Gene Therapy in a Patient with Sickle Cell Disease

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Summary

Sickle cell disease results from a homozygous missense mutation in the β-globin gene that causes polymerization of hemoglobin S. Gene therapy for patients with this disorder is complicated by the complex cellular abnormalities and challenges in achieving effective, persistent inhibition of polymerization of hemoglobin S. We describe our first patient treated with lentiviral vector-mediated addition of an antisickling β-globin gene into autologous hematopoietic stem cells. Adverse events were consistent with busulfan conditioning. Fifteen months after treatment, the level of therapeutic antisickling β-globin remained high (approximately 50% of β-like–globin chains) without recurrence of sickle crises and with correction of the biologic hallmarks of the disease. (Funded by Bluebird Bio and others; HGB-205 ClinicalTrials.gov number, NCT02151526.)

SICKLE CELL DISEASE IS AMONG THE MOST PREVALENT INHERITED MONOGENIC disorders. Approximately 90,000 people in the United States have sickle cell disease, and worldwide more than 275,000 infants are born with the disease annually.¹,² Sickle cell disease was the first disease for which the molecular basis was identified: a single amino acid substitution in “adult” βA-globin (Glu6Val) stemming from a single base substitution (A→T) in the first exon of the human βA-globin gene (HBB) was discovered in 1956.³ Sickle hemoglobin (HbS) polymerizes on deoxygenation, reducing the deformability of red cells. Patients have intensely painful vaso-occlusive crises, leading to irreversible organ damage, poor quality of life, and reduced life expectancy. Hydroxyurea, a cytotoxic agent that is capable of boosting fetal hemoglobin levels in some patients, is the only disease-modifying therapy approved for sickle cell disease.⁴

Allogeneic hematopoietic stem-cell transplantation currently offers the only curative option for patients with severe sickle cell disease.⁵,⁶ However, fewer than 18% of patients have access to a matched sibling donor.⁷,⁸ Therapeutic ex vivo gene transfer into autologous hematopoietic stem cells, referred to here as gene therapy, may provide a long-term and potentially curative treatment for sickle cell disease.⁹

We previously reported proof of effective, sustained gene therapy in mouse mod-
els of sickle cell disease by lentiviral transfer of a modified HBB encoding an antisickling variant (β<sup>α87Thr:Gln</sup>[β<sup>β-T87Q</sup>]). Here we report the results for a patient who received lentiviral gene therapy in the HGB-205 clinical study and who had complete clinical remission with correction of hemolysis and biologic hallmarks of the disease.

**CASE REPORT**

A boy with the β<sup>α</sup>/β<sup>α</sup> genotype, a single 3.7-kb α-globin gene deletion, and no glucose 6-phosphate dehydrogenase deficiency received a diagnosis of sickle cell disease at birth and was followed at the Reference Centre for Sickle Cell Disease of Necker Children’s Hospital in Paris. He had a history of numerous vaso-occlusive crises, two episodes of the acute chest syndrome, and bilateral hip osteonecrosis. He had undergone cholecystectomy and splenectomy. During screening, a cerebral hypodensity without characteristics of cerebral vasculopathy was detected.

Because hydroxyurea therapy administered when the boy was between 2 and 9 years of age did not reduce his symptoms significantly, a prophylactic red-cell transfusion program was initiated in 2010, including iron chelation with deferasirox (at a dose of 17 mg per kilogram of body weight per day). He had had an average of 1.6 sickle cell disease–related events annually in the 9 years before transfusions were initiated.

In May 2014, he was enrolled in our clinical study. His verbal assent and his mother’s written informed consent were obtained. In October 2014, his verbal assent and his mother’s written informed consent were obtained. In October 2014, when the patient was 13 years of age, he received an infusion of the drug product LentiGlobin BB305.

**METHODS**

**STUDY OVERSIGHT**

The study protocol, which is available with the full text of this article at NEJM.org, was designed by the last two authors and Bluebird Bio, the study sponsor. The protocol was reviewed by the French Comité de Protection des Personnes and relevant institutional ethics committees. Clinical data were collected by the first author, and laboratory data were generated by the sponsor, the last author, and other authors. The authors had access to all data, and data analysis was performed by them. The first author and one author employed by the sponsor wrote the first draft of the manuscript, which was substantively revised by the last two authors and further edited and approved by all the authors with writing assistance provided by an employee of the sponsor. The authors vouch for the accuracy and completeness of the data and adherence to the protocol.

**ANTISICKLING GENE THERAPY VECTOR**

The structure of the LentiGlobin BB305 vector has been previously described (see Fig. S1 in the Supplementary Appendix, available at NEJM.org). This self-inactivating lentiviral vector encodes the human HBB variant β<sup>α-T87Q</sup>. In addition to inhibiting HbS polymerization, the T87Q substitution allows for the β-globin chain of adult hemoglobin (HbA<sup>T87Q</sup>) to be differentially quantified by means of reverse-phase high-performance liquid chromatography.

**GENE TRANSFER AND TRANSPLANTATION PROCEDURES**

Bone marrow was obtained twice from the patient to collect sufficient stem cells for gene transfer and backup (6.2×10<sup>8</sup> per kilogram and 5.4×10<sup>8</sup> per kilogram, respectively, of total nucleated cells obtained). Both procedures were preceded by exchange transfusion, and bone marrow was obtained without clinical sequelae. Anemia was the only grade 3 adverse event reported during these procedures. Bone marrow–enriched CD34+ cells were transduced with LentiGlobin BB305 vector (see the Methods section in the Supplementary Appendix). The mean vector copy numbers for the two batches of transduced cells were 1.0 and 1.2 copies per cell.

The patient underwent myeloablation with intravenous busulfan (see the Methods section in the Supplementary Appendix). The total busulfan area under the curve achieved was 19,363 μmol per minute. After a 2-day washout period, transduced CD34+ cells (5.6×10<sup>6</sup> CD34+ cells per kilogram) were infused. Red-cell transfusions were to be continued after transplantation until a large proportion of HbA<sup>T87Q</sup> (25 to 30% of total hemoglobin) was detected.

The patient was followed for engraftment; toxic effects (graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03); vector copy number in total nucleated blood cells and in different lineages; quantification of HbA<sup>T87Q</sup>, HbS, and fetal hemoglobin levels by means of high-performance liquid chromatography; DNA integration-site map-
ping by linear amplification–mediated polymerase chain reaction in nucleated blood cells; and replication-competent lentivirus analysis by p24 antibody enzyme-linked immunosorbent assay. Red-cell analyses were performed at month 12 (see the Methods section in the Supplementary Appendix).

**Results**

**Engraftment and Gene Expression**

Neutrophil engraftment was achieved on day 38 after transplantation, and platelet engraftment was achieved on day 91 after transplantation. Figure 1A shows the trajectory of vector copy numbers and Figure 1B shows production of HbAT87Q. Gene marking increased progressively in whole blood, CD15 cells, B cells, and monocytes (Fig. S2 in the Supplementary Appendix), stabilizing 3 months after transplantation. Increases in levels of vector-bearing T cells were more gradual.

HbAT87Q levels also increased steadily (Fig. 1B) and red-cell transfusions were discontinued, with the last transfusion on day 88. Levels of HbAT87Q reached 5.5 g per deciliter (46%) at month 9 and continued to increase to 5.7 g per deciliter (48%) at month 15, with a reciprocal decrease in HbS levels to 5.5 g per deciliter (46%) at month 9 and 5.8 g per deciliter (49%) at month 15. Total hemoglobin levels were stable between 10.6 and 12.0 g per deciliter after post-transplantation.
The patient was discharged on day 50. More than 15 months after transplantation, no sickle cell disease-related clinical events or hospitalization had occurred; this contrasts favorably with the period before the patient began to receive regular transfusions. All medications were discontinued, including pain medication. The patient reported full participation in normal academic and physical activities. Magnetic resonance imaging (MRI) of the head at 8 months showed unchanged subcortical white-matter hypodensities. Lower limb MRI at 14 months showed no recent bone or tissue damage.

Changes in sickle cell disease-related biologic measures are shown in Table 1. Complete blood counts were stable, reticulocyte counts decreased substantially (Fig. S4 in the Supplementary Appendix), laboratory values including uric acid, albumin, and lipids were normal, and circulating white and red blood cells were not detected in blood counts. The patient reported no adverse events related to the LentiGlobin BB305-transduced stem cells were reported (Table S1 in the Supplementary Appendix). Test results for the presence of replication-competent lentiviruses were uniformly negative. Serial monitoring of integration sites in peripheral-blood samples showed a consistently polyclonal profile without detection of a dominant clone (defined as a single clone accounting for >30% of unique integration events) through month 12 (Fig. S3 in the Supplementary Appendix).
Sickled/Red Cells (%)  
- Patient 6 Mo
- Patient 12 Mo
- Control 1
- Control 2
- Control 3
- Control 4
- Control 5

A Sickled Red Cells, Normoxic Conditions

B Sickled Red Cells, Hypoxic Conditions

C Oxygen Equilibrium Curves (37°C, pH 7.4)

D Red-Cell Deformability

E Red-Cell Density
Because the patient received a regular transfusion regimen for 4 years before this study and because of the exchange transfusion before transplantation, meaningful comparative studies before and after transplantation could not be conducted. However, the proportions of sickled red cells in the patient’s blood at months 6 and 12 were significantly lower than those in untreated patients with sickle cell disease ($\beta^6/\beta^6$) (Fig. 2A). At month 12, the sickling rate in hypoxic conditions was not significantly different from that of the patient’s asymptomatic, heterozygous ($\beta^6/\beta^8$) mother (Fig. 2B). Oxygen dissociation studies, which quantify oxygen saturation relative to the partial pressure of oxygen, showed that results in the patient at month 12 and results in a heterozygous ($\beta^6/\beta^8$) control were similar (Fig. 2C and 2D).

**Discussion**

This case report of a patient with sickle cell disease who received gene therapy with the use of lentiviral gene addition of an antisickling $\beta$-globin variant provides proof of concept for this approach and may help to guide the design of future clinical trials of gene therapy for sickle cell disease. Once the transduced stem cells engrafted, normal blood-cell counts were ultimately attained in all lineages. Increasing levels of vector-bearing nucleated cells in the blood over the first 3 months after transplantation and general vector copy number stability through month 15 suggest engraftment of transduced stem cells that were capable of long-term repopulation. No adverse events that were considered by the investigators to be related to the BB305-transduced cells were observed, and the pattern of vector integration remained polyclonal without clonal dominance.

Insertional oncogenesis has been reported in clinical gene-transfer studies with gamma retroviral vectors but not with lentiviral vectors. Unlike gamma retroviruses, lentivirus tends to insert in transcriptionally active regions rather than near transcriptional start sites. In addition, the BB305 vector is an enhancer-deleted vector and is self-inactivating. Reported data from this and other ongoing studies of the BB305 vector involving patients with sickle cell disease (7 patients) and $\beta$-thalassemia (22 patients) show a consistent safety profile, with no evidence of insertional mutagenesis through 4 to 30 months.
The appearance of vector-bearing cells in the periphery corresponds to the time frame for engraftment of long-term progenitors and stem cells repopulating the space of nucleated cells. In contrast, the slower pace for the increase of HbAT87Q expression reflects the more gradual time course of replacement of transfused red cells from the pretransplantation and peritransplantation periods by newly matured, graft-derived red cells.

In mice models of sickle cell disease, therapeutic globin expression after gene addition was difficult to obtain, presumably because of competition with endogenous β-globin messenger RNAs. In the current study, a high concentration of therapeutic HbAT87Q (ratio of HbAT87Q to HbS, approximately 1) was achieved. HbAT87Q expression appears to be sufficient to suppress hemolysis, resulting in stable hemoglobin concentrations of 11 to 12 g per deciliter and major improvement in all measurable sickle cell disease–specific biologic markers and blocking sickle cell disease–related clinical events.

Additional data on LentiGlobin treatment in sickle cell disease is currently being collected in HGB-206, a multicenter, phase 1/2 clinical study in the United States. Follow-up is more limited for these patients than for the patient in our study, but initial reports in seven patients have not included any new safety findings. Gene-transfer efficiency was lower than reported here, although therapeutic gene expression remained correlated with vector copy number values.

Outcomes in this patient provide further supportive evidence to our previously reported results of patients who underwent a similar ex vivo gene therapy procedure for β-thalassemia with the same BB305 vector or the previous HPV569 vector. In addition to the patient with sickle cell disease described here, under this same clinical protocol, 4 patients with transfusion-dependent β-thalassemia have received LentiGlobin BB305. These participants had no clinically significant complications and no longer require regular transfusions. These findings are consistent with early results reported with 18 other patients with thalassemia who received LentiGlobin BB305 in clinical study HGB-204. Longer follow-up is required to confirm the durability of the efficacy and safety profile observed, and data from additional evaluations of gene therapy in a larger cohort of patients to confirm the promise of gene therapy for sickle cell disease are lacking.

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APPENDIX

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