Sickle Cell Disease

- 240,000 children born annually in Africa with SCD
  80% die by their second birthday
- Estimated 80,000 affected in USA, 6,900 in Ca.
- 1/400 African American, 1/36,000 Hispanics
- 97% of children in the US survive to age 18
- Average US lifespan ~40 yrs (unchanged 3-4 decades)
Single Amino Acid Change In β-Globin of SCD Leads to Hb Polymerization and RBC Sickling

- Continual low-grade ischemia and episodic exacerbations or “crises”.
- Results in tissue ischemia, organ damage, premature death.

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

The Human β-Globin Gene Complex

--RBC’s have mostly adult HbA (β₂α₂ >90%), but variable amounts of fetal HbF (γ₂α₂ 1-10%).

--Patients with SCD who express >8.6% HbF (i.e. <90% sickle HbS βˢ₂α₂) have milder disease and improved survival.

Lentiβ\textsuperscript{AS3} Vector Expresses “Anti-Sickling” Globin
Prior Work by T. Townes (UAB) (Blood 2003, JBC 2004)

\[ \beta^{\text{AS3}} = \text{Thr87Gln} - \gamma\text{-like “anti-sickling”} \]
\[ \text{Gly16Asp} - \text{increases affinity for } \alpha \]
\[ \text{Glu22Ala} - \text{blocks axial contacts} \]

In sickle cell \textbf{mouse} model, gene transfer/BMT with Lentiβ\textsuperscript{AS3} vector corrected hematologic and systemic disease manifestations.
Clinical Trial of Stem Cell Gene Therapy for Sickle Cell Disease

1. **Autologous Bone Marrow Harvest**
2. **Isolate BM Stem Cells**
3. **Add a Normal B-globin Gene**
4. **Test Cells. Freeze.**
5. **Condition with chemotherapy**
6. **Transplant BM Cells Back to Patient**
7. **Follow: Safety Efficacy**
Stem Cell Gene Therapy for Sickle Cell Disease

This is a prospective, non-controlled, non-randomized Phase I/II clinical trial to assess the safety and efficacy of cellular therapy in patients with sickle cell disease, using autologous bone marrow CD34+ cells transduced ex vivo by the Lenti $\beta^{AS3}$-FB lentiviral vector to express an anti-sickling ($\beta^{AS3}$) gene.
Stem Cell Gene Therapy for Sickle Cell Disease

Perform efficacy studies of vector in BM CD34+ cells from SCD donors to determine whether sufficient $\beta_{AS^3}$-globin gene transfer and expression can be achieved to reverse adverse effects of HbS on RBC properties/physiology.

(Year 1)

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

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

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

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<tr>
<th>Milestone</th>
<th>Goal</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
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<td>1</td>
<td>PRE-IND Meeting with FDA</td>
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<tr>
<td>2</td>
<td>Stem Cell Product Meets Spec</td>
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<td>3</td>
<td>Submit IND</td>
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<td>4</td>
<td>Ready to Open Clinical Trial</td>
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You Are Here
Stem Cell Gene Therapy for Sickle Cell Disease
Disease Team Organizational Plan

Clinical Trial Design:
- Patient Selection
- Clinical End-points

Human Subjects
- Thomas Coates, MD - Co-PI
- Hisham Abdel-Azim MD
- CHLA
- Victor Marder, MD - Co-PI
- Gary Schiller MD
- Satiro De Oliveira, MD
- UCLA
- Mark Walters, MD Site Leader
- CHRCO

Administrative Core
- Donald B. Kohn, M.D.
- Project Manager—Fabrizia Urbinati, PhD
- Clinical Coordinator—Kit Shaw, PhD
- IRB Specialist—Sally Shupien
- Biostatistics—David Gjerston

Advisory Committee
- Robertson Parkman, MD - CHLA
- Edward McCabe, MD/PhD - UCLA
- Elliott Vichinsky, MD - CHRCO
- Tim Townes, PhD - UAB
- Mary Brown, SCDFC

Clinical Trial Design:
- Stem Cell Processing
- Lab End-points

Laboratory Studies
- Hematopoietic Stem Cells
  - Gay Crooks, MD - UCLA
  - Elizabeth J.Read, MD - UCSF
- Lentiviral Vector
  - Donald Kohn, MD – UCLA
  - Kenneth Cornetta MD - IUPUI
- Hemorheology
  - Herbert Meiselman, SCD - USC

Bone Marrow Samples
From Sickle Cell Patients

University of California, Los Angeles (UCLA); Children's Hospital Los Angeles (CHLA)
University of Southern California (USC); Children's Hosp & Research Center, Oakland (CHRCO)
Indiana University-Purdue University-Indiana (IUPUI);
**In Vitro Erythroid Differentiation of Human CD34+ Cells To Assess Expression of β^{AS3} in Human RBC**

**End-point Assays:**
- CFU (#, types, % PCR(+)) from day 2
- Cell numbers, immunophenotype
- Gene Transfer (VCN by qPCR)
- Hb^{AS3} production (IEF, qRT-PCR)
- RBC morphology (sickling)
Hemoglobin Types Expressed in RBC Made *In Vitro* from SCD BM CD34+ Cells

### Hemoglobin electrophoresis

<table>
<thead>
<tr>
<th>Hb Type</th>
<th>AA 6</th>
<th>Charge</th>
<th>AA 16</th>
<th>Charge</th>
<th>AA 22</th>
<th>Charge</th>
<th>AA 87</th>
<th>Charge</th>
<th>Net Total Charge</th>
<th>X2 β-chains/ Hb Tetramer</th>
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<tbody>
<tr>
<td>HbA</td>
<td>Glu</td>
<td>-1</td>
<td>Gly</td>
<td>0</td>
<td>Glu</td>
<td>-1</td>
<td>Thr</td>
<td>0</td>
<td>-2</td>
<td>-4</td>
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<tr>
<td>HbS</td>
<td>Val</td>
<td>0</td>
<td>Gly</td>
<td>0</td>
<td>Glu</td>
<td>-1</td>
<td>Thr</td>
<td>0</td>
<td>-1</td>
<td>-2</td>
</tr>
<tr>
<td>HbAS3</td>
<td>Glu</td>
<td>-1</td>
<td>Asp</td>
<td>-1</td>
<td>Ala</td>
<td>0</td>
<td>Gln</td>
<td>0</td>
<td>-2</td>
<td>-4</td>
</tr>
</tbody>
</table>

### Hemoglobin Types Made In Vitro from SCD BM CD34+ Cells

- Std. +
- Mock: 22.8%
- 1.5 vcn: 16.6%
- 1.3 vcn: 15.5%
- 0.9 vcn: 9.3%

### Lenti/βAS3 VCN:
- 0.4
- 1.0
- 1.6
- 1.4
- 1.0
- 0.6
- 1.6
- 1.0

### % βAS3 / VCN:
- 18.8
- 16.6
- 14.8
- 14.7
- 20.4
- 22.0
- 15.9
- 17.9

### Age of donor:
- 12
- 12
- 17
- 17
- 17
- 8
- 8
- mix

**Average 18% HbAS3 of total Hb / VCN**
Summary of Pre-clinical Data

- Optimized LV transduction of SCD BM CD34+ cells to obtain the desired VCN (0.5-3 VCN) and % transduced CD34+ cells (>30%).

- Assessed *in vitro* hematologic potential of SCD-BM CD34+ cells transduced with βAS3 LV vector (not impaired)

- Obtained sufficient level of βAS3-globin expression in SCD BM CD34+ cells *in vitro* (average 18% AS3/total globin by IEF and 14% by qRT-PCR per VCN) and *in vivo* in NSG mice BM transplant (average 20%AS3/total globin per VCN).

- Evaluated morphologic correction (up to 25% corrected Sickle RBC)

- Preliminary results showing lack of insertional oncogenesis by gene modification by βAS3 LV vector (IVIM)
Key Goals for Years 3-4

- Finalize Clinical-Scale Cell Processing Methods. SOP
- Perform In Vitro and In Vivo Toxicology Studies
- Complete clinical trial protocol and associated documents
- Undergo regulatory reviews (IRB, IBC, NIH RAC, FDA IND)
- Produce clinical-grade vector, obtain other essential reagents (cytokines, etc)
- Apply for clinical trial grant
U.C.L.A. Stem Cell Gene Therapy Group

Funding:
- ADA - FDA, NHLBI, NIAID
- SCD - CIRM
- ZFN - NHLBI, DDCF
- MART - NCI

Home and Support:
- BSCRC
- JCCC
- DGSOM-HGMP
- MIMG
- Pediatrics

CIRM Disease Team Collaborators
- UCLA - Victor Marder, Gay Crooks
- CHLA - Tom Coates
- USC - Herb Meiselman
- CHRCO - Mark Walters
- UCSF - E.J. Read
- IUPUI - Ken Cornetta
Eligible subjects will be:

1. adults age 18 or older at the time of enrollment
2. diagnosis of SCD (SS or S/βthal°)
3. lacking a medically eligible HLA-identical sibling donor or a 10/10 allele-matched adult unrelated donor and
4. meeting defined inclusion/exclusion criteria.

Per FDA pre-IND – limit enrollment to subjects who do not show improvement from HU
Primary Objective

Examine the safety of the lentiviral-mediated gene transfer in patients who receive the Lenti $\beta^{AS3}$-FB–transduced CD34+ cells through autologous bone marrow transplants (BMT) after myeloablative conditioning with Busulfan.

The primary endpoints for this objective include identification of grade III/IV procedure-related severe adverse events (SAEs) and estimation of event free-survival by 24 months, where failure is defined by occurrence of grade III/IV SAE, or one of the following endpoints: 1) death or 2) need for performance of an allogeneic BMT due to non-engraftment.
1. Assess the efficacy of stem cell transduction/engraftment through serial examination of peripheral blood cells for percentages of cells containing anti-sickling ($\beta^{AS3}$) vector using quantitative PCR (qPCR).

2. Quantify $\beta^{AS3}$-globin gene expression by Isoelectric Focusing, qRT-PCR.

3. Examine the effects of $\beta^{AS3}$-globin gene expression on the sickling of the red blood cells by evaluating RBC function/rheology in vitro.

4. Determine the effect of $\beta^{AS3}$-globin gene expression on clinical and laboratory manifestations of sickle cell disease.