Gene Therapy and Gene Editing as a Treatment Option for Patients with Hemoglobinopathies

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Misty Evans, APRN, CPNP-AC, BMTCN
Conflict of Interest

• Haydar Frangoul has the following disclosures:
  o Vertex Pharmaceutical (Consulting)
• Misty Evans has no disclosures.
Objectives

- Provide a general overview of gene therapy.
- Review the mechanism of disease correction using viral vector to treat sickle cell disease and thalassemia.
- Discuss gene therapy for the treatment of sickle cell disease and thalassemia.
- Discuss the use of CRISPR-Cas9 gene editing for the treatment of sickle cell disease and thalassemia.
- Review the outcomes of gene therapy and gene editing trials.
Why Gene Therapy

• The AMA reports that around 4000 diseases have been linked to gene disorders (including blood diseases, cancers, cystic fibrosis, Parkinson’s and Alzheimer’s diseases)

• This therapy can potentially help millions of people

• The field is years from making this therapy commercially available to many patients
What is Gene Therapy

• The AMA describes gene therapy as “a novel approach to treat, cure or ultimately prevent disease by changing the expression of person’s genes”

• Gene therapy works by addressing defective genes
  o Repairing
  o Deactivating “turning off”
  o Replacing
How Does Gene Therapy Work

• Gene therapy involves the administration of specific genetic material (i.e., DNA or RNA) via a carrier, known as a “vector,” that enables the foreign genetic material to enter the target cells.

• Most gene therapies use modified versions of natural viruses as vectors, as they are an efficient way of introducing DNA or RNA into a cell.

Gonzalez A et al. hem Onc Clin N Am 2019
How Does Gene Therapy Work

• The gene therapy agent can be injected into the body (in vivo gene therapy) or used to modify cells taken from the body, which are then re-infused (ex vivo gene therapy)

• Replacement gene therapy aims to:
  o Provide a working copy of the damaged gene(s)
  o Boost the availability of a disease-modifying gene
  o Suppress the production of a damaged gene

Gonzalez A et al. hem Onc Clin N Am 2019
In Vivo Gene Therapy

• Involves direct injection of the gene therapy agent into the body.
• Depending upon the vector and the target, in vivo gene therapy can be administered intravenously, injected into the muscles, infused or injected into an organ or bodily structure, or injected directly into a tumor.

Shim G et al. Acta Phar 2017
Ex Vivo Delivery System

- Cells are harvested (autologous or allogeneic). They are then modified using genetic engineering tools outside the body and purified, enriched, and/or activated before being transplanted back into the patient.
- These modified cells then further replicate and spread in the body.
In vivo gene editing

Ex vivo gene editing

Viral vector
Liposome
Polymers
Peptides

Isolation

Target cells

Non-viral delivery

Viral delivery
Electroporation
Liposome
Polymers

Gene edited cells

Shim G et al. Acta Phar 2017
# Viral Vectors

<table>
<thead>
<tr>
<th></th>
<th>Adeno-Associated Virus</th>
<th>Adenovirus</th>
<th>Retrovirus/Lentivirus</th>
<th>Herpesvirus</th>
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</thead>
<tbody>
<tr>
<td>Genome</td>
<td>ssDNA</td>
<td>dsDNA</td>
<td>ssRNA</td>
<td>dsDNA</td>
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<tr>
<td>Allowable size of foreign DNA</td>
<td>~5 kb</td>
<td>7.5 kb</td>
<td>8 kb</td>
<td>&gt; 25 kb</td>
</tr>
<tr>
<td>Type of cells targeted</td>
<td>Nondividing cells</td>
<td>Nondividing cells</td>
<td>Dividing and nondividing cells</td>
<td>Dividing and nondividing cells</td>
</tr>
<tr>
<td>Integration into genome</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Duration of expression</td>
<td>Potentially long duration</td>
<td>Transient</td>
<td>Long duration</td>
<td>Potentially long duration</td>
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<tr>
<td>Immunogenicity</td>
<td>Presence of antibodies varies by serotype</td>
<td>Antibodies prevalent</td>
<td>Used ex vivo</td>
<td>Antibodies prevalent</td>
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</tbody>
</table>
History of Gene Therapy

1968
Successful allogeneic HSCT in SCID-X1 and WAS

1983
Successful retroviral gene transfer to murine HSCs

1990-1996
First attempted HSC gene therapy in CGD

1997
Successful HSC gene therapy in SCID-X1 and ADA-SCID

2000-2002
First report of LTR-mediated insertional mutagenesis leading to leukaemia

2003
Development of SIN gammaretroviral and lentiviral vector systems for application to PID

2013-2015
Successful SIN gammaretroviral and lentiviral gene therapy in several PID

2016
First licensed ex vivo gene therapy (StrimvelisTM for ADA-SCID)

2017
Gene Therapy for Hemoglobinopathies
Hemoglobin

- Hemoglobin (Hb), the protein that carries oxygen from the lungs to the tissues, is a tetramer composed of 2 α-like globin chains and 2 β-like globin chains.

Sankaran VG et al. Cold Spr Presp 2013
In humans, β-like globin genes are sequentially expressed throughout development.

Cavazzana et al. *Molecular Ther* 2017
Beta Thalassemia

• β-Thalassemia is caused by more than 200 different β-globin gene mutations that reduce or abrogate production of the β-globin chains.

• Severe β-Thalassemia results in the following:
  o ineffective erythropoiesis
  o intramedullary apoptosis of erythroid precursors
  o hemolytic anemia
  o severe anemia
  o iron overload
  o hepatosplenomegaly
  o cardiopathies and endocrine disorders
Treatment of Beta Thalassemia

• Allogeneic bone marrow transplant remain the only curative option.

• Results in patients with HLA identical siblings are excellent, recent data using unrelated donors is encouraging.

• Gene therapy is an appealing alternative to transplant
  - Use autologous stem cells
  - Avoid risk of GVHD

Taher AT et al. Blood 2018
Gene Therapy for Beta Thalassemia

• First report in 18-year-old with $\beta^0/\beta^E$ transfusion dependent since age 3 years.

• Used a self-inactivating Lentiviral vector. It encodes a mutated adult $\beta$-globin ($\beta^{A(T87Q)}$).

• Patient received conditioning with myeloablative doses of busulfan (3.2 mg/kg) for 4 days

Cavazzana-Cavo M et al. *Nature* 2010
Gene Therapy for Beta Thalassemia

Cavazzana-Cavo M et al. *Nature* 2010
Gene Therapy for Beta Thalassemia

Cavazzana-Cavo M et al. Nature 2010
Gene Therapy for Beta Thalassemia

- Further improvements in the lentiviral vector leading to greater transduction efficiency and higher vector copy numbers

- Report on 22 patients ages (12-35).
  - 9 patients with $\beta^0/\beta^0$
  - 9 patients with $\beta^E/\beta^0$
  - 4 other

- LentiGlobin BB305 vector, which encodes adult hemoglobin (HbA) with a T87Q amino acid substitution (HbA$^{T87Q}$)

Thompson AA et al. *NEJM* 2018
Gene Therapy for Beta Thalassemia

- Patients mobilized with GCSF+Plerixafor

- Conditioning regimen was with daily busulfan with daily dosing AUC of 4000 μM.

- Median CD34 cell dose infused is $8.1 \times 10^6$/Kg
Gene Therapy for Beta Thalassemia

Results:

• 13 patients with non- β⁰/β⁰
  o 12 are transfusion independent
  o Median hemoglobin A^{T87Q} was 6.0 g/dL. Median total hemoglobin 11.2 d/dL

• 9 patients with β⁰/β⁰
  o 6 are transfusion dependent median hemoglobin A^{T87Q} was 4.2 g/dL. (74% less transfusions)
  o 3 are transfusion independent median hemoglobin A^{T87Q} was 6-8 g/dL

Thompson AA et al. NEJM 2018
Sickle Cell Disease

- One of the most prevalent inherited blood disorders worldwide.
- β-globin gene that causes polymerization of hemoglobin S resulting in stiff, sickle shaped hemoglobin that cause blockages and damage organs/tissue.
- Associated with significant mortality and morbidity:
  - Vaso-occlusive pain crisis
  - Splenic sequestration
  - Acute chest syndrome
  - Hemolytic crisis
  - Infection
  - Priapism
  - Stroke
  - Organ damage
Sickle Cell Disease Treatment

- Hydroxyurea is the mainstay of management to reduce complications
- Voxelotor, crizanluzumab, L-glutamine are alternative options
- Allogeneic bone marrow transplant is the only curative option.
- Patients with HLA identical siblings have excellent outcomes
- Haplo-identical transplantation for those without fully matched donors
- Gene therapy offers an alternative to transplant
  - Use autologous stem cells
  - Avoid risk of GVHD
Gene Therapy for Sickle Cell Disease

- One patient treated using identical approach to the thalassemia trial
- Stem cell source was bone marrow (harvested twice)
- Patient received busulfan conditioning regimen
- Engrafted neutrophils at day 38 and platelets at day 88

Ribeil et al. NEJM 2017
Gene Therapy for Sickle Cell Disease

Ribeil et al. NEJM 2017
Gene Therapy for Sickle Cell Disease

• Same group enrolled 22 patients with Sickle Cell Disease and preliminary results are encouraging

• Main changes include using plerixafor mobilized PBSC
  o Shorter hospital stay
  o More rapid engraftment

• Higher stem cell dose translated in improved hemoglobin

Walters MC et al. TCT abstract 2019
# Gene Therapy for Sickle Cell Disease

### Table 1. DP and Treatment Characteristics

Values are presented as median (min-max)

<table>
<thead>
<tr>
<th></th>
<th>Group A (n=7)</th>
<th>Group B (n=2)</th>
<th>Group C (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Dose (x 10⁶ CD34+ cells/kg)</td>
<td>2.1 (1.6–5.1)</td>
<td>2.7 (2.2–3.2)</td>
<td>7.1 (3.0–8.0)</td>
</tr>
<tr>
<td>DP VCN (copies/diploid genome)</td>
<td>0.6 (0.3–1.3)</td>
<td>3.1 (1.4–5.0)</td>
<td>4.0 (2.8–5.6)</td>
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<tr>
<td>Transduced Cells (%)</td>
<td>25 (8–42)</td>
<td>87 (46–95)</td>
<td>81 (78–88)</td>
</tr>
<tr>
<td>Neutrophil engraftment (days)</td>
<td>22 (17–29)</td>
<td>26 (23–28)</td>
<td>19 (18–20)</td>
</tr>
<tr>
<td>Platelet engraftment (days)</td>
<td>56 (29–63)</td>
<td>46 (31–61)</td>
<td>28 (12–64)*</td>
</tr>
<tr>
<td>Hospitalization time from conditioning to discharge (days)</td>
<td>37 (29–54)</td>
<td>41 (36–46)</td>
<td>34 (30–65)</td>
</tr>
<tr>
<td>Follow-up (months)</td>
<td>24.2 (22.8–32.9)</td>
<td>11.4 (8.5–14.3)</td>
<td>3 (1.2–6.0)</td>
</tr>
</tbody>
</table>

Walters MC et al. *TCT abstract* 2019
Gene Editing
Gene Editing

- Definition: Group of technologies that give scientists the ability to change an organism's DNA. These technologies allow genetic material to be added, removed, or altered at particular locations in the genome.

- These technologies include the zinc fingers nucleases (ZFN) and the transcription activator-like effector nucleases (TALENS) and more recently, the clustered regularly interspaced short palindromic repeats (CRISPR) with Cas9 nuclease system.
Gene Editing Using CRISPR-Cas9

• The CRISPR-Cas9 system has generated a lot of excitement in the scientific community because it is faster, cheaper, more accurate, and more efficient than other existing genome editing methods.

• This technology allows molecular ‘cut and paste’ approach to gene correction.
Gene Editing Using CRISPR-Cas9

• CRISPR-Cas9 was adapted from a naturally occurring genome editing system in bacteria.

• The bacteria capture snippets of DNA from invading viruses and use them to create DNA segments known as CRISPR arrays.

• The CRISPR arrays allow the bacteria to "remember" the viruses (or closely related ones).

Gammon K et al, *Nature* 2014
[https://www.broadinstitute.org](https://www.broadinstitute.org)
Gene Editing Using CRISPR-Cas9

- If the viruses attack again, the bacteria produce RNA segments from the CRISPR arrays to target the viruses' DNA.

- The bacteria then use Cas9 enzyme to cut the DNA apart, which disables the virus.

Gammon K et al, *Nature* 2014
https://www.broadinstitute.org

Srivastava et al, *Hematologica* 2017

Gammon K et al, *Nature* 2014
https://www.broadinstitute.org
Srivastava et al, *Hematologica* 2017
Clinical Application of Gene Editing Using CRISPR-Cas9

Chinese Scientist Claims to Use Crispr to Make First Genetically Edited Babies

New York Times 2019
Gene Editing Using CRISPR-Cas9 to Treat Hemoglobinopathies

Wienert B et al, Trends in Gen 2018
Protective Effect of Fetal Hemoglobin

• Janet Watson in 1948 observed that the blood of young children with SCD showed less sickling and attributed this to the residual HbF that exists in early life.

• Adults affected by SCD express unusually high levels of HbF and that these individuals present with a much milder course of SCD

• The phenomenon was named hereditary persistence of fetal hemoglobin (HPFH)

Watson J, Am J of Med 1948
Jacob GF et al. Br J of Hem 1958
Protective Effect of Fetal Hemoglobin

• In 2007, a series of genome-wide association studies (GWAS) identified BCL11A gene as a modulator of HbF levels

• Targeted deletion of BCL11A gene by CRISPR-Cas9 system for fetal hemoglobin reactivation

• Khosravi et using CRISPR-Cas9 genome-editing strategy they deleted a 200bp genomic region within the human erythroid-specific BCL11A resulting strong induction of γ-hemoglobin expression

Menzel Set al. Nat Gen 2007
Wu Y et al. nat Med 2019
Khosravi MA et al. Eur J Phar 2019
Gene Editing Using CRISPR-Cas9 for patients with Sickle Cell Disease and Thalassemia Major

• "A Safety and Efficacy Study Evaluating CTX001 in Subjects With Severe Sickle Cell Disease" (NCT03745287)

• "A Safety and Efficacy Study Evaluating CTX001 in Subjects With Transfusion-Dependent β-Thalassemia" (NCT03655678)

• The studies are evaluating the safety and efficacy of autologous CRISPR-Cas9 Modified CD34+ Human Hematopoietic Stem and Progenitor Cells (hHSPCs) using CTX001.

https://www.clinicaltrial.gov
CTX001 Infusion Process

Stage 1

Screening

Stage 2

Blood stem cells collected

Cells returned ready for use

Stage 3

Preparative chemotherapy (busulfan)

CTX001 infusion

Stage 4

Engraftment and discharge

Follow-up

Central manufacturing facility

CRISPR-Cas9 editing

Cells frozen and tested for safety

- Patients are monitored for stem cell engraftment, hematopoietic recovery, adverse events (AEs), hemoglobin (Hb) production, hemolysis, HbF and F-cell expression, packed red blood cell (pRBC) transfusion requirements (TDT), and VOCs (SCD)
# TDT Patient Baseline and Treatment Characteristics

## Patient baseline

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>β₀ / β⁺ (IVS-I-110)</td>
<td>β₀ / β⁺ (IVS-II-745)</td>
</tr>
<tr>
<td>Age at consent, years</td>
<td>19</td>
<td>26</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Pre-study pRBC transfusions&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Units /year</td>
<td></td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>61</td>
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</table>

## Treatment characteristics

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell dose, CD34+ cells/kg</td>
<td>17.0×10⁶</td>
<td>12.3×10⁶</td>
</tr>
<tr>
<td>Neutrophil engraftment&lt;sup&gt;b&lt;/sup&gt;, Study Day&lt;sup&gt;c&lt;/sup&gt;</td>
<td>33</td>
<td>36</td>
</tr>
<tr>
<td>Platelet engraftment&lt;sup&gt;d&lt;/sup&gt;, Study Day&lt;sup&gt;c&lt;/sup&gt;</td>
<td>37</td>
<td>34</td>
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## TDT: Adverse Events

<table>
<thead>
<tr>
<th></th>
<th>TDT patient 1</th>
<th>TDT patient 2</th>
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<tbody>
<tr>
<td><strong>Screening to CTX001 infusion</strong></td>
<td></td>
<td></td>
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<tr>
<td>AEs</td>
<td>12</td>
<td>8</td>
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<tr>
<td>Serious AEs</td>
<td>0</td>
<td>0</td>
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<tr>
<td><strong>Post CTX001 infusion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AEs</td>
<td>32</td>
<td>34</td>
</tr>
<tr>
<td>Serious AEs</td>
<td>2(^a)</td>
<td>2(^b)</td>
</tr>
<tr>
<td>Weeks of follow-up</td>
<td>66.6</td>
<td>24.7</td>
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<tr>
<td><strong>AE relationship</strong>(^c)</td>
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<tr>
<td>Related to filgrastim only</td>
<td>4(^d)</td>
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<tr>
<td>Related to plerixafor and filgrastim</td>
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<td>2</td>
</tr>
<tr>
<td>Related to busulfan only</td>
<td>8(^e)</td>
<td>15(^f)</td>
</tr>
<tr>
<td>Related to CTX001 only</td>
<td>0</td>
<td>1(^g)</td>
</tr>
<tr>
<td>Related to busulfan and CTX001</td>
<td>0</td>
<td>3(^h)</td>
</tr>
<tr>
<td>Not related to any study drug</td>
<td>32</td>
<td>19</td>
</tr>
</tbody>
</table>

AEs were generally consistent with myeloablation and autologous stem cell transplant.
HbF Levels Maintained >10 g/dL Starting at Month 4 in Both TDT Patients

Hb fractionation over time (Hb [g/dL])

**Patient 1**

<table>
<thead>
<tr>
<th>Months post-CTX001 infusion</th>
<th>Baseline</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbF</td>
<td>9.0</td>
<td>12.0</td>
<td>11.6</td>
<td>12.1</td>
<td>12.0</td>
<td>12.3</td>
<td>11.9</td>
<td>12.7</td>
<td>14.2</td>
<td></td>
</tr>
<tr>
<td>HbA</td>
<td>0.3</td>
<td>6.5</td>
<td>8.4</td>
<td>10.1</td>
<td>10.2</td>
<td>10.4</td>
<td>10.1</td>
<td>12.4</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td>HbA2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
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<td>0.1</td>
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**Patient 2**

<table>
<thead>
<tr>
<th>Months post-CTX001 infusion</th>
<th>Baseline</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbF</td>
<td>10.1</td>
<td>9.8a</td>
<td>11.5</td>
<td>12.9</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>HbA</td>
<td>0.0</td>
<td>5.1</td>
<td>9.5</td>
<td>12.5</td>
<td>12.2</td>
<td></td>
</tr>
<tr>
<td>HbA2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

HbA, adult hemoglobin

*aTotal Hb from local laboratory and Hb fraction from central laboratory; *bHb adducts and other variants
Both TDT Patients Have Stopped Receiving pRBC Transfusions

- **Patient 1**: Pre-study RBC transfusions: 34 units/year. 14.2 months post-CTX001 infusion. Total Hb at last visit: 14.2 g/dL.

- **Patient 2**: Pre-study RBC transfusions: 61 units/year. 3.5 months post-CTX001 infusion. Total Hb at last visit: 12.5 g/dL.

- **Legend**:
  - Light grey: Patient receiving pRBC transfusions
  - Dark grey: Patient not receiving pRBC transfusions
**SCD Patient Baseline and Treatment Characteristics**

<table>
<thead>
<tr>
<th>Patient baseline</th>
<th>Treatment characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>Cell dose, CD34+ cells/kg</td>
</tr>
<tr>
<td>βS / βS</td>
<td>3.3×10^6</td>
</tr>
<tr>
<td>Age at consent, years</td>
<td>Neutrophil engraftment^c, Study Day^d</td>
</tr>
<tr>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>Gender</td>
<td>Platelet engraftment^e, Study Day^d</td>
</tr>
<tr>
<td>Female</td>
<td>30</td>
</tr>
<tr>
<td>Pre-study VOCs, VOCs/year^b</td>
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</tr>
<tr>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

**Patient baseline**

- **Genotype**: βS / βS
- **Age at consent, years**: 33
- **Gender**: Female
- **Pre-study VOCs, VOCs/year^b**: 7

**Treatment characteristics**

- **Cell dose, CD34+ cells/kg**: 3.3×10^6
- **Neutrophil engraftment^c, Study Day^d**: 30
- **Platelet engraftment^e, Study Day^d**: 30
# SCD: Adverse Events

<table>
<thead>
<tr>
<th>n</th>
<th>SCD patient</th>
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<tbody>
<tr>
<td><strong>Screening to CTX001 infusion</strong></td>
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<tr>
<td>AEs</td>
<td>35</td>
</tr>
<tr>
<td>Serious AEs</td>
<td>11</td>
</tr>
<tr>
<td><strong>Post CTX001 infusion</strong></td>
<td></td>
</tr>
<tr>
<td>AEs</td>
<td>91&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serious AEs</td>
<td>3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weeks of follow-up</td>
<td>45.1</td>
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</table>

<table>
<thead>
<tr>
<th>AE relationship&lt;sup&gt;c&lt;/sup&gt;</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Related to plerixafor only</td>
<td>6</td>
</tr>
<tr>
<td>Related to busulfan only</td>
<td>21&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>Related to CTX001 only</td>
<td>0</td>
</tr>
<tr>
<td>Related to busulfan and CTX001</td>
<td>5&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Not related to any study drug</td>
<td>94</td>
</tr>
</tbody>
</table>

*Most common Grade ≥3 AEs (occurring ≥2 times) post CTX001: headache, neck pain, cholelithiasis, esophagitis, leukopenia, musculoskeletal chest pain, non-cardiac chest pain and stomatitis; <sup>a</sup>Sepsis (related to busulfan), cholelithiasis, and abdominal pain (both not related to any study drug); all resolved; <sup>b</sup>Includes related and possibly related AEs. Only those AEs which occurred ≥2 times are described in the footnote except for “Related to CTX001” AEs where all are listed; <sup>c</sup>Oesophagitis (x3), leukopenia (x2), vulvovaginal inflammation (x2), stomatitis (x2); <sup>d</sup>Lymphopenia (x5), all recovered with the exception of 1 instance of lymphopenia; all attributed to the CD34+ hematopoietic stem cell enrichment before stem cell transplant; <sup>e</sup>Lymphopenia (x5), all recovered with the exception of 1 instance of lymphopenia; all attributed to the CD34+ hematopoietic stem cell enrichment before stem cell transplant.
## HbF Levels Maintained >40% Starting at Month 4 in the First SCD Patient

Hb fractionation over time (Hb [g/dL])

<table>
<thead>
<tr>
<th>Months post-CTX001 infusion</th>
<th>Baseline</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTX001 infusion</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>7.2</td>
<td>8.3</td>
<td>8.9</td>
<td>10.1</td>
<td>11.3</td>
<td>11.9</td>
<td>11.3</td>
<td>11.8</td>
</tr>
<tr>
<td>HbF</td>
<td>74.1%</td>
<td>25.9%</td>
<td>37.2%</td>
<td>46.6%</td>
<td>48.6%</td>
<td>47.3%</td>
<td>47.3%</td>
<td>46.1%</td>
</tr>
<tr>
<td>HbS</td>
<td>9.1%</td>
<td>21.3%</td>
<td>32.6%</td>
<td>41.2%</td>
<td>47.3%</td>
<td>49.7%</td>
<td>50.6%</td>
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<tr>
<td>HbA</td>
<td>0.8%</td>
<td>0.8%</td>
<td>0.8%</td>
<td>0.8%</td>
<td>0.8%</td>
<td>0.8%</td>
<td>0.8%</td>
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</tbody>
</table>

**Hb, other**: [Not specified]
SCD: Highly Pancellular HbF in Peripheral RBCs

Peripheral F-cells (% RBCs expressing Hb)

<table>
<thead>
<tr>
<th>Months post-CTX001 infusion</th>
<th>Baseline</th>
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<th>2</th>
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<th>5</th>
<th>6</th>
<th>9</th>
</tr>
</thead>
<tbody>
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<td>CTX001 infusion</td>
<td>33.9</td>
<td>4.3</td>
<td>43.8</td>
<td>70.2</td>
<td>94.7</td>
<td>99.9</td>
<td>99.6</td>
<td>99.7</td>
</tr>
</tbody>
</table>

Allelic editing in CD34+ bone marrow cells (%) 81.4
The First SCD Patient Has Not Experienced Any VOCs Post-CTX001 Infusion

Prior to screening
2 years

On-Study/pre-CTX001 period
6.5 months

Treatment period
9 months

- Simple transfusions unrelated to SCD; post transplant support
- Simple transfusions related to SCD
- Exchange transfusions per protocol

No pRBC transfusions have occurred since Study Day 19
Conclusion

• Gene therapy is a promising therapy for patients with sickle cell disease and thalassemia

• Preliminary results of gene editing clinical trials are promising.

• Additional studies and larger number of patients are needed to evaluate efficacy and safety of this therapy
Thank you

Stein, R. (Host). (2020, June 23). < A Year In, First Patient to Get Gene Editing for Sickle Cell Disease is Thriving . [Radio broadcast episode]. https://www.npr.org/transcripts/877543610