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Gene Therapy in Patients with Transfusion-Dependent β-Thalassemia

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ABSTRACT

BACKGROUND

Donor availability and transplantation-related risks limit the broad use of allogeneic hematopoietic-cell transplantation in patients with transfusion-dependent β-thalassemia. After previously establishing that lentiviral transfer of a marked β-globin ($β^{A-T87Q}$) gene could substitute for long-term red-cell transfusions in a patient with β-thalassemia, we wanted to evaluate the safety and efficacy of such gene therapy in patients with transfusion-dependent β-thalassemia.

METHODS

In two phase 1–2 studies, we obtained mobilized autologous CD34+ cells from 22 patients (12 to 35 years of age) with transfusion-dependent β-thalassemia and transduced the cells ex vivo with LentiGlobin BB305 vector, which encodes adult hemoglobin (HbA) with a T87Q amino acid substitution (HbA^{T87Q}). The cells were then reinfused after the patients had undergone myeloablative busulfan conditioning. We subsequently monitored adverse events, vector integration, and levels of replication-competent lentivirus. Efficacy assessments included levels of total hemoglobin and HbA^{T87Q}, transfusion requirements, and average vector copy number.

RESULTS

At a median of 26 months (range, 15 to 42) after infusion of the gene-modified cells, all but 1 of the 13 patients who had a non–$β^0/β^0$ genotype had stopped receiving red-cell transfusions; the levels of HbA^{T87Q} ranged from 3.4 to 10.0 g per deciliter, and the levels of total hemoglobin ranged from 8.2 to 13.7 g per deciliter. Correction of biologic markers of dyserythropoiesis was achieved in evaluated patients with hemoglobin levels near normal ranges. In 9 patients with a $β^0/β^0$ genotype or two copies of the IVS1-110 mutation, the median annualized transfusion volume was decreased by 73%, and red-cell transfusions were discontinued in 3 patients. Treatment-related adverse events were typical of those associated with autologous stem-cell transplantation. No clonal dominance related to vector integration was observed.

CONCLUSIONS

Gene therapy with autologous CD34+ cells transduced with the BB305 vector reduced or eliminated the need for long-term red-cell transfusions in 22 patients with severe β-thalassemia without serious adverse events related to the drug product. (Funded by Bluebird Bio and others; HGB-204 and HGB-205 ClinicalTrials.gov numbers, NCT01745120 and NCT02151526.)

THE β-HEMOGLOBINOPATHIES, WHICH include β-thalassemia and sickle cell disease, are among the most prevalent monogenic disorders worldwide.[1] β-thalassemia is caused by more than 200 mutations in the *HBB* globin gene, which encodes the beta subunit of the most common form of adult hemoglobin, HbA. These mutations either abolish (β^0) or reduce (β^+) β-globin synthesis, which results in intracellular hemichrome precipitation, ineffective erythropoiesis, chronic hemolysis, and profound anemia.[2,3] In the most severe clinical form of the disease, transfusion-dependent β-thalassemia, patients require long-term red-cell transfusions for survival and the prevention of serious complications.[4] Coinheritance of the genetic variant β^E (HBB:c.79G→A) with any β^0 mutation results in a β^E/β^0 genotype, a condition of varying severity that is responsible for approximately half of all cases of transfusion-dependent β-thalassemia worldwide.[5]

The only potentially curative option for β-thalassemia is allogeneic hematopoietic-cell transplantation, but owing to risks of graft rejection, graft-versus-host disease, and other treatment-related toxic effects, transplantation is primarily reserved for young children with an HLA-identical sibling donor.[6-8] Thus, the current standard of care for patients with β-thalassemia consists of lifelong, regular red-cell transfusions and iron chelation.[9] The risks of serious complications from transfusion-related iron toxicity and viral infections persist despite improvements in care.[10,11] Therefore, gene therapy is being evaluated as a new option in patients with β-thalassemia.[12-15]

Lentiviral vectors have the ability to transfer complex genetic structures into quiescent hematopoietic stem cells.[16] After establishing that β-globin lentiviral vectors had successfully corrected models of β-thalassemia and sickle cell disease in mice,[17,18] we initiated a human clinical study of ex vivo gene therapy for the β-hemoglobinopathies (called the LG001 study) using a lentiviral vector,[19,20] which was followed by other studies.[12-15] We used the HPV569 vector[18] to transfer an extended β-globin gene structure, including segments of the human β-globin locus control region,[21,22] into hematopoietic stem cells obtained from patients with β-thalassemia and transplanted the gene-modified cells back into the patients. Vector-encoded $\beta^{A\text{-}T87Q}$-globin was

engineered with a single amino acid substitution (T87Q) that strongly inhibits the polymerization of sickle hemoglobin in patients with sickle cell disease and also permits precise quantification of vector-derived therapeutic globin expression in vivo.[23] A patient with a severe β^E/β^0 genotype safely discontinued transfusions for more than 6 years with sustained $\beta^{A\text{-}T87Q}$-globin expression.[13,20]

Here, we report interim results from two companion phase 1/2 clinical studies that evaluated the safety and efficacy of gene therapy for β-thalassemia using the LentiGlobin BB305 vector,[13,24] which is similar to our HPV569 vector. In the international HGB-204 (Northstar) study, 18 patients underwent infusion of BB305-transduced autologous hematopoietic stem cells, with follow-up ranging from 15 to 38 months. The second study, HGB-205, which was conducted at a single site in Paris, treated 3 patients with sickle cell disease[25] and 4 with β-thalassemia, with follow-up ranging from 20 months to more than 3 years. We report the outcomes to date in all 22 patients with β-thalassemia who were treated in the two studies.

METHODS

PATIENTS

Patients with β-thalassemia of any genotype who were 35 years of age or younger were eligible for enrollment in one of the two studies. Enrollment was restricted to patients who were at least 12 years of age in HGB-204 and at least 5 years of age in HGB-205. Transfusion dependence was defined as the receipt of at least eight transfusions or at least 100 ml per kilogram of body weight of packed red cells per year in the 2 years before enrollment. Patients with advanced organ damage were not eligible to participate (Fig. 1). Patients who were 18 years of age or older provided written informed consent. For patients under the age of 18 years, written informed consent was provided by a parent or guardian, and the patient provided assent, according to institutional standards. Details regarding eligibility are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.

VIRAL VECTOR

LentiGlobin BB305 vector is similar to the HPV569 vector from the Leboulch laboratory.[18,20] The BB305 vector was modified to remove the

chicken cHS4 insulator core to increase vector stability and titers and to incorporate the cytomegalovirus promoter to drive vector transcription in packaging cells. In the two studies, high-titer, large-scale, clinical-grade BB305 vector production and purification by ion-exchange chromatography were performed by Bluebird Bio with the use of packaging plasmids from the Leboulch laboratory[26] at a single site in the United States.
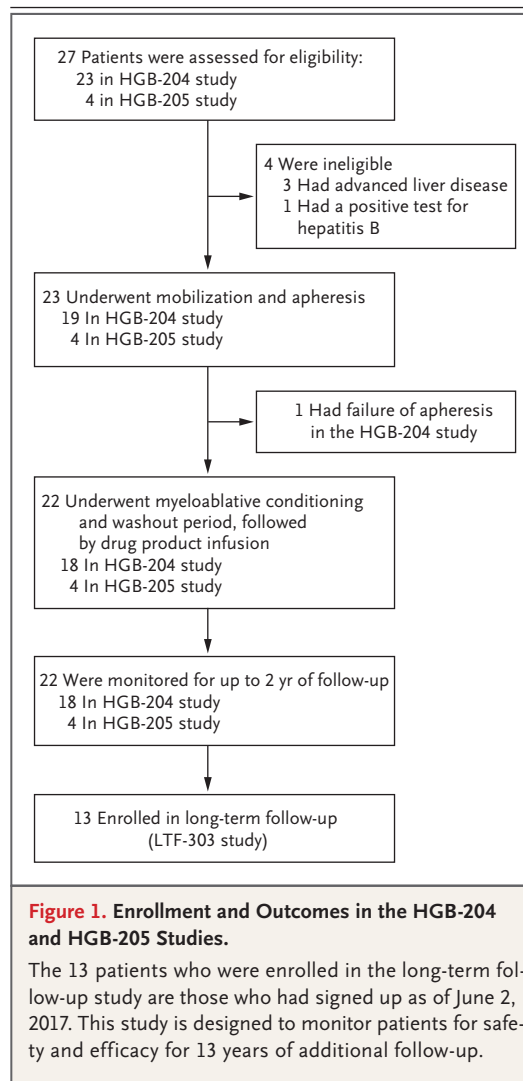
STUDY DESIGN

The two nonrandomized, open-label, single-dose phase 1/2 studies were initiated in 2013. The protocols for the two studies are available in a single document at NEJM.org.

The HGB-204 study was conducted at six sites — four in the United States (with 14 patients), one in Australia (2 patients), and one in Thailand (2 patients) — where patients' conditioning and transplantation occurred. In all the patients, the harvesting of hematopoietic stem cells and progenitor cells after mobilization with the use of filgrastim and plerixafor was performed in the United States. Unmanipulated hematopoietic stem cells and progenitor cells were transported to a central manufacturing facility, where CD34+ cells were enriched and transduced with BB305 to manufacture the LentiGlobin drug product.[24] After cryopreservation and testing and release of the drug product, patients underwent myeloablative conditioning with intravenous busulfan for 4 consecutive days at an initial dose of 3.2 mg per kilogram per day. The dose was adjusted on the basis of first-dose pharmacokinetic analysis to achieve a target area under the curve of 1000 μM per minute (range, 900 to 1200) for doses administered every 6 hours and 4000 μM per minute (range, 3600 to 5000) for once-daily administration. The LentiGlobin drug product was infused after a 72-hour washout period, and patients were monitored until it was determined that their condition was medically stable, with an absolute neutrophil count of at least 500 cells per cubic millimeter for 3 consecutive days. An additional aliquot of hematopoietic stem cells and progenitor cells (minimum, 2 million CD34+ cells per kilogram) was collected by apheresis in each patient and stored at the clinical site for rescue use in the event of engraftment failure.

The HGB-205 study was conducted at Necker Children's Hospital in Paris under a similar protocol to that used in HGB-204, except for some procedural differences. In HGB-205, enhanced red-cell transfusion (called hypertransfusion) was performed for at least 3 months before stem-cell mobilization and harvesting to maintain a hemoglobin level of more than 11.0 g per deciliter in an effort to enrich for bona fide hematopoietic stem cells in the harvested CD34+ cell compartment by suppressing the erythroid lineage expansion and the skewing that is seen in β-thalassemia. In addition, the dose of busulfan was adjusted on the basis of daily pharmacokinetic analysis.

After the 24-month primary study period, patients in the two studies were asked to transition to a long-term follow-up study (ClinicalTrials.gov number, NCT02633943) for 13 years of additional

Figure 1. Enrollment and Outcomes in the HGB-204 and HGB-205 Studies.

The 13 patients who were enrolled in the long-term follow-up study are those who had signed up as of June 2, 2017. This study is designed to monitor patients for safety and efficacy for 13 years of additional follow-up.

monitoring. Additional details regarding the two studies are provided in the Supplementary Appendix.

SAFETY AND EFFICACY ASSESSMENTS

In the two studies, safety end points included hematopoietic engraftment and kinetics, the rate of transplantation-related death at 100 days, overall survival, adverse events and serious adverse events (both graded according to international standards), detection of vector-derived replication-competent lentivirus, and analysis of vector insertion sites. The observation of a limited number of integration sites may suggest clonal outgrowth, a potential precursor to oncogenesis. Details are provided in the Supplementary Appendix.

Efficacy end points included the quantification of vector-derived HbA^{T87Q} in the peripheral blood after infusion of the drug product and the discontinuation of red-cell transfusions. In patients with at least 12 months of follow-up who had received transfusions 6 or more months after treatment, we determined the reduction in the number of transfusions by comparing the average annualized number and volume of transfusions for the 2 years before enrollment with average annualized values, beginning 6 months after infusion of the drug product. Additional pharmacodynamic measures of treatment efficacy included vector copy number in peripheral-blood mononuclear cells (PBMCs). In HGB-205, investigators also monitored iron metabolism, hemolysis, and biologic markers of dyserythropoiesis.

STUDY OVERSIGHT

The HGB-204 and HGB-205 studies were designed by the lead investigators in collaboration with the study sponsor, Bluebird Bio. All the other authors also participated in data collection and analysis. Laboratory data were generated by the authors and by the study sponsor. Clinical data were generated and collected by the authors, who performed all treatment procedures and follow-up assessments and were responsible for all clinical decisions. Independent data and safety monitoring committees regularly reviewed safety data. The authors had confidential access to all the data from the studies, which were provided by the study sponsor. The first two authors and last two authors wrote the first draft of the manuscript, which was substantively edited and approved by all the authors for submission for publication. No one who is not an author contributed to the writing of the manuscript.

STATISTICAL ANALYSIS

The treatment population, which included all the patients who had received the LentiGlobin BB305 drug product, was the primary population for this interim analysis, and data were analyzed separately for each study. For continuous variables, the median, minimum, and maximum values are presented. For categorical variables, the proportion of treated patients in each category is presented. Specific statistical tests are described in figure legends and in the Supplementary Appendix.

RESULTS

CHARACTERISTICS OF THE PATIENTS

Data through June 2, 2017, are presented for all 22 treated patients with β-thalassemia who were enrolled in the two studies. The duration of follow-up after transplantation ranged from 15 to 42 months.

In HGB-204, a total of 23 patients between the ages of 12 and 35 went through the consenting process. Of these patients, mobilization was initiated in 19, and 18 received the infusion. All the treated patients met the criteria for transfusion dependence and included 8 patients with a β^0/β^0 genotype, 6 patients with a β^E/β^0 genotype, and 4 patients with other β-thalassemia genotypes (Table 1 and Fig. 1, and Table S1 in the Supplementary Appendix). Patients had started receiving red-cell transfusions at a median age of 3.5 years, and 6 had undergone splenectomy. In the 2 years before study enrollment, the median annual red-cell transfusion volume was 164 ml per kilogram per year (range, 124 to 261), which included 183 ml per kilogram per year in the 8 patients with a β^0/β^0 genotype and 147 ml per kilogram per year in the 10 patients with other genotypes.

In HGB-205, 4 patients between the ages of 16 and 19 years went through the consenting process and received an infusion of the drug product. Of these patients, 3 had a β^E/β^0 genotype, and 1 was homozygous for the IVS1-110 mutation, a β^+ genotype with only trace endogenous β^A-globin expression (0.9 g per deciliter in this patient), a severe clinical presentation equiv-

Table 1. Characteristics of the Patients and Cellular Products.*

Characteristic	HGB-204 Study (N = 18)	HGB-205 Study (N = 4)
Sex — no. (%)		
Female	13 (72)	2 (50)
Male	5 (28)	2 (50)
Race — no. (%)†		
Asian	14 (78)	2 (50)
White	4 (22)	2 (50)
Median age at initiation of regular transfusions (range) — yr	3.5 (0–26.0)	1.8 (0–14.0)
Median age (range) — yr	20 (12–35)	18 (16–19)
Genotype		
β^0/β^0 or IVS1-110 mutation	8 (44)	1 (25)
β^E/β^0	6 (33)	3 (75)
Other‡	4 (22)	0
Median monthly transfusion volume for 2 yr before enrollment (range) — ml/kg	13.6 (10.4–21.8)	15.2 (11.6–15.7)
Patients who had undergone splenectomy — no. (%)	6 (33)	3 (75)
Drug product		
Median vector copy no. (range)	0.7 (0.3–1.5)	1.3 (0.8–2.1)
Median percentage of cells positive for vector (range)	32 (17–58)	ND
Median no. of CD34+ cells (range) — million/kg	8.1 (5.2–18.1)	10.5 (8.8–13.6)

* A complete description of the mutations is provided in the Supplementary Appendix. Percentages may not total 100 because of rounding. ND denotes not done.
† Race was reported by the patients.
‡ Other genotypes were β^+/β^+, β^0/β^+, β^E/β^+, and β^0/β^X, in which "x" indicates that the specific mutation was not identified.
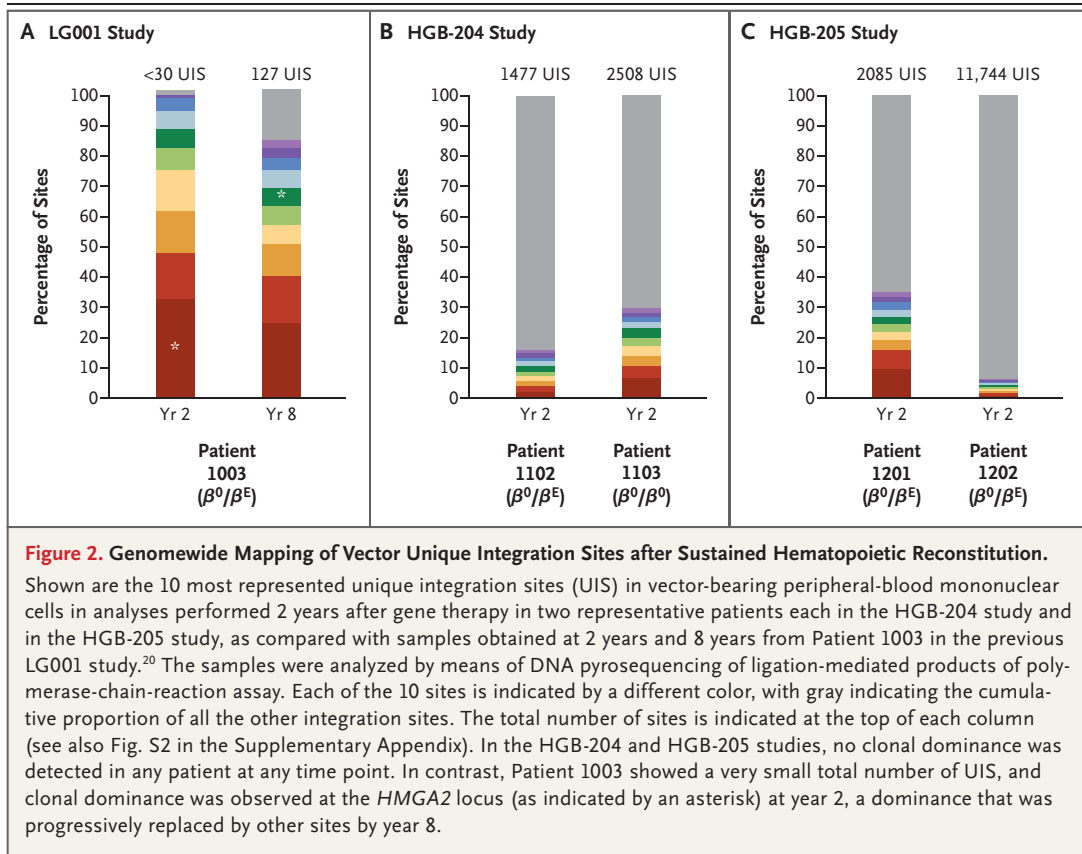
alent to that seen in patients with a β^0/β^0 genotype (Table 1 and Fig. 1). Three of the patients had started receiving red-cell transfusions before the age of 3 years, and 3 had undergone splenectomy. In the 2 years before enrollment, the median red-cell transfusion volume was 182 ml per kilogram per year (range, 139 to 197).

DRUG PRODUCT CHARACTERISTICS AND PRETRANSPLANTATION CONDITIONING

Patients underwent conditioning with a single agent, intravenous busulfan. Because complete myeloablation is essential for high-level engraftment with ex vivo transduced hematopoietic stem cells[27,28] and increased busulfan metabolism had been reported in some patients with β-thalassemia,[29] plasma busulfan pharmacokinetic analysis was performed in all the patients, either after the first busulfan injection (in HGB-204) or daily (in HGB-205).[30] Such analysis was

followed by dose adjustments to achieve appropriately targeted drug exposure. The average daily plasma busulfan area-under-the-curve values ranged from 3029 to 4714 μM per minute in HGB-204 (estimated values) and from 4670 to 5212 μM per minute in HGB-205 (actual values).

Among the HGB-204 study patients, the median dose of the drug product was 11.0 million CD34+ cells per kilogram (range, 6.1 million to 18.1 million) in patients with a β^0/β^0 genotype and 7.1 million CD34+ cells per kilogram (range, 5.2 million to 13.0 million) in those with other genotypes. The vector copy number in the drug products ranged from 0.3 to 1.5 (Table 1). In HGB-205, the dose was 8.8 million CD34+ cells per kilogram in the IVS1-110 homozygote and 12.0 million CD34+ cells per kilogram (range, 8.9 million to 13.6 million) in the 3 patients with a β^E/β^0 genotype. The vector copy number in the drug products ranged from 0.8 to 2.1 (Table 1).

Figure 2. Genomewide Mapping of Vector Unique Integration Sites after Sustained Hematopoietic Reconstitution.

Shown are the 10 most represented unique integration sites (UIS) in vector-bearing peripheral-blood mononuclear cells in analyses performed 2 years after gene therapy in two representative patients each in the HGB-204 study and in the HGB-205 study, as compared with samples obtained at 2 years and 8 years from Patient 1003 in the previous LG001 study.[20] The samples were analyzed by means of DNA pyrosequencing of ligation-mediated products of polymerase-chain-reaction assay. Each of the 10 sites is indicated by a different color, with gray indicating the cumulative proportion of all the other integration sites. The total number of sites is indicated at the top of each column (see also Fig. S2 in the Supplementary Appendix). In the HGB-204 and HGB-205 studies, no clonal dominance was detected in any patient at any time point. In contrast, Patient 1003 showed a very small total number of UIS, and clonal dominance was observed at the *HMGA2* locus (as indicated by an asterisk) at year 2, a dominance that was progressively replaced by other sites by year 8.

SAFETY

No safety issues were attributed to the BB305 vector in either study. In HGB-204, five mild adverse effects (all grade 1) were characterized as related or possibly related to the drug product. Other than the hematologic alterations that commonly occur after busulfan conditioning, all adverse events of grade 3 or higher that occurred in two or more patients are listed in Table S2 in the Supplementary Appendix. Nine serious adverse events were reported, including two episodes of veno-occlusive liver disease attributed to busulfan conditioning. In HGB-205, no serious adverse events were considered to be related to the drug product. All nonhematologic adverse events of grade 3 or higher are listed in Table S3 in the Supplementary Appendix. In the two studies, all adverse events were treated with standard measures.

No replication-competent lentivirus has been detected in the patients in either study, and serial monitoring of vector integration sites in blood samples has consistently shown polyclonal profiles of unique integration sites without domi-

nant clones. At 12 months after infusion, the median number of unique integration sites was 1646 per patient (range, 202 to 5501) in HGB-204 and 5322 (range, 756 to 8685) in HGB-205. Integration data for representative patients are shown in Figure 2, and in Figure S2 in the Supplementary Appendix.

These integration data include a comparison with findings in Patient 1003, who was enrolled in the previous LG001 study[20] and had initial partial clonal dominance at the *HMGA2* locus. This clonal expansion was followed to determine whether its presence predicted any adverse event. At the time of this report (year 12), the *HMGA2* locus was no longer dominant in Patient 1003 and had not been associated with serious adverse events. After receiving the drug product, Patient 1003 had received no red-cell transfusions during year 2 through year 8 while maintaining a total hemoglobin level of approximately 8 g per deciliter. During year 9 through year 12, the patient resumed sporadic transfusions, although the HbA^{T87Q} levels had remained stable above 2 g per deciliter since month 18[20] (data not shown).

Figure 3. Kinetics of Engraftment with Vector-Transduced Hematopoietic Stem Cells.

Shown is the average vector copy number (VCN) per diploid genome in peripheral-blood mononuclear cells (PBMCs) after drug product infusion in the HGB-204 and HGB-205 studies among patients with a non–β^0/β^0 genotype (Panel A) and in those with a β^0/β^0 genotype or a genotype of equivalent clinical severity (homozygous IVS1-110) (Panel B). The data for Patient 1121, the only patient with a non–β^0/β^0 genotype who had not yet stopped receiving red-cell transfusions at the last study visit, are indicated with a dotted line in Panel A. Data for three patients with clinically severe genotypes who had stopped receiving transfusions — Patient 1106 (blue squares) and Patient 1123 (blue triangles) with a β^0/β^0 genotype and Patient 1203 (red triangles) who was homozygous for the IVS1-110 mutation — are shown in Panel B. In a pooled analysis, there was significant correlation between the VCN in the drug product and the VCN in PBMCs at 6 months (Panel C).

HEMATOPOIETIC RECOVERY AND GENE MARKING OF BLOOD CELLS

After the intravenous infusion of the thawed LentiGlobin drug product, neutrophil engraftment occurred within a median of 18.5 days (range, 14.0 to 30.0) in HGB-204 and 16.5 days (range, 14.0 to 29.0) in HGB-205. Platelet engraftment occurred within a median of 39.5 days (range, 19.0 to 191.0) in HGB-204 and 23.0 days (range, 20.0 to 26.0) in HGB-205, during which time there were no bleeding complications resulting in serious adverse events.

The average vector copy numbers in PBMCs over time after drug product infusion in the two studies are shown in Figure 3. The median vector copy number at 15 months was 0.3 copies per diploid genome (range, 0.1 to 0.9) in HGB-204 and 2.0 copies per diploid genome (range, 0.3 to 4.2) in HGB-205. In a pooled analysis, there was significant correlation between the vector copy number in PBMCs at 6 months and the initial vector copy number in the drug product ($r^2=0.69$, P<0.001) (Fig. 3C).
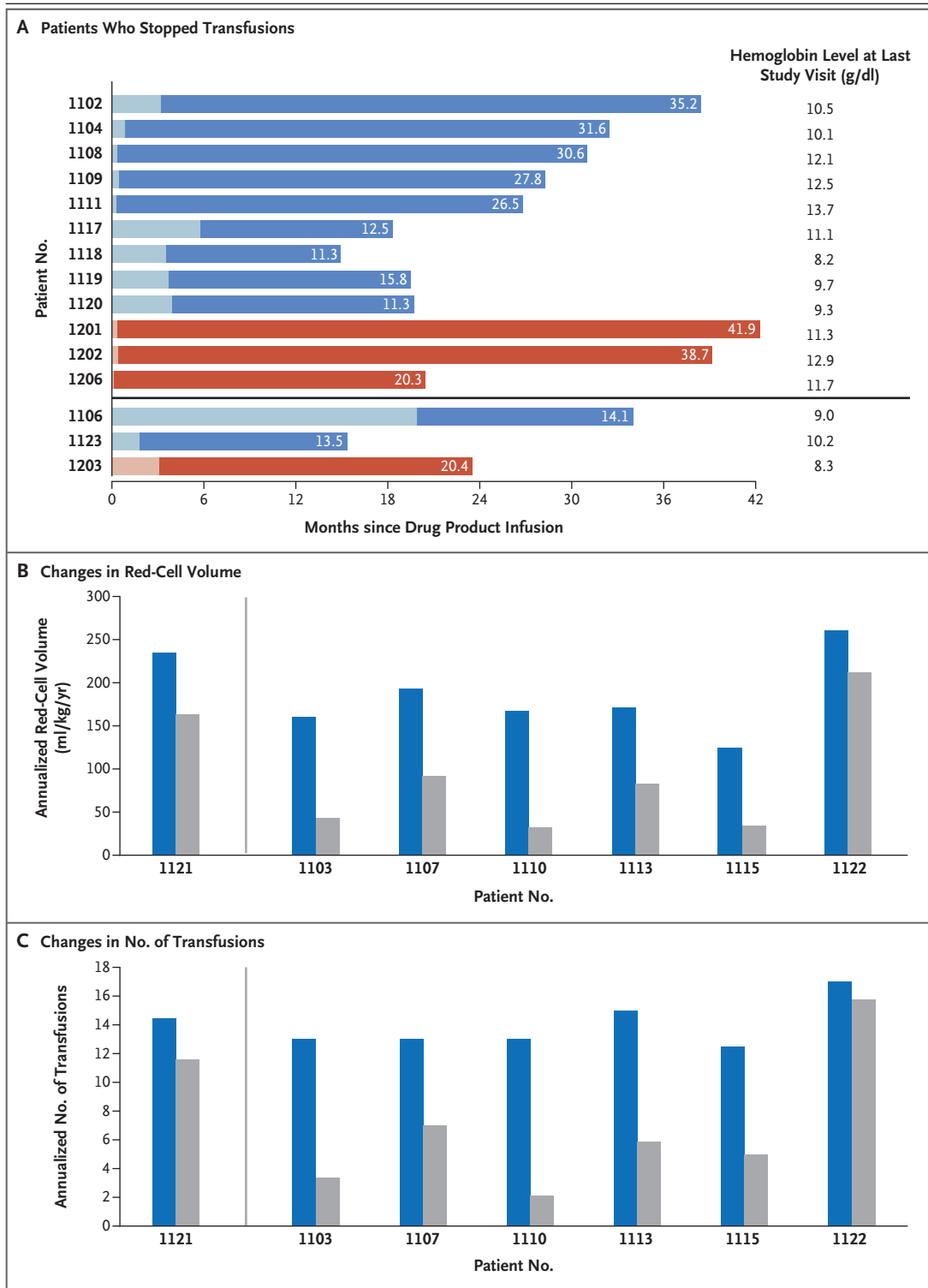
BLOOD HbA^{T87Q} LEVELS AND CHANGES IN TRANSFUSION REQUIREMENTS

Of the 22 patients who were treated, 13 had a non–β^0/β^0 genotype. Of these 13 patients, all but 1 stopped receiving red-cell transfusions after gene therapy. At the last study visit (12 to 36 months after infusion), the median HbA^{T87Q} level was 6.0 g per deciliter (range, 3.4 to 10.0), and the median total hemoglobin level was 11.2 g per
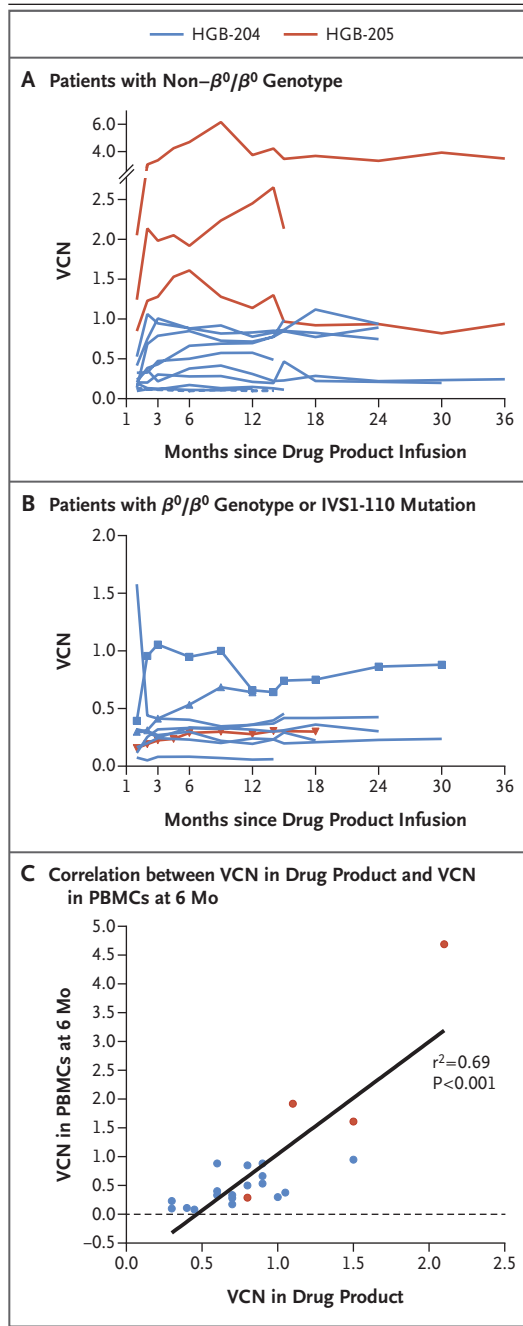
deciliter (range, 8.2 to 13.7) (Fig. 4A). Patient 1121, who had a vector copy number of 0.3 in the drug product, had a blood vector copy number of 0.10 at the last follow-up and continued to receive red-cell transfusions. In addition, Patient 1118 received a single transfusion 13 months after drug product infusion during an acute viral illness.

At the last study visit, of the 9 patients with a

A Patients Who Stopped Transfusions

Hemoglobin Level at Last
Study Visit (g/dl)

Patient No.	Value	Hemoglobin
1102	35.2	10.5
1104	31.6	10.1
1108	30.6	12.1
1109	27.8	12.5
1111	26.5	13.7
1117	12.5	11.1
1118	11.3	8.2
1119	15.8	9.7
1120	11.3	9.3
1201	41.9	11.3
1202	38.7	12.9
1206	20.3	11.7
1106	14.1	9.0
1123	13.5	10.2
1203	20.4	8.3

Months since Drug Product Infusion

B Changes in Red-Cell Volume

Annualized Red-Cell Volume (ml/kg/yr)

Patient No.: 1121, 1103, 1107, 1110, 1113, 1115, 1122

C Changes in No. of Transfusions

Annualized No. of Transfusions

Patient No.: 1121, 1103, 1107, 1110, 1113, 1115, 1122

β^0/β^0 genotype or homozygosity for the IVS1-110 mutation, 6 had a median HbA^{T87Q} level of 4.2 g per deciliter (range, 0.4 to 8.7) and continued to receive transfusions. However, there was a median reduction of 74% (range, 7 to 100) in the annual number of transfusions and a 73% reduction

(range, 19 to 100) in the annual transfusion volume, as compared with transfusion support in the 2 years before enrollment (Fig. 4B and 4C). The remaining 3 patients with a β^0/β^0 genotype or two copies of the IVS1-110 mutation had not received transfusions for 14 to 20 months (Fig. 4A). At the most recent follow-up (12 to 30 months), the patients' HbA^{T87Q} level ranged from 6.6 to 8.2 g per deciliter, and the total hemoglobin level ranged from 8.3 to 10.2 g per deciliter. Additional characteristics of this subgroup of patients are summarized in Table S5 in the Supplementary Appendix. Table 2 provides a summary of the outcomes in the 22 patients.

In the two studies, blood HbA^{T87Q} levels correlated with blood vector copy number levels ($r^2 = 0.75$, P<0.001) (Fig. 5C). Other factors, such as age, genotype, and splenectomy status, did not appear to correlate with gene expression. The studies were not powered to conclusively assess determinants of response. The hemoglobin fractions that contributed to total hemoglobin levels over time and the timing of red-cell transfusions in representative patients with β^0/β^0, β^E/β^0, and other non–β^0/β^0 genotypes are shown in Figure S1 in the Supplementary Appendix. Hemoglobin fractions in erythroid burst-forming units in the four patients in HGB-205 are shown in Table S4 in the Supplementary Appendix.

EFFECT ON HEMOLYSIS AND DYSERYTHROPOIESIS

In HGB-205, in an exploratory analysis that was performed among patients who had stopped receiving red-cell transfusions after gene therapy, the degree of hemolysis at first stabilized relative to pretransplantation levels and was fully corrected in Patients 1201 and 1202 by 36 months after treatment (Table S6 in the Supplementary Appendix). Because strict adherence to iron chelation therapy was difficult in the 3 patients with a β^E/β^0 genotype, they underwent regular phlebotomy, in which 200 ml of blood was withdrawn each month. At the time of the last study visit, the patients' hemoglobin levels were stable, despite a cumulative phlebotomy volume of more than 1 liter per patient. Patient 1203 was receiving deferiprone to remove excess iron. The tissue iron content in the patient's heart and liver is being followed with the use of magnetic resonance imaging (MRI), and normalization of values was awaited before the discontinuation of iron chelation or phlebotomy.[9] Such discontinuation of treatment was reported in Patient 1202, who is no longer receiving iron chelation therapy and who completed phlebotomy 36 months after gene therapy.

In addition, we investigated biologic markers of dyserythropoiesis (Fig. 6, and Table S6 in the Supplementary Appendix).[31] Plasma levels of two markers of ineffective erythropoiesis or erythroid expansion, soluble transferrin receptor[32] and erythroid protoporphyrin IX,[33] were within normal ranges for the 3 patients with a β^E/β^0 genotype after they had stopped receiving transfusions. In addition, the plasma ratio of hepcidin to ferritin increased substantially over time in these patients. The normalization in markers of dyserythropoiesis correlated with the hemoglobin levels that were achieved. Together, these results point to the elimination of dyserythropoiesis in the 3 patients with a β^E/β^0 genotype who were treated. In contrast, complete normalization of biologic markers of ineffective erythropoiesis was not observed in Patient 1203 (IVS1-110 homozygote) (Fig. 6, and Table S6 in the Supplementary Appendix).

DISCUSSION

Gene therapy in patients with transfusion-dependent β-thalassemia requires efficient transduction of hematopoietic stem and progenitor cells

Table 2. Summary of Outcomes in the 22 Study Patients.*						
Patient No.	Genotype	Last Study Visit	HbA^{T87Q} at Last Visit	Total Hemoglobin at Last Visit	Still Receiving Transfusions	Time since Last Transfusion†
		mo after infusion	*g/dl*			*mo*
1102	β^E/β^0	36	5.4	10.5	No	35.2
1103	β^0/β^0	24	8.7	9.8	Yes	NA
1104	β^E/β^0	30	4.8	10.1	No	31.6
1106	β^0/β^0	30	8.2	9.0	No	14.1
1107	β^0/β^0	30	4.1	8.6	Yes	NA
1108	β^0/β^+	24	9.6	12.1	No	30.6
1109	β^0/β^X	24	5.7	12.5	No	27.8
1110	β^0/β^0	24	4.3	8.8	Yes	NA
1111	β^E/β^0	24	8.0	13.7	No	26.5
1113	β^0/β^0	21	2.6	11.3	Yes	NA
1115	β^0/β^0	18	7.2	9.0	Yes	NA
1117	β^E/β^0	15	6.3	11.1	No	12.5
1118	β^E/β^0	12	3.4	8.2	No	11.3
1119	β^+/β^+	18	5.6	9.7	No	15.8
1120	β^E/β^0	18	3.6	9.3	No	15.8
1121	β^+/β^+	15	1.1	10.6	Yes	NA
1122	β^0/β^0	15	0.4	10.3	Yes	NA
1123	β^0/β^0	12	6.8	10.2	No	13.5
1201	β^E/β^0	36	8.2	11.3	No	41.9
1202	β^E/β^0	36	10.0	12.9	No	38.7
1203	β^+/β^+	21	6.6	8.3	No	20.4
1206	β^E/β^0	18	8.4	11.7	No	20.3

* Listed are the values a median of 26 months (range, 15 to 42) after drug product infusion. NA denotes not applicable.
† The time since the last red-cell transfusion was calculated according to the data cutoff date, which might have been later than the last study visit.

as well as stable, erythroid-specific expression of the globin gene at a level that is clinically effective. The feasibility of the addition of a gene to autologous hematopoietic cells using a lentiviral vector was shown previously in a single patient with β-thalassemia who safely discontinued transfusions for more than 6 years with ongoing, sustained β^{A-T87Q}-globin expression.[14,20] Here, in two studies involving 22 treated patients — HGB-204 (on three continents) and HGB-205 (in France) — we have expanded on this proof of concept by investigating the safety and efficacy of gene therapy for β-thalassemia with a lentiviral vector (BB305) that is similar to our initial HPV569 vector. The use of a marked human

β^{A-T87Q}-globin gene allowed for quantification of vector-encoded globin in all β-thalassemia genotypes. Although the two studies were conducted independently, they were broadly similar with slight differences in design (Table S7 in the Supplementary Appendix). The BB305 vector is currently being used in studies of gene therapy in sickle cell disease.[25,34]

With follow-up extending to 3 years (median, 26 months) after infusion, no serious adverse events related to the drug product have been detected, and there were no significant unexpected safety issues. Although the theoretical possibility of genotoxicity by semirandom vector integration in the genome remains, the risk ap-
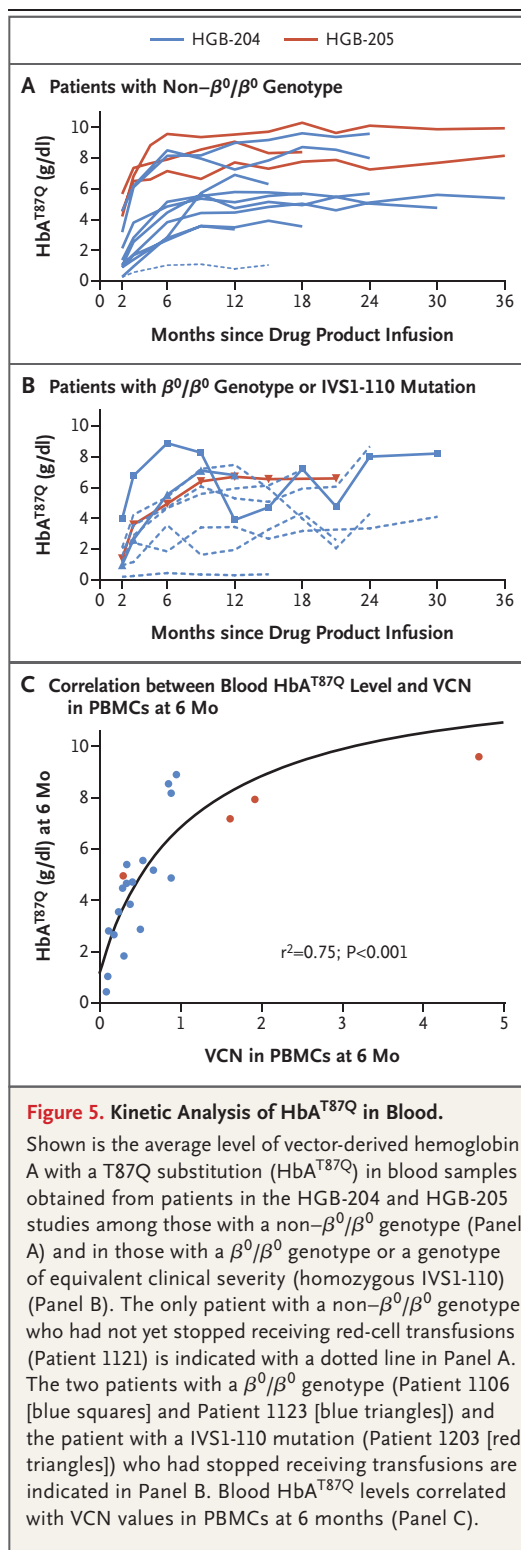
pears to be reduced with self-inactivating retroviral[35] and lentiviral[36] vectors and lineage-restricted promoters.[36] The relative clonal dominance at the *HMGA2* locus that was initially observed in 1 patient in the LG001 study of the HPV569 vector spontaneously subsided while that patient maintained stable HbA^{T87Q} levels. BB305 and other lentiviral vectors have been used in more than 50 patients thus far without vector-related adverse events. Nonetheless, long-term follow-up is critical, and we intend to follow all the patients for a total of 15 years or more in the LTF-303 study (ClinicalTrials.gov number, NCT02633943).

In the HGB-205 study, all the steps were performed at Necker Children's Hospital in Paris, whereas in the HGB-204 study, a central manufacturing facility prepared the drug product for all 18 patients. The latter process enabled high-quality, high-capacity production with minimal procedural variability across the clinical sites, and our experience suggests that these procedures could be adapted for worldwide clinical use.

In the two studies, the vector copy number in the drug product was found to correlate with the vector copy number in PBMCs, which in turn correlated with the production of vector-encoded β^{A-T87Q}-globin. Consequently, the vector copy number in the drug product appears to be a key determinant of HbA^{T87Q} levels in blood, which were similar across β-thalassemia genotypes. The pre-enrollment transfusion requirement, spleen status, and age were not observed to have an effect on the study outcome.

Although the studies were not designed to test specific hypotheses about differences in the characteristics of the patients or the drug products, clinical outcomes appeared to vary according to the underlying genotype. All 12 patients with a non–β^0/β^0 genotype who achieved and maintained a vector copy number of more than 0.1 in PBMCs, including the 9 patients with a β^E/β^0 genotype, stopped receiving red-cell transfusions, with steady-state hemoglobin levels of at least 9 g per deciliter (>8 g per deciliter in Patient 1118) and normal or nearly normal total hemoglobin levels in several patients (Table 2).

In contrast, in the HGB-204 study, among the 8 patients with a β^0/β^0 genotype (for whom the threshold HbA^{T87Q} level that was necessary for discontinuation of red-cell transfusion was higher in the absence of endogenous production of β-like globin chains), transfusion support after

Figure 5. Kinetic Analysis of HbA^{T87Q} in Blood.

Shown is the average level of vector-derived hemoglobin A with a T87Q substitution (HbA^{T87Q}) in blood samples obtained from patients in the HGB-204 and HGB-205 studies among those with a non–β^0/β^0 genotype (Panel A) and in those with a β^0/β^0 genotype or a genotype of equivalent clinical severity (homozygous IVS1-110) (Panel B). The only patient with a non–β^0/β^0 genotype who had not yet stopped receiving red-cell transfusions (Patient 1121) is indicated with a dotted line in Panel A. The two patients with a β^0/β^0 genotype (Patient 1106 [blue squares] and Patient 1123 [blue triangles]) and the patient with a IVS1-110 mutation (Patient 1203 [red triangles]) who had stopped receiving transfusions are indicated in Panel B. Blood HbA^{T87Q} levels correlated with VCN values in PBMCs at 6 months (Panel C).

treatment was reduced but not eliminated in 6 patients, and 2 of these patients have stopped receiving transfusions. In addition, in HGB-205,
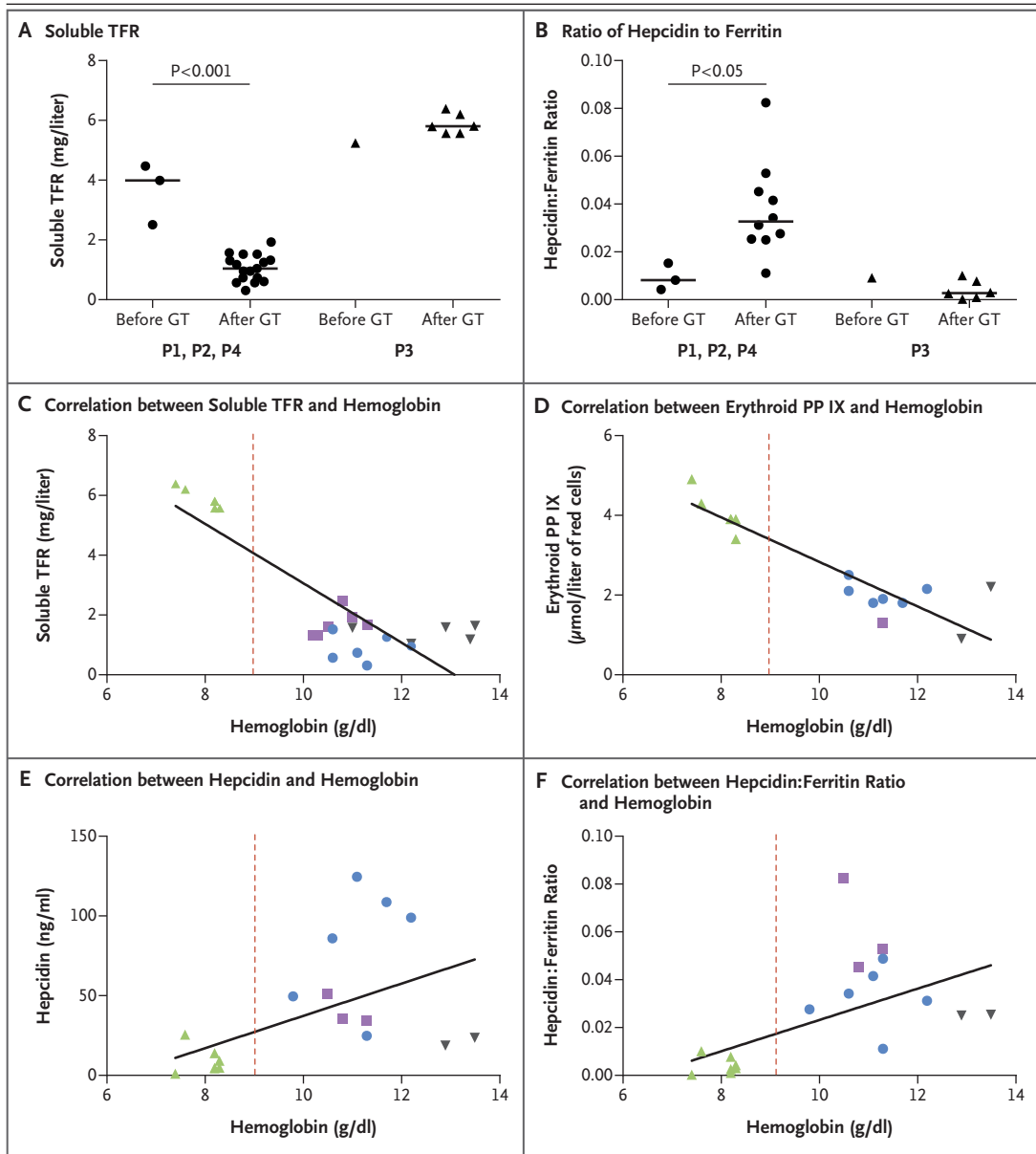
Figure 6. Iron Metabolism and Abatement of Dyserythropoiesis in the HGB-205 Study.

Shown is the progressive decrease in the plasma level of soluble transferrin receptor (TFR) (Panel A) and increase in the hepcidin:ferritin ratio (Panel B), as indicated by solid circles representing data for three patients — Patient 1201 (P1), Patient 1202 (P2), and Patient 1206 (P4), all of whom had a β^E/β^0 genotype — before and after gene therapy (GT). Data for Patient 1203 (P3, triangles), who was homozygous for the IVS1-110 mutation, showed no change in either marker. The entire follow-up data for the three patients with a β^E/β^0 genotype (from 3 to 36 months) are shown with horizontal bars indicating medians. The data were pooled and compared with pooled values at screening with the use of a two-tailed Wilcoxon rank-sum test. Also shown are the correlations between hemoglobin levels and markers of ineffective erythropoiesis and iron homeostasis, including soluble TFR (Panel C), erythroid protoporphyrin (PP) IX (Panel D), hepcidin (Panel E), and the ratio of hepcidin to ferritin (Panel F). In Panels C through F, data for Patient 1201 are indicated by squares, Patient 1202 by gray triangles, Patient 1203 by green triangles, and Patient 1206 by circles. The Spearman test was used to assess correlations between biologic variables. Hemoglobin levels had a negative correlation with soluble TFR levels (Spearman $r=-0.63$, $P<0.001$) and erythroid protoporphyrin IX levels in red cells (Spearman $r=-0.85$, $P<0.001$) and a positive correlation with hepcidin levels and hepcidin:ferritin ratios (Spearman $r=0.57$, $P=0.01$ for both calculations). At values of more than 9.5 g per deciliter of hemoglobin, the erythroid mass was reduced and peripheral iron control was restored by hepcidin. Below 8.5 g per deciliter, ineffective erythropoiesis was maintained and hepcidin repressed, as indicated by the vertical red dotted line separating the two performance levels.

the patient with two copies of the IVS1-110 mutation, in whom the clinical severity mirrored that of patients with a β^0/β^0 genotype, has also discontinued receiving transfusions.

Of the 22 treated patients, 7 expressed at least 8 g per deciliter of HbA[T87Q], including 3 patients with a β^0/β^0 genotype or the severe IVS1-110 mutation; all have been free from transfusion for more than 1 year. These patients had a vector copy number of more than 0.6 in the drug product. These results suggest that achieving transfusion independence is an attainable goal for most patients with a β^0/β^0 genotype if the rates of hematopoietic-cell transduction can be further improved.

Preliminary assessments of dyserythropoiesis in the 3 patients with a β^E/β^0 genotype who were enrolled in HGB-205 showed stabilization and then correction of hemolysis, and biologic hallmarks of dyserythropoiesis were largely eliminated. One patient (Patient 1202) who no longer had any evidence of dyserythropoiesis and iron overload was able to discontinue both iron chelation therapy and therapeutic phlebotomy.

Limited changes in the LentiGlobin vector (HPV569 vs. BB305) did not significantly alter the expression of β^{A-T87Q}-globin per integrated vector copy, but they contributed (along with changes in vector manufacturing) to an increase in vector titers by a factor of approximately 4.[14,24,37] In addition, an important advance over the previous LG001 study was the high degree of purification of vector particles by ion-exchange chromatography. Consequently, the numbers of unique integration sites that were found in the patients in HGB-204 and HGB-205 were higher by a factor of 150 to 500 than the numbers of sites in patients who were enrolled in the LG001 study at the same time points. The very low dose of vector-transduced hematopoietic cells that was received by Patient 1003 in the LG001 study was a major contributor to the development of oligoclonality. In the current studies, genomewide mapping of chromosomal sites of vector integration showed a highly polyclonal pattern with no dominance observed.

In addition, two other protocol-related features may have had an effect on efficacy: an enhanced series of red-cell transfusions before harvesting of CD34+ cells to mitigate the dilution of hematopoietic-cell targets in the expanded erythroid CD34+ cell compartment in patients with β-thalassemia and monitoring of plasma busulfan concentrations with dose adjustments to optimize myeloablation.[30] The number of patients who were treated remains relatively small, so extended follow-up is required to establish the durability of transduction hematopoietic stem cells and progenitor cells after a single infusion. Long-term surveillance of treatment-related toxicity related to the transfer of genes into hematopoietic stem cells and progenitor cells, busulfan conditioning, or both will also be necessary to define the therapeutic profile of this new treatment approach.

Another phase 1/2 study of lentiviral vector–mediated gene therapy in patients with β-thalassemia is ongoing in Italy and has shown reductions in transfusion requirements,[14] whereas an earlier study at Memorial Sloan Kettering Cancer Center was suspended to allow for testing of a revised lentiviral vector.[13] Also under investigation are alternative approaches for the treatment of β-thalassemia, such as activin receptor ligand trap and Janus kinase inhibitors,[38] allogeneic transplantation with alternative conditioning regimens,[28,39] and gene editing.[40]

In conclusion, we found that gene therapy with LentiGlobin drug product succeeded in overcoming a principal limitation of allogeneic hematopoietic-cell transplantation, which is a lack of a histocompatible donor. The safety profile after infusion was consistent with that associated with myeloablative conditioning with single-agent busulfan. In several treated patients, hemoglobin levels reached or approached normal ranges, thereby correcting dyserythropoiesis. One patient also had normalization of iron overload and was able to discontinue both iron chelation and therapeutic phlebotomy. Although several patients with a β^0/β^0 genotype or equivalent disease have stopped receiving red-cell transfusions, and ongoing improvements in manufacturing of the drug product hold promise for achieving similar results in most patients with a β^0/β^0 genotype, even the partial reduction in transfusion requirements that we observed in these patients may result in a reduction in iron loading (and thereby long-term damage to target organs) and in increased life expectancy.

APPENDIX

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REFERENCES

1. Piel FB. The present and future global burden of the inherited disorders of hemoglobin. Hematol Oncol Clin North Am 2016;30:327-41.

2. Cao A, Galanello R. Beta-thalassemia. Genet Med 2010;12:61-76.

3. Arlet JB, Ribeil JA, Guillem F, et al. HSP70 sequestration by free α-globin promotes ineffective erythropoiesis in β-thalassaemia. Nature 2014;514:242-6.

4. Thein SL. Pathophysiology of beta thalassemia — a guide to molecular therapies. Hematology Am Soc Hematol Educ Program 2005;1:31-7.

5. Olivieri NF, Pakbaz Z, Vichinsky E. Hb E/beta-thalassaemia: a common & clinically diverse disorder. Indian J Med Res 2011;134:522-31.

6. Lucarelli G, Isgrò A, Sodani P, Gaziev J. Hematopoietic stem cell transplantation in thalassemia and sickle cell anemia. Cold Spring Harb Perspect Med 2012;2(5): a011825.

7. Locatelli F, Kabbara N, Ruggeri A, et al. Outcome of patients with hemoglobinopathies given either cord blood or bone marrow transplantation from an HLA-identical sibling. Blood 2013;122:1072-8.

8. Baronciani D, Angelucci E, Potschger U, et al. Hemopoietic stem cell transplantation in thalassemia: a report from the European Society for Blood and Bone Marrow Transplantation Hemoglobinopathy Registry, 2000-2010. Bone Marrow Transplant 2016;51:536-41.

9. Engert A, Balduini C, Brand A, et al. The European Hematology Association Roadmap for European Hematology Research: a consensus document. Haematologica 2016;101:115-208.

10. Tubman VN, Fung EB, Vogiatzi M, et al. Guidelines for the standard monitoring of patients with thalassemia: report of the Thalassemia Longitudinal Cohort. J Pediatr Hematol Oncol 2015;37(3):e162-e169.

11. Bonifazi F, Conte R, Baiardi P, et al. Pattern of complications and burden of disease in patients affected by beta thalassemia major. Curr Med Res Opin 2017; 33:1525-33.

12. Malik P. Gene therapy for hemoglobinopathies: tremendous successes and remaining caveats. Mol Ther 2016;24:668-70.

13. Mansilla-Soto J, Riviere I, Boulad F, Sadelain M. Cell and gene therapy for the beta-thalassemias: advances and prospects. Hum Gene Ther 2016;27:295-304.

14. Negre O, Eggimann AV, Beuzard Y, et al. Gene therapy of the β-hemoglobinopathies by lentiviral transfer of the β(A(T87Q))-globin gene. Hum Gene Ther 2016;27:148-65.

15. Ferrari G, Cavazzana M, Mavilio F. Gene therapy approaches to hemoglobinopathies. Hematol Oncol Clin North Am 2017;31:835-52.

16. Naldini L, Blömer U, Gallay P, et al. In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science 1996;272:263-7.

17. May C, Rivella S, Callegari J, et al. Therapeutic haemoglobin synthesis in beta-thalassaemic mice expressing lentivirus-encoded human beta-globin. Nature 2000; 406:82-6.

18. Pawliuk R, Westerman KA, Fabry ME, et al. Correction of sickle cell disease in transgenic mouse models by gene therapy. Science 2001;294:2368-71.

19. Bank A, Dorazio R, Leboulch P. A phase I/II clinical trial of beta-globin gene therapy for beta-thalassemia. Ann N Y Acad Sci 2005;1054:308-16.

20. Cavazzana-Calvo M, Payen E, Negre O, et al. Transfusion independence and HMGA2 activation after gene therapy of human β-thalassaemia. Nature 2010;467: 318-22.

21. Tuan D, Solomon W, Li Q, London IM. The "beta-like-globin" gene domain in human erythroid cells. Proc Natl Acad Sci U S A 1985;82:6384-8.

22. Grosveld F, van Assendelft GB, Greaves DR, Kollias G. Position-independent, high-level expression of the human beta-globin gene in transgenic mice. Cell 1987;51:975-85.

23. Takekoshi KJ, Oh YH, Westerman KW, London IM, Leboulch P. Retroviral transfer of a human beta-globin/delta-globin hybrid gene linked to beta locus control region hypersensitive site 2 aimed at the gene therapy of sickle cell disease. Proc Natl Acad Sci U S A 1995;92:3014-8.

24. Negre O, Bartholomae C, Beuzard Y, et al. Preclinical evaluation of efficacy and safety of an improved lentiviral vector for the treatment of β-thalassemia and sickle cell disease. Curr Gene Ther 2015; 15:64-81.

25. Ribeil JA, Hacein-Bey-Abina S, Payen E, et al. Gene therapy in a patient with sickle cell disease. N Engl J Med 2017;376:848-55.

26. Westerman KA, Ao Z, Cohen EA, Leboulch P. Design of a trans protease lentiviral packaging system that produces high titer virus. Retrovirology 2007;4:96.

27. Hayakawa J, Ueda T, Lisowski L, et al. Transient in vivo beta-globin production after lentiviral gene transfer to hematopoietic stem cells in the nonhuman primate. Hum Gene Ther 2009;20:563-72.

28. Chandrasekaran D, Nakamoto B, Watts KL, Kiem HP, Papayannopoulou T. Modeling promising nonmyeloablative conditioning regimens in nonhuman primates. Hum Gene Ther 2014;25:1013-22.

29. Shteyer E, Nitzan I, Godfarb A, Hemed N, Revel-Vilk S. Activity of cytochrome P450 1A2 in relation to hepatic iron accumulation in transfusion-dependent β-thalassaemia major patients. Vox Sang 2015;108:268-73.

30. Bourget P, Falaschi L, Suarez F, et al. A medical-pharmaceutical partnership model as a contributor to the success in conditioning regimen for allogenic hematopoietic stem cell transplantation in adults: a cross-reflection on our organizations. Bull Cancer 2012;99:643-53. (In French.)

31. Porter JB, Cappellini MD, Kattamis A, et al. Iron overload across the spectrum of non-transfusion-dependent thalassaemias: role of erythropoiesis, splenectomy and transfusions. Br J Haematol 2017;176:288-99.

32. Ricchi P, Ammirabile M, Costantini S, et al. Soluble form of transferrin receptor as a biomarker of overall morbidity in patients with non-transfusion-dependent thalassaemia: a cross-sectional study. Blood Transfus 2016;14:538-40.

33. Graham EA, Felgenhauer J, Detter JC, Labbe RF. Elevated zinc protoporphyrin associated with thalassemia trait and hemoglobin E. J Pediatr 1996;129:105-10.

34. Kanter J, Walters MC, Hsieh MM, et al. Interim results from a phase 1/2 clinical study of LentiGlobin gene therapy for severe sickle cell disease. Blood 2016;128: 1176. abstract.

35. Hacein-Bey-Abina S, Pai S-Y, Gaspar HB, et al. A modified γ-retrovirus vector for X-linked severe combined immunodeficiency. N Engl J Med 2014;371:1407-17.

36. Schambach A, Zychlinski D, Ehrnstroem B, Baum C. Biosafety features of lentiviral vectors. Hum Gene Ther 2013; 24:132-42.

37. Walters MC, Thompson A, Hongeng S, et al. A phase 3 study to evaluate safety and efficacy of LentiGlobin gene therapy for transfusion-dependent beta-thalassemia in patients with non-beta0/beta0 genotypes: the Northstar-2 (HGB-207) trial. Haematologica 2017;102:335. abstract.

38. Motta I, Scaramellini N, Cappellini MD. Investigational drugs in phase I and phase II clinical trials for thalassemia. Expert Opin Investig Drugs 2017;26:793-802.

39. Anurathapan U, Pakakasama S, Mekjaruskul P, et al. Outcomes of thalassemia patients undergoing hematopoietic stem cell transplantation by using a standard myeloablative versus a novel reduced-toxicity conditioning regimen according to a new risk stratification. Biol Blood Marrow Transplant 2014;20:2066-71.

40. Dever DP, Bak RO, Reinisch A, et al. CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. Nature 2016;539:384-9.