GENE THERAPY

Safe mobilization of CD34+ cells in adults with β-thalassemia and validation of effective globin gene transfer for clinical investigation

Farid Boulad,1,2 Xiuyan Wang,1,3,4 Jinrong Qu,4 Clare Taylor,4 Leda Ferro,4 Garyfalia Karponi,4 Shirley Bartido,4 Patricia Giardina,5 Glenn Heller,5 Susan E. Prockop,2 Aurelio Maggio,7 Michel Sadelain,1,3 and Isabelle Rivière1,3,4

1Center for Cell Engineering, 2Bone Marrow Transplant Service, Department of Pediatrics, 3Molecular Pharmacology and Chemistry Program, and 4Cell Therapy and Cell Engineering Facility, Memorial Sloan-Kettering Cancer Center, New York, NY; 5Department of Pediatrics, New York-Presbyterian Hospital-Weill Cornell Medical College, New York, NY; 6Department of Epidemiology & Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY; and 7Ospedale V. Cervello, Palermo, Italy

Key Points
- Safe mobilization of CD34+ cells in adults with β-thalassemia and effective transduction with a globin vector under cGMP conditions.
- Stable vector copy number and β-globin expression in BFU-Es derived from engrafted CD34+ HPCs 6 months post-transplant in NSG mice.

Introduction

The β-thalassemias are hereditary anemias caused by the deficient production of the β-chain of hemoglobin.1 The standard of care for patients with β-thalassemia major consists in lifelong transfusion therapy combined with pharmacologic iron chelation 1-3 The only curative treatment is allogeneic bone marrow transplantation from a matched, related donor.4,5 Most patients, however, lack such a donor.6 The goal of therapeutic globin gene transfer is to stably insert a functional globin gene into the patient’s own hematopoietic progenitor cells (HPCs) to achieve transfusion independence.7 We previously demonstrated successful globin gene therapy in murine thalassemia models, using a lentiviral vector that includes the human β-globin gene.8-10 The vector termed TNS9 increased hemoglobin levels by an average 4 to 6 g/dL per vector copy.8-10 Several groups have confirmed and extended these results in models of thalassemia and sickle cell disease, using variant vectors encoding β-, γ-, or mutated β-globin genes.7,11,12 For the past decade, the inability to transduce patient CD34+ HPCs at potentially therapeutic levels under clinically relevant conditions has precluded effective implementation of this therapy.12-15

We conducted a pilot trial to investigate the safety and effectiveness of mobilizing CD34+ hematopoietic progenitor cells (HPCs) in adults with β-thalassemia major. We further assessed whether thalassemia patient CD34+ HPCs could be transduced with a globin lentiviral vector under clinical conditions at levels sufficient for therapeutic implementation. All patients tolerated granulocyte colony-stimulating factor well with minimal side effects. All cell collections exceeded 8 × 10⁶ CD34+ cells/kg. Using clinical grade TNS9.3.55 vector, we demonstrated globin gene transfer averaging 0.53% in 3 validation runs performed under current good manufacturing practice conditions. Normalized to vector copy, the vector-encoded β-chain was expressed at a level approximating normal hemizygous protein output. Importantly, stable vector copy number (0.2-0.6) and diminished vector expression were obtained in NSG mice 6 months posttransplant. Thus, we validated a safe and effective procedure for β-globin gene transfer in thalassemia patient CD34+ HPCs, which we will implement in the first US trial in patients with severe inherited globin disorders. This trial is registered at www.clinicaltrials.gov as #NCT01639690. (Blood. 2014;123(10):1483-1486)

Study design

CD34+ cell collection and clinical grade TNS9.3.55 vector stocks

We used granulocyte colony-stimulating factor (G-CSF) (10 μg/kg, once daily subcutaneously for 6 days) to mobilize HPCs as specified in the Memorial Sloan-Kettering Cancer Center’s Institutional Review Board-approved protocol. This study was conducted in accordance with the Declaration of Helsinki. CD34+ cells were selected using an ISOLEX TM cell collection and clinical grade TNS9.3.55 vector stocks, manufactured under current good manufacturing practice (cGMP) conditions at the Center for Biomedicine and Genetics (CBG, Duarte, CA) had a HeLa titer of 3.5 and 6.6 × 10³ TU/mL, respectively.

Transduction and VCN quantification

CD34+ HPCs were cultured for 18 to 24 hours in serum-free X-VIVO 10 supplemented with human stem cell factor, Fms-like tyrosine kinase 3 ligand (Flt3-L), thrombopoietin, and interleukin-3. Fractions were subsequently cultured for 14 to 16 days in liquid erythroid cultures (see supplemental Methods available on the Blood Web site) or hematopoietic colony assays for vector copy number (VCN) quantification by quantitative polymerase chain reaction using the Applied Biosystems 7500...


F.B and X.W. contributed equally to this study.

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real-time polymerase chain reaction system (see supplemental Methods for details).

Analysis of human cells engrafted in NSG mice

Murine studies were conducted under a Memorial Sloan-Kettering Cancer Center’s Institutional Animal Care and Use Committee-approved protocol. Non-obese diabetic (NOD) Cg-Prkdc scid IL2R-null (NOD/severe combined immunodeficiency-g-null, NSG) mice were conditioned with 35 mg/kg busulfan 24 hours prior to receiving TNS9.3.55-transduced HPCs. Bone marrow was analyzed 3.5 to 7 months posttransplantation (see supplemental Methods for details).

Globin expression studies

Globin chain expression was analyzed by high-performance liquid chromatography as previously described. Total RNA was isolated from peripheral blood and from erythroid burst-forming units (BFU-Es) generated from pre-infusion CD34\(^+\) cell cultures or posttransplant NSG bone marrow. Primers and probes were previously described (see supplemental Methods for details).

Results and discussion

Here, we demonstrate safe and efficacious CD34\(^+\) cell collection in transfusion-dependent β-thalassemia major patients and robust globin gene transfer under cGMP conditions. All 5 enrolled adults were on a hypertransfusion and chelation regimen (supplemental Table 1). Throughout the 6-day mobilization process, the maximum white blood cell counts and absolute neutrophil counts reached 46 to 65 \(\times\) 10\(^9\)/L and 43 to 55 \(\times\) 10\(^9\)/L on day 6 for the 2 patients with intact spleen, and 75 to 93 \(\times\) 10\(^9\)/L and 60 to 84 \(\times\) 10\(^9\)/L on days 3 to 5 for the splenectomized patients (supplemental Table 2). Hemoglobin levels decreased slightly during mobilization and leukapheresis (from 10.3-11.3 to 9.2-10.6 g/dL). The harvested CD34\(^+\) cell dose ranged from 8 to 12 \(\times\) 10\(^6\) / kg in 4 subjects who completed both leukaphereses (supplemental Table 2). One patient did not complete the second leukapheresis due to anxiety.

In previation studies, we determined that the highest transduction efficiency utilizing cGMP TNS9.3.55 vector was obtained by performing 2 transduction cycles in presence of 100 ng/mL of TPO, Flt3-L, SCF and 20 U/mL interleukin-3. Approximately 80 CFUs per validation run were screened for the TNS9.3.55 by quantitative polymerase chain reaction. Avg, average. CFU, colony forming unit; EC, erythroid culture (d 14 bulk); NA, not applicable; SCF, stem cell factor; TPO, thrombopoietin.

![Figure 1. Restoration of β-globin chain synthesis in patient hematopoietic cells.](image-url)
Mice were infused with 5.0e5 to 2.0e6 transduced CD34⁺ HPCs. The average VCN and the frequency of BFU-E colonies positive for the vector were determined as well as the level of β/α chain expression in the BFU-Es derived from the BM of engrafted mice. β/α⁺ expression was measured in 2 independent pools of transduced BFU-Es and averaged. Untransduced pool 1, n = 30; transduced with TNS9.3.55 pool 1, n = 32; average VCN = 1.2. Untransduced pool 2, n = 30; transduced with TNS9.3.55 pool 2, n = 30; average VCN = 1.1. Experiments 4 and 5 were conducted with TNS9.3.55-transduced CD34⁺ cells derived from validation 2 and validation 3, respectively. CFU-GM, granulocyte-macrophage CFU; ND, not done; +ve, positive.

Table 2. Long-term engraftment in vivo in NOD SCID mice

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. mice (mo post-BMT)</th>
<th>CD34⁺/mouse</th>
<th>% huCD45 in BM</th>
<th>Avg VCN bulk BM</th>
<th>Frequency vector +ve BFU-E/BM (%)</th>
<th>β/α⁺ Expression BFU-E/BM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postinfusion (in vivo)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1 (6)</td>
<td>5.0 × 10⁵</td>
<td>54</td>
<td>0.17</td>
<td>54.0</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>6 (7)</td>
<td>1.8 × 10⁶</td>
<td>13.6 ± 9.8</td>
<td>0.50 ± 0.3</td>
<td>31.0</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>3 (6)</td>
<td>2.0 × 10⁵</td>
<td>0.8 ± 0.2</td>
<td>0.54 ± 0.2</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>3 (6)</td>
<td>6.7 × 10⁵</td>
<td>2.5 ± 2.6</td>
<td>0.60 ± 0.9</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>4 (3)</td>
<td>1.6 × 10⁶</td>
<td>3.2 ± 4.6</td>
<td>0.60 ± 0.3</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

The average VCN in liquid EC and the frequency of BFU-E colonies positive for the vector were determined in 5 independent experiments. β/α⁺ expression was measured in pooled BFU-Es: untransduced pool, n = 30; transduced with TNS9.3.55 n = 30; average VCN = 1.6. ND, not done.
vector copy/cell of TNS9.3.55 will effectively treat β-thalassemia and may be sufficient in β-thalassemia.

In summary, we demonstrate that CD34+ HPCs from adult thalassemic patients can be safely collected and effectively transduced at large scale in clinically relevant manner. These data provide the basis for a clinical trial, which was registered at ClinicalTrials.gov as #NCT01639690.

Acknowledgments

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References