene Therapy for Sickle Cell Disease

Autologous Bone Marrow Harvest

Isolate BM Stem Cells

ADD ANTI-SICKLING GENE OR EDIT TO NORMAL B-GLOBIN GENE

Test Cells. Freeze.

Condition with chemotherapy

Transplant BM Cells Back to Patient

Follow: Safety Efficacy
Why might gene therapy be better than BMT?

1. Every patient has a perfectly matched donor - themself.
2. No immune mismatches → no GVHD, rejection unlikely.
3. Less chemotherapy and immune suppression may be needed for gene therapy to be beneficial than is needed for successful BMT from another person.

So, some of the most severe side-effects from BMT may be absent or lessened with gene therapy.
Why might gene therapy be worse than BMT?

1. May not get the new gene into enough stem cells.
2. The new gene may not make enough hemoglobin to fix the red blood cells.
3. Handling the stem cells could hurt them so that they can’t continue to make new blood cells for decades.
4. The gene manipulation of the stem cells could make them grow too much, even cause leukemia (cancer of the blood stem cells).

So, some new problems and side-effects could occur.
Lenti\(\beta^{AS3}\) Vector Expresses “Anti-Sickling” Globin
Prior Work by T. Townes (UAB) (Blood 2003, JBC 2004)

\[ \beta^{AS3} = \text{Thr}87\text{Gln} - \gamma\text{-like “anti-sickling”} \]
\[ \text{Gly}16\text{Asp} - \text{blocks axial contacts} \]
\[ \text{Glu}22\text{Ala} - \text{increases affinity for } \alpha \]

In sickle cell mouse model, gene transfer/BMT with Lenti\(\beta^{AS3}\) vector corrected hematologic and systemic disease manifestations.
Stem Cell Gene Therapy for Sickle Cell Disease  
CIRM DR1-01452 (03/01/10)

1) Perform efficacy studies of vector in BM CD34+ cells from SCD donors to determine whether sufficient $\beta^{AS3}$-globin gene transfer and expression can be achieved to reverse adverse effects of HbS on RBC properties/physiology. (Year 1)

2) Perform IND-enabling pre-clinical studies and qualify end-point assays. (Year 2-3)

3) Develop clinical protocol and associated documents for regulatory applications. (Year 1-3)

4) Obtain regulatory approvals (IND, NIH-RAC, IRB, IBC, etc). (Year3-4)

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<td>Ready to Open Clinical Trial</td>
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β-Globin Gene Transfer To Human Bone Marrow For Sickle Cell Disease


Dept. of Microbiology, Immunology & Molecular Genetics and Pediatrics  
Eli and Edythe Broad Center for Stem Cells and Regenerative Medicine  
University of California, Los Angeles

Journal of Clinical Investigation – July 1, 2013
(Funded by CIRM DR1-01452)
Phase I trial

Eligible subjects (n=6) will be:

- adults age 18 or older
- Dx of SCD (SS or S/βthal<sup>o</sup>) with complications
- lacking a medically eligible HLA-identical sibling donor or a 10/10 allele-matched adult unrelated donor and
- meeting defined inclusion/exclusion criteria.
SCD 101 Timeline

Enroll 12/2104
Confirm Eligibility
Marrow harvest 1
Manufacture FCP
Cryopreserve back-up
(2x10^6 CD34+/kg)
Marrow harvest 2
Manufacture FCP
Busulfan Conditioning
Transplant
Cells (BM CD34+ cells/Lenti/βAS3-transduced) thawed and infused for:

- Total dose of ~4x10^6 cells/Kg.
- VCN average 0.25.
SCD 101 - ANC

Transplant

Busulfan

No G-CSF
SCD 101 Blood Cell VCN
Gene Therapy in a Patient with Sickle Cell Disease

Jean-Antoine Ribeil, M.D., Ph.D., Salima Hacein-Bey-Abina, Pharm.D., Ph.D., Emmanuel Payen, Ph.D., Alessandra Magnani, M.D., Ph.D., Michaela Semeraro, M.D., Ph.D., Elisa Magrin, Ph.D., Laure Caccavelli, Ph.D., Benedicte Neven, M.D., Ph.D., Philippe Bourget, Pharm.D., Ph.D., Wassim El Nemer, Ph.D., Pablo Bartolucci, M.D., Ph.D., Leslie Weber, M.Sc., Hervé Puy, M.D., Ph.D., Jean-François Meritet, Ph.D., David Grevent, M.D., Yves Beuzard, M.D., Stany Chrétien, Ph.D., Thibaud Lefebvre, M.D., Robert W. Ross, M.D., Olivier Negre, Ph.D., Gabor Veres, Ph.D., Laura Sandler, M.P.H., Sandeep Soni, M.D., Mariane de Montalembert, M.D., Ph.D., Stéphane Blanche, M.D., Philippe Leboulch, M.D., and Marina Cavazzana, M.D., Ph.D.

N Engl J Med
Volume 376(9):848-855
March 2, 2017
Engraftment with Transduced Cells and Therapeutic Gene Expression in the Patient.

Why is Sickle Cell Disease So Much Harder to Treat than ADA-SCID?

1. The Vector: The larger size and complexity of the beta-globin gene unit needed for high-level, erythroid-specific expression of beta-globin. ADA is simpler to express.

   a. MUCH more raw vector is needed to produce a patient dose.

      ADA: 1 30L lot treats ~ 20 patients;
      SCD: 1 30L lot treats 1-2 patient.

   b. Gene transfer to human HSC is much less efficient by the bigger vector
EFS-ADA

Lenti/$\beta^{AS3}$-FB

**Raw Titers (HT29)**

- Titer 10 x $10^6$ TU/ml
- Titer 5 x $10^6$ TU/ml
- Titer 0.7 x $10^6$ TU/ml
Vector Provirus Size vs. Raw Titer

- EFS-ADA
- chimGP91
- βAS3-FB
We’re Gonna Need a Smaller Vector!
HONEY, I SHRUNK THE VECTOR
Gene Editing
Repair of Sickle Mutation in Beta-Globin Gene

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Gene Editing Using Site-Specific Endonucleases and DNA Repair

**Patient’s Gene**

**Site-Specific Endonuclease:**
- Zinc finger nuclease
- Homing endonuclease
- TALEN
- CRISPR/Cas9

**M** = mutant bp  
**N** = normal bp

**Gene Disruption**

- No Donor (NHEJ)

**Gene Correction**

- Homologous Donor (HDR)
Site-specific Gene Editing of Autologous Hematopoietic Stem Cells for Gene Therapy

- **Autologous HSC (BM, PBSC or CB)**
  - CD34 select
  - Pre-stimulate S/F/T (24-48 hr.)

- **Electroporation**
  - Site-Specific Endonuclease (as mRNA or RNP)
  - Homologous Donor (as Oligo or by Viral Vector)

- **Formulate Graft. Infuse. (Fresh or Cryopreserve)**

Kohn & Kuo, JACI 2017.
Human β-Globin Gene ZFNs and HDR Donors

Human β-globin Gene

ATG

Sickle Mutation
A→T

ZFNs

Pr

1

2

3

pA

Oligo Donor (101 bp)

T/A

SMS

Hhal

RFLP

Hemoglobin HPLC

HbF

HbS

HbA

Hoban et al, Blood, 2015
Gene Correction at the β-globin Locus in CD34+ Cells from Cord Blood

HDR: Has the sickle SNP, and could have other SNPs, but no indels

NHEJ: Indels, one or more, could have SNPs. On Target Indels Only

Outcomes from Editing the Sickle Mutation in the Human Beta-Globin Gene

HBB°/HBB°

Edit with Nuclease and HDR Donor

(HDR → Correct)
(NHEJ → Disrupt)

No Modification  Correct 1  Correct Both  Disrupt 1  Disrupt Both  Correct 1  Disrupt 1

Sickle Cell  Sickle Trait  Normal  Sickle/Thal  Thal Major  Thal Trait

No Effect  ++  +/‐  ‾  ++
NHEJ Occurs Throughout the Cell Cycle, but HDR is Restricted to S/G2 Phases
Conclusions

Advances and improved outcomes are occurring with application of hematopoietic stem cell transplantation for sickle cell disease:

1. Improved unrelated donor transplants by graft manipulation (e.g. CD34-selection, TCR α/β-depletion)

2. Improved haplo-identical (parent/sib) transplants using post-transplant Cytoxan and other immune modulation

3. Improved autologous transplants using gene therapy to correct the sickle mutation, induce fetal globin, etc.
Conclusions

Advances and improved outcomes are occurring with application of hematopoietic stem cell transplantation for sickle cell disease:

BUT NOT FAST ENOUGH........
Support and Funding

UCLA BSCRC, JCCC, HGCTP/SOM, CDI, MIMG/Pediatrics/MMP, SRL/αSCC

ADA: Doris Duke Charitable Foundation; FDA OOPD; NIAID, NIH; CIRM

Sickle Cell: CIRM, DDCF, Hina Patel Foundation, BioMarin

XCGD: CIRM, NHLBI, NIH