Understanding the Complexities of Patient Selection, Enrollment, and the Consent Process: Gene Therapy for Sickle Cell Disease

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Bone marrow stem cells produce all types of blood cells for the life of a patient.

We have sought to develop curative strategies based upon replacing or repairing bone marrow stem cells.
Bone marrow transplant strategies for SCD

1. Allogeneic transplantation
   Bone marrow transplant from someone who does not have SCD
   Donor is usually an HLA-matched sibling, but could include cord blood, matched unrelated, or half-matched family member

2. Autologous gene therapy
   Bone marrow transplant from patient’s own bone marrow
   β-globin gene transfer with an engineered virus to transfer or gene editing with an engineered endonuclease

Sickle cell disease patients
Comparison between NIH transplant results and mathematical modeling demonstrates that only 20% donor level needed and is dependent only on red blood cell life span differences

\[ f_M = \frac{f_P t_D}{f_P t_D + (1 - f_P) t_H} \]

In our model the fraction of mature donor erythrocytes in the periphery \( f_M \) is a function of Progenitor chimerism, \( f_P \), Donor and recipient erythrocyte half-lives, \( t_D \) and \( t_H \), respectively.

Can we achieve this modest 20% correction level with gene addition using the patients’ own bone marrow HSCs?

Fitzhugh, Cordes et al, Blood, 2017 Oct 26;130(17):1946-1948
1. How do we introduce the experimental vector?

Patients with sickle cell disease when confronted with our experimental trial that employs HIV as the delivery vector may think back on Tuskegee experiments.
2. How do we determine that the first trial patients get a potentially therapeutic dose?
HGB-206: Further evolution of the protocol allowed refinements during the course of the study.

**HSC collection**  
*Bone marrow harvest or mobilization with plerixafor & apheresis*

**Busulfan myeloablative conditioning**

**DP infusion**

**Transduced HSCs engraft and contribute to reconstitution of functional RBCs**

2-yr follow-up  
**Long-Term Follow-Up Study**

**Key Enrollment Criteria**

- 18+ years of age
- History of symptomatic SCD
- Adequate organ function
- No previous HSCT or gene therapy

**Study Objectives**

- Primary objective: Safety
- Key Secondary Objectives:
  - Frequency of VOCs and ACS
  - Total Hb and Hb fractions
  - Vector copies in peripheral blood

**Group A**  
- Pre-collection transfusion regimen: Optional
- HSC source: Bone marrow
- Manufacturing process: Original

**Group B**  
- Pre-collection transfusion regimen: Required
- HSC source: Bone marrow
- Manufacturing process: Refined

**Group C**  
- Pre-collection transfusion regimen: Required
- HSC source: Mobilized PB
- Manufacturing process: Refined

**LentiGlobin DP centralized manufacturing**

*Select CD34+ cells*  
*Transduce with BB305 lentiviral vector*  
*Cryopreserve, test, release DP*
HGB-206: Refinements to manufacturing and cell harvesting improved product characteristics

**Vector copy number**

<table>
<thead>
<tr>
<th>Group</th>
<th>N = 7</th>
<th>N = 2</th>
<th>N = 9</th>
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<tbody>
<tr>
<td>A</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>3.1‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>3.8</td>
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**% Transduced cells**

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<tbody>
<tr>
<td>A</td>
<td>25</td>
<td>87‡</td>
<td>81</td>
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**CD34+ cell dose**

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<tbody>
<tr>
<td>A</td>
<td>2.1</td>
<td>2.7</td>
<td>6.5</td>
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</table>

1 Number of DP exceeds number of patients since some patients were harvested or mobilized more than once; 
2 % Transduced cells not available for 1 DP at time of analyses;  † Group B DP lot was made using original manufacturing process, while the other 3 DP lots were made using refined manufacturing process

BM, bone marrow; DP, drug product; HSC, hematopoietic stem cell; PB, peripheral blood; VCN, vector copy number
HGB-206: Gene therapy-derived hemoglobin mirrors the carrier state at ≥ 3 months in Group C

At 3 months study visit

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<th>Group</th>
<th>Total Hb</th>
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<tbody>
<tr>
<td>A</td>
<td>9.2</td>
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<tr>
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<td>2</td>
</tr>
<tr>
<td>C</td>
<td>11.0</td>
<td>7</td>
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</table>

At 6 months study visit

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<tr>
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<td>8.9</td>
<td>7</td>
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<tr>
<td>B</td>
<td>11.5</td>
<td>2</td>
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<tr>
<td>C</td>
<td>11.7</td>
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At 9 months study visit

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</thead>
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<td>7</td>
</tr>
<tr>
<td>B</td>
<td>11.6</td>
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<tr>
<td>C</td>
<td>12.6</td>
<td>2</td>
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% represent median Hb fractions as % of total; Hb, hemoglobin
Further issues that arose along the course of the first gene therapy trial for sickle cell disease

3. How do we enroll pre-symptomatic patients?

4. How do we follow up with participants on the results of clinical trials?

5. What are the scientific and clinical reasons a patient might be precluded from gene therapy trials after participating in a trial for a gene therapy investigational agent?
Autologous bone marrow stem cell-targeted gene editing

Harvest

Patients’ own bone marrow stem cells

Reactivate fetal hemoglobin by cutting repressor genes or correct the mutation by cutting and repairing

Transplantation back to patients

SCD patients

- Patients serve as their own donor
- Available for all patients
- No need for immunosuppression
- No risk of GVHD
CRISPR/Cas9 system for genome editing

• Arose from basic science studies of bacteria
• Achieves targeted disruption of genomes with enzyme + guide RNA
  – Initial approaches to create double strand breaks
  – Can serve a “find and replace” function when delivered with template DNA
• Has revolutionized basic molecular biology due to accuracy and ease of use
• Paves the way for new therapeutics
CRISPR/Cas9 system for genome editing, just a click away....
CRISPR/Cas9 system for genome editing

Genomic DNA break

Sickle mutation

Promoter

Donor DNA

Promoter

Correct gene

NHEJ: Non-homologous end joining “Break”

HDR: Homology directed repair “Fix”

β-globin gene

1. DNA repair

Sickle mutation

Promoter

Correct gene

2. Indel (NHEJ)

Deletion

Promoter

3. Gene correction (HDR)

Correct gene

Promoter

Single guide RNA
Gene correction with CRISPR/Cas9 in SCD bone marrow stem cells

Guide RNA targeting the β-globin gene
Cas9 mRNA or Cas9 protein
Donor ssDNA: 80, 120, or 200 µg/ml

SCD CD34+ cells → Electroporation → Grow red blood cells in flasks → Electrophoresis
RP-HPLC
SNP PCR
Targeted sequence

Colony assay → Targeted sequence
~30% of gene correction evaluated by DNA sequencing

The chart shows the percentage of WT, SCD, and Indel for different treatments of Donor DNA (µg/ml) at 80, 120, 200, and 200 µg/ml. The treatments include No electroporation, Cas9 mRNA, and Cas9 protein. The chart indicates that the percentage of WT is highest, followed by SCD, and finally Indel.
Robust hemoglobin formation following red blood cell production

<table>
<thead>
<tr>
<th>SCD CD34+ cell gene correction</th>
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<tbody>
<tr>
<td>No electro poration</td>
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[Image of three centrifuge tubes with liquid and red drops]
High-efficiency gene correction from $\beta_s$-globin to $\beta$-globin

Electrophoresis after erythroid differentiation of CD34+ cells with electroporation

<table>
<thead>
<tr>
<th></th>
<th>No electroporation</th>
<th>Cas9 mRNA</th>
<th>Cas9 protein</th>
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<tbody>
<tr>
<td>Donor DNA (µg/ml)</td>
<td></td>
<td>80</td>
<td>120</td>
</tr>
<tr>
<td>HbC</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HbS</td>
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<td></td>
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<td></td>
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<tr>
<td>HbA</td>
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Cas9 mRNA and Cas9 protein were added to the cultures to facilitate gene correction.
Detection of normal globin peaks in red blood cells by HPLC

- No electroporation
- Gene correction: 120 µg/ml DNA
- Gene correction: 200 µg/ml DNA

SCD CD34+ cell gene correction

- βS-globin
- Aγ-globin
- Gγ-globin
~70% β-globin production in gene-corrected red blood cells *in vitro*

Control: no electroporation
A CRISPR focus on attitudes and beliefs toward somatic genome editing from stakeholders within the sickle cell disease community

Anitra Persaud, BA1, Stacy Desine, BA1, Katherine Bilzinsky, PhD1,2,3, and Vence L. Bonham, JD1

INTRODUCTION
One of the first targets of CRISPR-mediated somatic genome editing will likely be sickle cell disease (SCD, OSMIM 60305). SCD affects millions of people, particularly those in regions where malaria is highly prevalent, such as sub-Saharan Africa, India, and the Mediterranean. SCD is caused by a single pathogenic variation (A→T) in the sixth codon of the β-globin gene. Affected individuals inherit two abnormal copies of the gene, resulting in the production of misfolded hemoglobin. This diminishes the oxygen carrying capacity of erythrocytes, resulting in medical complications, including pain crises, strokes, pulmonary hypertension, leg ulcers, priapism, and acute chest syndrome. Despite being identified over a century ago and posing a significant global health burden, those living with SCD have limited treatments available to them. Hematopoietic stem cell transplantation (HSCT) remains the only nonexperimental cure for SCD. However, while the event-free survival rate of HSCT exceeds 90%, few patients can access this curative therapy due in part to stringent eligibility criteria. Further, while the life expectancy of the general adult SCD population has increased over the past 40 years, premature death continues.

Because SCD is a well-studied molecular disorder impacting the blood system, it comprises an ideal candidate for gene editing therapies, with different approaches under current investigation. One mechanism involves promoting fetal hemoglobin (HbF) levels, which can reduce the disease’s severity by inhibiting HbS polymerization. However, HbF expression is typically suppressed after birth. Genome editing can be used to dectivate the B-cell lymphoma/leukemia 11a (BCL11A) transcription factor promoter, allowing HbF to persist. Other researchers have displayed proof of principle success in removing hematopoietic stem and progenitor cells (HSPC) from the bone marrow, correcting the pathogenic variation itself with CRISPR, and repopulating the bone marrow with the edited cells.

Given these preliminary results, clinical trials are soon expected. On 13 September 2018, the National Heart, Lung,

Motivators included hope in technology, altruism, shortcomings of current treatment, increased awareness of the importance of clinical trials

Deterrents included uncertainty about consequences, permanence of change, trial burden, mistrust, reproductive risk, cost, lack of access

Mediators included religiosity, capacity to manage disease and life

Information desired included specific details, expected interpatient variability, optimal timing, track record of treatment

Educational video, 2-part survey, 15 moderated focus groups in 7 U.S. cities
“Lest there be any doubt, and as we have stated previously, NIH does not support the use of gene-editing technologies in human embryos.”
1. Sickle cell disease is a single-gene disorder.

2. Clinical trials have established bone marrow transplant as a one time cure for SCD.
   • Bone marrow transplantation can cure >90% of SCD patients.

3. Gene therapy trials are open at NIH.
   • Early results demonstrate efficacy with gene addition

4. Gene editing trials are now being developed.

5. Access to and participation in clinical trials should improve the outlook for patients with SCD.