Gene Therapy for Hemoglobinopathies

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Disclosures

• Consultant: bluebird bio, Celgene (Bristol Myers Squibb), Imara, Agios

• Site principal investigator: bluebird bio, Sangamo, Bioverativ, CRISPR, ApoPharma (Chiesi), Terumo BCT
Overview

• Background

• Gene therapy methods
  – Gene addition
  – Gene editing
  – Post-transcriptional silencing of *BCL11A* for SCD

• Efficacy of gene therapy

• Safety of gene therapy
Sickle Cell Anemia and β-Thalassemia

**Adult Hemoglobin**

- **Mutations that alter the structure**
  - (Single mutation, β6 glu → val)

- **Mutations that reduce the synthesis**
  - (>200 mutations
    - β0 - severe; β+, HbE - milder)

**Consequences of Abnormal β-Globin Chain Structure**
- Vaso-occlusion
- Hemolysis
- Anemia

**Consequences of Reduced β-Globin Chain Production**
- Ineffective erythropoiesis
- Hemolysis
- Anemia

Similar approaches to gene therapy for both disorders with disease-specific modifications in treatment and assessment of efficacy.
Gene Addition

- Self-inactivating lentiviral vector

- Beta globin gene addition
  - Lentiglobin (beti-cel, bb1111): Single amino acid substitution T87Q that has anti-sickling properties and can be distinguished from HbA by HPLC
  - GLOBE
  - Others in earlier phase of study

- Gamma globin gene addition
  - RVT-1801: Modified gamma globin with anti-sickling properties
  - Also utilizes non-myeloablative conditioning
Gene Editing

- **Rationale:** raise HbF levels by reducing BCL11A, a HbF repressor, or editing gamma globin promoter region
  - Improves alpha:beta-like imbalance
  - Reduces Hb S polymerization

- **Ongoing clinical trials**
  - CRISPR
  - Zinc finger

**Double strand break**
- Non-homologous end rejoining
- Homologous recombination
- Insertions or deletions “indels”
- Donor DNA
- Gene replacement/correction
Lentiviral vector targeting BCL11A (BCH-BB694)

- Gene therapy approach using a lentiviral vector which encodes a short hairpin RNA (shRNA) targeting BCL11A mRNA embedded in a microRNA
  - Downregulation of BCL11A
  - Regulated erythroid expression: avoids off-target toxicity in HSCs and B Cells

HSC, hematopoietic stem cells
**Ex-Vivo Gene therapy: Schema**

**HSC Collection:** HSCs harvested from bone marrow or by mobilization & apheresis

**Transduction or Gene Editing:** HSC modified by transduction (gene addition) or gene editing

**Reinfusion:** Conditioning (partial or full myeloablation) and reinfusion of genetically modified HSC

**Hematopoietic stem cells**

**Modified hematopoietic stem cells**

**Informed Consent**
Efficacy of Gene Therapy
HGB-207 and HGB-212 interim results: transfusion status following Beti-cel infusion

- **HGB-207**
  - 91% (20/22) with > 3mo follow-up stopped transfusion

- **HGB-212**
  - 85% (11/13) have been off transfusions for > 6mo

*Supported by transfusions; †Patient’s total Hb level at Month 22 was 13.2 g/dL. Following a planned orthopedic surgery, the patient had blood loss, which required 1 packed RBC transfusion; ‡Transfusion within 60 days. Hb, haemoglobin; RBC, red blood cell; TDT, transfusion-dependent β-thalassemia. Data as of 3 March 2020
Sickle Cell Disease (HGB-206 Group C)
Improvement in Hemoglobin; HbA<sup>T87Q</sup> ≥ 40%

<table>
<thead>
<tr>
<th>Months post-LentiGlobin infusion</th>
<th>Baseline</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
<th>18</th>
<th>21</th>
<th>24</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>N&lt;sup&gt;+&lt;/sup&gt;</td>
<td>15</td>
<td>25</td>
<td>22</td>
<td>22</td>
<td>17</td>
<td>13</td>
<td>10</td>
<td>8</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

Improvement in markers of hemolysis (LDH, bilirubin, reticulocyte count)

% represents median Hb fraction as % of total Hb; *Number of patients with data available. Hb, hemoglobin; max, maximum; min, minimum.
HGB-206 Group C: Complete resolution of VOEs ≥6 months post-LentiGlobin treatment

Protocol VOE are shown; Patients with ≥ 4 sVOE at baseline before IC and with ≥ 6 months of follow-up post-DP infusion are included. A VOE includes episodes of acute pain with no medically determined cause other than a vaso-occlusion, lasting more than 2 hours and severe enough to require care at a medical facility, a VOE includes acute episodes of pain, acute chest syndrome, acute hepatic sequestration, and acute splenic sequestration; \(^*\)HbA\(^{T87Q}\) expression stabilizes within 6 months; \(^\dagger\)One death, unlikely related to LentiGlobin, > 18 months post treatment in a patient with significant baseline SCD-related cardiopulmonary disease.

Note: In the last dataset, one patient had a non-serious VOC at Day 107. The event is recorded as an investigator reported VOE but does not meet the definition of a protocol VOE.

Data as of 20 August 2020
CRISPR in Transfusion Dependent Thalassemia (CTX001): Rapid improvement in HbF, Total Hb

Median Hb fractionation\textsuperscript{a}, Hb g/dL

<table>
<thead>
<tr>
<th>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>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA\textsuperscript{d}</td>
<td>0.3 (0.0 – 0.6)</td>
<td></td>
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<tr>
<td>HbF\textsuperscript{d}</td>
<td>0.1 (0.1 – 1.8)</td>
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<tr>
<td>HbA2</td>
<td>5.1 (1.9 – 7.0)</td>
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</tr>
<tr>
<td>Hb, other\textsuperscript{a}</td>
<td>8.8 (6.6 – 12.1)</td>
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<td></td>
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<tr>
<td>Total Hb, Median (range), g/dL</td>
<td>10.1 (8.4 – 12.0)</td>
<td>8.8 (6.6 – 13.2)</td>
<td>10.5\textsuperscript{b} (6.6 – 12.1)</td>
<td>11.5 (8.5 – 13.1)</td>
<td>12.1 (11.0 – 12.9)</td>
<td>12.0 (11.1 – 13.6)</td>
<td>11.6 (10.3 – 13.4)</td>
<td>12.2 (11.9 – 12.5)</td>
<td>12.7</td>
<td>13.5</td>
<td>14.2</td>
</tr>
<tr>
<td>Months after CTX001 infusion</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

n = 7


\textsuperscript{a}Hb adducts and other variants. \textsuperscript{b}With respect to Patient 2, Total Hb from local laboratory and Hb fraction from central laboratory.

Franjoul, et al, ASH 2020
### CRISPR in Sickle Cell Disease (CTX001): Improvement in Fetal and Total Hemoglobin

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hb fractionation, Hb g/dL</th>
<th>CTX001 infusion</th>
<th>Months after CTX001 infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HbA</td>
<td>HbF</td>
<td>HbS</td>
</tr>
<tr>
<td>Patient 1</td>
<td>7.2</td>
<td>9%</td>
<td>74%</td>
</tr>
<tr>
<td>Patient 2</td>
<td>6.0</td>
<td>5%</td>
<td>90%</td>
</tr>
<tr>
<td>Patient 3</td>
<td>9.2</td>
<td>4%</td>
<td>43%</td>
</tr>
</tbody>
</table>

\(^a\)Hb adducts and other variants.

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Fetal hemoglobin expression is pancellular

Franjoul, et al, ASH 2020
CRISPR in Sickle Cell Disease (CTX001): Resolution of Vasoocclusive Pain Episodes following Treatment

Pre-study VOC burden
Average number per year over the previous 2 years

- **Patient 1**
  - Pre-study VOC burden: 7.0
  - Total Hb at last visit: 12.0 g/dL

- **Patient 2**
  - Pre-study VOC burden: 7.5
  - Total Hb at last visit: 11.5 g/dL

- **Patient 3**
  - Pre-study VOC burden: 4.0
  - Total Hb at last visit: 13.2 g/dL

All patients have detectable haptoglobin and improved LDH, indicating no evidence of hemolysis.

SCD: sickle cell disease; VOCs: vaso-occlusive crises.

Franjoul, et al, ASH 2020
### Post-transcriptional Genetic Silencing of BCL11A (BCH-BB694)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Follow-up (mo)</th>
<th>% Hb F</th>
<th>F-cells (%)</th>
<th>Recent Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>24</td>
<td>22.7</td>
<td>71</td>
<td>11.4</td>
</tr>
<tr>
<td>3*</td>
<td>18</td>
<td>20.4</td>
<td>58.9</td>
<td>9.5</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>31.9</td>
<td>81.9</td>
<td>11.1</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>38.8</td>
<td>65.3</td>
<td>11.0</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>29</td>
<td>70.6</td>
<td>11.0</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>41.3</td>
<td>93.6</td>
<td>9.3</td>
</tr>
</tbody>
</table>

*Remains on transfusions

- No vaso-occlusive pain events or acute chest
- 1 episode recurrent priapism >5 months post gene tx

Safety of Gene Therapy

- Adverse effects typical of myeloablative chemotherapy
  - Beticel: VOD in 5/41 subjects with thalassemia; bb1111: no VOD in SCD
  - HLH reported in 1 patient with TDT treated with CTX011 - though secondary to conditioning
  - Long-term: infertility risk

- Lentiglobin: slow platelet engraftment

- No vector mediated replication competent LV
  - One subject with thalassemia treated with beticel acquired HIV infection, 23 mo after drug product infusion – Wildtype HIV-1 documented

- Sudden death in one subject with SCD, Group C, 20 months after treatment, thought unrelated to the gene therapy
<table>
<thead>
<tr>
<th>Patient: SCD Pt 1</th>
<th>Patient: SCD Pt 2</th>
<th>Patient: SCD Pt 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year of Diagnosis:</td>
<td>2018</td>
<td>2021</td>
</tr>
<tr>
<td>Age</td>
<td>45 year old</td>
<td>Adult</td>
</tr>
<tr>
<td>Study/Group</td>
<td>HGB-206/Group A</td>
<td>HGB-206/Group C</td>
</tr>
<tr>
<td>Time from dosing to diagnosis</td>
<td>3 years</td>
<td>6 months</td>
</tr>
<tr>
<td>Presenting diagnosis</td>
<td>MDS (progressed to AML)</td>
<td>MDS?</td>
</tr>
<tr>
<td>Relevant findings</td>
<td>Monosomy 7, No vector in blast cells</td>
<td>Trisomy 8, No blasts/dysplasia identified therefore MDS cannot be confirmed</td>
</tr>
<tr>
<td></td>
<td>Pre-tx BM negative for monosomy 7 and NGS for 54 mutations associated with AML</td>
<td>Follow-up BMA without genetic abnormality</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diagnosis revised to transfusion-dependent anemia</td>
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<td></td>
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</tbody>
</table>

Lentiglobin SCD trials placed on hold. Clinical use of Zynteglo for thalassemia (Europe) on hold.
Summary/Conclusions

- Beta globin gene addition trials have achieved hemoglobin levels that enable transfusion independence in β-thalassemia and improvement in anemia and vasoocclusive events in SCD.

- CRISPR gene editing targeting BCL11a also with early results showing robust HbF production, transfusion independence in thalassemia and improvement in anemia and vasoocclusive events.

- Safety profile generally consistent with myeloablative conditioning, autologous transplant, and the underlying blood disorder.
  - Cases of MDS/AML in SCD have resulted in clinical trial hold.

- Gene therapy may offer an alternative curative/disease modifying treatment option but a better understanding of leukemia risk especially for patients with SCD is needed.