ORIGINAL ARTICLE

Betibeglogene Autotemcel Gene Therapy for Non–β^0/β^0 Genotype β-Thalassemia

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ABSTRACT

BACKGROUND

Betibeglogene autotemcel (beti-cel) gene therapy for transfusion-dependent β-thalassemia contains autologous CD34+ hematopoietic stem cells and progenitor cells transduced with the BB305 lentiviral vector encoding the β-globin ($\beta^{A\text{-}T87Q}$) gene.

METHODS

In this open-label, phase 3 study, we evaluated the efficacy and safety of beti-cel in adult and pediatric patients with transfusion-dependent β-thalassemia and a non–β^0/β^0 genotype. Patients underwent myeloablation with busulfan (with doses adjusted on the basis of pharmacokinetic analysis) and received beti-cel intravenously. The primary end point was transfusion independence (i.e., a weighted average hemoglobin level of ≥9 g per deciliter without red-cell transfusions for ≥12 months).

RESULTS

A total of 23 patients were enrolled and received treatment, with a median follow-up of 29.5 months (range, 13.0 to 48.2). Transfusion independence occurred in 20 of 22 patients who could be evaluated (91%), including 6 of 7 patients (86%) who were younger than 12 years of age. The average hemoglobin level during transfusion independence was 11.7 g per deciliter (range, 9.5 to 12.8). Twelve months after beti-cel infusion, the median level of gene therapy–derived adult hemoglobin (HbA) with a T87Q amino acid substitution (HbA^{T87Q}) was 8.7 g per deciliter (range, 5.2 to 10.6) in patients who had transfusion independence. The safety profile of beti-cel was consistent with that of busulfan-based myeloablation. Four patients had at least one adverse event that was considered by the investigators to be related or possibly related to beti-cel; all events were nonserious except for thrombocytopenia (in 1 patient). No cases of cancer were observed.

CONCLUSIONS

Treatment with beti-cel resulted in a sustained HbA^{T87Q} level and a total hemoglobin level that was high enough to enable transfusion independence in most patients with a non–β^0/β^0 genotype, including those younger than 12 years of age. (Funded by Bluebird Bio; HGB-207 ClinicalTrials.gov number, NCT02906202.)

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β-thalassemia is caused by β-globin gene (*HBB*) mutations that either reduce (β^+) or abrogate (β^0) production of functional β-globin.[1,2] In β-thalassemia, an excess of unpaired α-globin impedes red-cell development and survival, leading to ineffective erythropoiesis, hemolysis, chronic anemia, and compromised quality of life.[2-6] Patients with severe anemia receive lifelong red-cell transfusions and regular iron chelation to prevent iron overload.[2,6-8]

Allogeneic hematopoietic stem-cell transplantation is a potentially curative therapy for β-thalassemia,[1,9-11] with the best outcomes in patients younger than 14 years of age who have an HLA-identical donor.[12,13] However, hematopoietic stem-cell transplantation is limited by a lack of suitable, unaffected, histocompatible donors and by the risk of transplantation-related death, graft failure, and graft-versus-host disease.[14-16]

Gene therapy with betibeglogene autotemcel (beti-cel; Bluebird Bio) is being evaluated in patients with transfusion-dependent β-thalassemia. Beti-cel adds functional copies of a modified *HBB* containing an amino acid substitution (T→Q) at position 87 and β-globin regulatory elements into hematopoietic stem cells through autologous CD34+ cells transduced with the replication-defective, self-inactivating BB305 lentiviral vector.[17,18]

In two phase 1–2 clinical studies (HGB-204 and HGB-205) involving adolescents and adults, 11 of 14 patients with β-thalassemia and a non–β^0/β^0 genotype had transfusion independence after infusion of beti-cel.[19-21] In these patients, however, the weighted average hemoglobin levels after infusion, which ranged from 9.1 to 13.2 g per deciliter, were often lower than normal levels.[20,21] (Weighted average hemoglobin levels are defined in the Supplementary Appendix, available with the full text of this article at NEJM.org.) The vector copy number and the percentage of lentiviral vector–positive cells in beti-cel were shown to be associated with hemoglobin levels; therefore, the transduction process was refined to increase the vector copy number in beti-cel and, consequently, to increase the levels of gene therapy–derived adult hemoglobin (HbA) with a T87Q amino acid substitution (HbA^{T87Q}). We report the results of the ongoing phase 3 HGB-207 (Northstar-2) study, which involved the use of gene therapy with beti-cel that was manufactured with the use of the refined process. In addition to adolescents and adults, this study included 8 patients who were younger than 12 years of age.

METHODS

STUDY DESIGN AND OVERSIGHT

This single-group, open-label, single-dose, phase 3 efficacy and safety study was initiated in July 2016 in patients with transfusion-dependent β-thalassemia. The last patient received beti-cel in January 2020; follow-up is ongoing at nine centers in France, Germany, Italy, Thailand, the United Kingdom, and the United States. After the 2-year study period, the patients could participate in a long-term follow-up study (LTF-303; ClinicalTrials.gov number, NCT02633943) for 13 additional years.

The target enrollment was 23 patients. Cohort 1 consisted of 15 patients who were 12 to 50 years of age, and cohort 2 consisted of 8 patients who were younger than 12 years of age.

All the authors generated, collected, and had access to the clinical data and confirm that the study was conducted in accordance with the protocol (available at NEJM.org). The authors and the sponsor, Bluebird Bio, interpreted the data, and an independent data monitoring committee regularly reviewed all safety data. All the authors reviewed and approved the manuscript for publication and vouch for the accuracy and completeness of the data. Medical writing support was funded by Bluebird Bio.

PATIENTS

Patients with β-thalassemia who were 50 years of age or younger were eligible for enrollment in the study. Enrollment was restricted to patients with transfusion dependence (i.e., those who had received transfusions of ≥100 ml per kilogram of body weight of packed red cells per year or who had disease that had been managed under standard thalassemia guidelines,[6] with ≥eight transfusions per year in the 2 years before enrollment). Patients with β^0/β^0 genotypes were excluded. In this study, the *HBB* mutation IVS-I-110 was considered to be equivalent to a β^0 mutation. Patients were also excluded if they had T2*-weighted magnetic resonance imaging (MRI) measurements of myocardial iron of less than 10 msec or other evidence of severe iron overload or a known available HLA-matched family donor. If

required by a regional regulatory authority, patients with available matched unrelated donors were also excluded.

Complete eligibility criteria are listed in Table S1 in the Supplementary Appendix. Patients who were 18 years of age or older provided written informed consent. For patients younger than 18 years of age, written informed consent was provided by a parent or legal guardian, and the patient provided assent.

GENE THERAPY

Peripheral-blood hematopoietic stem cells for centralized manufacturing and potential rescue, if necessary, were collected through mobilization with granulocyte colony-stimulating factor and plerixafor, followed by apheresis. Details regarding cell collection and conditioning and the methods for assessing the percentage of lentiviral vector–positive cells in beti-cel are described in the Supplementary Methods section in the Supplementary Appendix. The CD34+ cell population was isolated with the use of a CliniMACS system (Miltenyi Biotec).

The CD34+ cells were then activated ex vivo with a mixture of recombinant human cytokines, fms-like tyrosine kinase receptor 3, stem-cell factor, and thrombopoietin. The cells were then washed and transduced ex vivo with BB305 lentiviral vector. The transduction medium used in the current study was refined by adding components to improve the efficiency of transduction; these components had not been included in the previous two studies.[19-21] The transduced cells were cryopreserved in a medium containing 5% dimethylsulfoxide and were stored in the vapor phase of liquid nitrogen. Testing for sterility, endotoxin, and mycoplasma was conducted before cryopreservation.

The patients underwent myeloablative conditioning with busulfan (with doses adjusted on the basis of pharmacokinetic analysis) over a period of 4 days. After a minimum 48-hour washout period, beti-cel was thawed and infused intravenously. The target cell dose was at least 5.0 million CD34+ cells per kilogram of body weight. Eligibility criteria for conditioning are presented in Table S2. Neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count of at least 500 cells per cubic millimeter. Platelet engraftment was defined as the first of three consecutive platelet counts of at least 20,000 cells per cubic millimeter for at least 7 days after the last platelet transfusion.

EFFICACY AND SAFETY EVALUATIONS

The primary end point of the study was transfusion independence. Transfusion independence was defined as a weighted average hemoglobin level of at least 9 g per deciliter starting 60 days after the last transfusion in patients who had not received red-cell transfusions for 12 months or longer.

The secondary efficacy end points included the characteristics of transfusion independence (e.g., the duration of transfusion independence, as well as the total hemoglobin and gene therapy–derived HbA^{T87Q} levels over time), a decrease in the number of transfusions, treatments to reduce iron levels after infusion, and the change in the iron burden over time. Pharmacodynamic end points included HbA^{T87Q} levels, the vector copy number in peripheral-blood mononuclear cells (PBMCs), the correlation between the vector copy number in PBMCs at 6 months and the vector copy number in beti-cel (measured by means of quantitative polymerase-chain-reaction assay), and the correlation between the vector copy number in PBMCs and beti-cel and HbA^{T87Q} levels. The change in ineffective erythropoiesis was evaluated with the use of reticulocyte, nucleated red-cell, serum transferrin receptor, hepcidin, and erythropoietin studies.

Safety evaluations included engraftment, survival, and adverse events. In addition, these evaluations included assessment of vector insertion sites and monitoring to detect vector-derived replication-competent lentivirus or cancer.

STATISTICAL ANALYSIS

The primary population for this analysis consisted of patients who received beti-cel. Protocol-defined success criteria were transfusion independence in 9 of 15 patients (60%) in cohort 1 and 5 of 8 patients (62%) in cohort 2. For categorical variables, summary tabulations of the number and percentage of patients are presented. For continuous variables, the number of patients and the median, minimum, and maximum values are presented. Selected parameters are described with summary statistics.

RESULTS

CHARACTERISTICS OF THE PATIENTS

Eight of 32 enrolled patients were deemed to be ineligible for the study or withdrew; 23 received beti-cel. Data are presented through March 9, 2021. The median duration of follow-up was 29.5 months (range, 13.0 to 48.2). The enrollment and treatment status and baseline characteristics of the patients are summarized in Table 1, and β-globin mutations are presented in Table S3.

Eighteen of 23 patients (78%) underwent one cycle of mobilization and apheresis, and 5 (22%) underwent two cycles (Table 1). The median estimated daily busulfan area-under-the-curve plasma value was 4337 μmol × minute (range, 3708 to 8947).

ENGRAFTMENT CHARACTERISTICS

Neutrophil engraftment occurred at a median of 23 days (range, 13 to 32) after beti-cel infusion. Neither primary nor secondary graft failure occurred. Platelet engraftment occurred at a median of 46 days (range, 20 to 94) after beti-cel infusion. Platelet counts of at least 100,000 per cubic millimeter were observed in 13 of 23 patients (57%) by day 90 and in 22 of 23 patients (96%) by day 365. We noted a trend toward more rapid neutrophil and platelet recovery in patients who had undergone splenectomy than in those with an intact spleen, even without splenomegaly or hypersplenism (Table S4). Mild-to-moderate deficits in lymphocyte counts recovered over time (Table S5). The median duration of hospitalization from conditioning through discharge was 45 days (range, 30 to 92).

PHARMACODYNAMIC ASSESSMENT

Vector was detected in all patients. The median vector copy number in PBMCs per diploid genome was stable over time (Fig. S1). Among patients who had transfusion independence, the total hemoglobin and HbA^{T87Q} levels reached a plateau by 6 months after infusion (Fig. 1A). Figure 1B shows the correlation of HbA^{T87Q} levels at 6 months with the percentage of CD34+ cells transduced, and Figure 1C shows the correlation between HbA^{T87Q} levels at 6 months and the vector copy number in beti-cel. The correlation between HbA^{T87Q} levels and the vector copy number in PBMCs at 6 months is shown in Figure S2, and the correlations between the vector copy number in PBMCs and the vector copy number in beti-cel at 6, 12, and 24 months are shown in Figure S3.

TRANSFUSION INDEPENDENCE

A total of 20 of 22 patients (91%) who could be evaluated for the primary end point had transfusion independence; the median time to the last transfusion was 0.9 months (range, 0.5 to 2.4) after the previous infusion. Transfusion independence was durable; the median duration was 20.4 months (range, 15.7 to 21.6). The average hemoglobin level during transfusion independence was 11.7 g per deciliter (range, 9.5 to 12.8). At 12 months, the median HbA^{T87Q} level in these patients was 8.7 g per deciliter (range, 5.2 to 10.6) (Fig. 1A), and the median endogenous hemoglobin level was 3.0 g per deciliter (range, 0.9 to 5.0). Five of these patients had homozygous severe β^+ mutations or severe β^+ mutations with a β^0 allele, resulting in endogenous β-globin production of less than 2 g per deciliter. A total of 14 of 15 evaluable patients who were 12 to 50 years of age (93%), as well as 6 of 7 patients younger than 12 years of age (86%), had transfusion independence. The transfusion status of all the patients is shown in Figure 2.

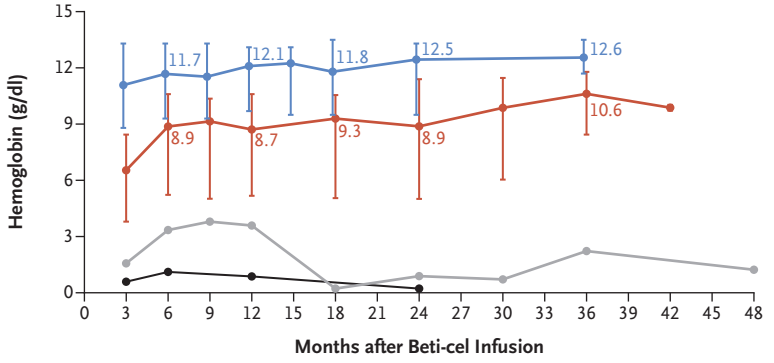
The two evaluable patients who did not have transfusion independence (Patients 2 and 20) (Fig. 2) had 67.4% and 22.7% reductions in transfusion volume from 6 months to the last follow-up (at 48.2 and 27.2 months, respectively). The vector copy number in beti-cel was 2.4 copies per diploid genome in Patient 2 and 2.3 copies per diploid genome in Patient 20 (as compared with 1.9 to 5.6 copies per diploid genome in patients who had transfusion independence); 53% of the CD34+ hematopoietic stem cells and progenitor cells were transduced in Patient 2 and 58% of these cells were transduced in Patient 20. The highest HbA^{T87Q} level was 3.8 g per deciliter at 9 months in Patient 2 and 1.1 g per deciliter at 6 months in Patient 20, at time points when both patients had a vector copy number in PBMCs of 0.2 copies per diploid genome, as compared with 0.4 to 4.5 at 6 months and 0.4 to 5.1 at 9 months in those who had transfusion independence. These two patients had unremarkable clinical characteristics as compared with those in whom transfusions were discontinued (Table S6).

Table 1. Characteristics of the Patients.*

Characteristic	Value
Enrollment and treatment status — no. of patients	
Provided consent	32†
Mobilization complete	24‡
Betibeglogene autotemcel (beti-cel) infused	23
Completed HGB-207 study	20
Enrolled in LTF-303 study	19§
Demographic and clinical characteristics at baseline	
Genotype — no. of patients/total no. (%)	
β^0/β^+¶	12/23 (52)
β^E/β^0	6/23 (26)
β^+/β^+‖	5/23 (22)
Sex — no. of patients/total no. (%)	
Female	12/23 (52)
Male	11/23 (48)
Race or ethnic group — no. of patients/total no. (%)**	
Asian	13/23 (57)
White	8/23 (35)
Other	2/23 (9)
Age at consent or assent	
Median (range) — yr	15 (4–34)
Distribution — no. of patients/total no. (%)	
<12 yr	8 (35)
12 to <18 yr	6 (26)
≥18 yr	9 (39)
Median age at first transfusion (range) — yr	1 (<1–7)
Median red-cell transfusion volume ≤2 yr before enrollment (range) — ml/kg of body weight/yr	207.9 (142.1–274.4)
Median no. of red-cell transfusions ≤2 yr before enrollment (range) — no./yr	16.0 (11.5–37.0)
Weighted average nadir hemoglobin level before transfusion (range) — g/dl††	9.6 (7.5–11.0)
Previous splenectomy — no. of patients/total no. (%)	4/23 (17)
Iron status	
Median liver iron concentration (range) — mg/g of dry weight	5.3 (1.0–41.0)
Median T2*-weighted MRI measurement of myocardial iron (range) — msec	36.7 (21.0–57.0)
Median serum ferritin level (range) — ng/ml	1975.2 (349.0–10,021)
Mobilization and apheresis cycle — no. of patients/total no. (%)	
1 cycle	18/23 (78)
2 cycles‡‡	5/23(22)

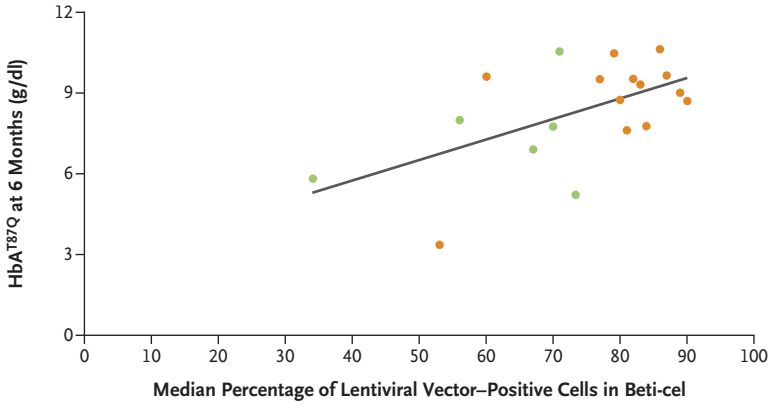
* Percentages may not total 100 because of rounding.
† At screening, 8 patients were deemed to be ineligible for the study because of advanced liver disease (in 3 patients), T2*-weighted magnetic resonance imaging (MRI) measurement of myocardial iron of less than 10 seconds (in 1 patient), β^0 mutation of both *HBB* alleles (in 1 patient), and withdrawal of consent (in 3 patients).
‡ One patient discontinued treatment because of pregnancy.
§ One patient who completed the HGB-207 study did not enroll in the LTF-303 study until after the data cutoff date of March 9, 2021.
¶ This category includes 4 patients who were heterozygous for severe β^+ and β^0 alleles.
‖ This category includes 2 patients who were homozygous for severe β^+ alleles and 2 patients who were heterozygous for severe β^+ alleles.
** Race or ethnic group was reported by the treating physicians.
†† The nadir hemoglobin level is defined as the most recent hemoglobin level before each packed red-cell transfusion, on the day of transfusion or within 3 days before the transfusion. The weighted average nadir hemoglobin level at baseline is the weighted average of the nadir hemoglobin level values in the 2-year period before enrollment.
‡‡ These patients received two cycles of mobilization and apheresis to meet the cell dose requirement of 5.0 million CD34+ cells per kilogram.

A



Patients with Transfusion Independence										
Unsupported total hemoglobin	12	20	20	19	18	17	NR	8	NR	NR
HbA^{T87Q}	20	18	20	20	19	18	11	7	2	NR
Patients without Transfusion Independence										
HbA^{T87Q}	2	2	1	2	1	2	1	1	NR	1

B



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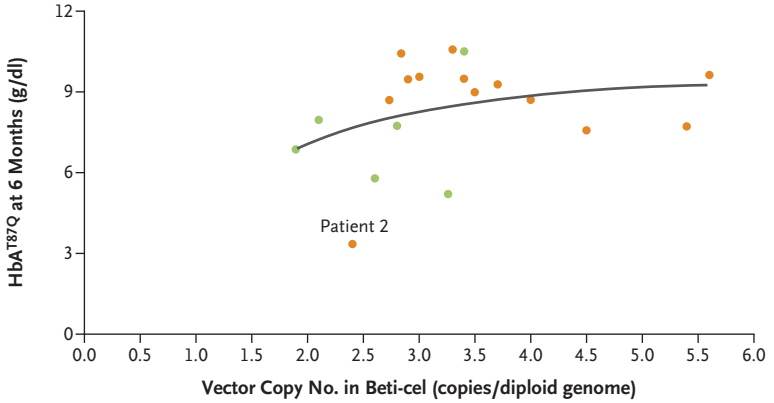


Figure 1 (facing page). Kinetics of HbA^{T87Q} and Characteristics of Betibeglogene Autotemcel (Beti-cel).

Panel A shows the median unsupported total hemoglobin levels (defined as the total hemoglobin levels in patients who had not received any packed red-cell transfusions ≤60 days before the measurement date) and the gene therapy–derived adult hemoglobin (HbA) with a T87Q amino acid substitution (HbA^{T87Q}) levels in patients who had transfusion independence as well as the HbA^{T87Q} levels in those who did not have transfusion independence. The numbers of patients with levels that were evaluated at given time points are shown at the bottom of the panel. In one patient who did not have transfusion independence, transfusions were discontinued for approximately 11 months beginning 3 months after beti-cel infusion, but after resumption of the transfusions, the HbA^{T87Q} level was lower at the 18-month visit. I bars represent minimum and maximum values. NR denotes not reported. Panel B shows the correlation between HbA^{T87Q} expression at 6 months and the percentage of transduced CD34+ cells in patients younger than 12 years of age and in those 12 years of age or older who received beti-cel and who were not receiving transfusions. Data from Patient 20 (who was transfusion dependent at 6 months) were excluded. Panel C shows the relationship between HbA^{T87Q} expression at 6 months and the vector copy number in beti-cel in patients younger than 12 years of age and those 12 years of age or older who received beti-cel and who were not receiving transfusions. This relationship was evaluated with the use of a nonlinear regression model in which $HbA^{T87Q} = HbA^{T87Q}$ max × $(1\text{-}e^{-K \times \text{vector copy number}})$ and in which HbA^{T87Q} max=9.47 and K=0.69 were determined from the line of best fit. Data from Patient 20 (who was transfusion dependent at 6 months) were excluded. HbA^{T87Q} levels at 6 months were missing for two patients.

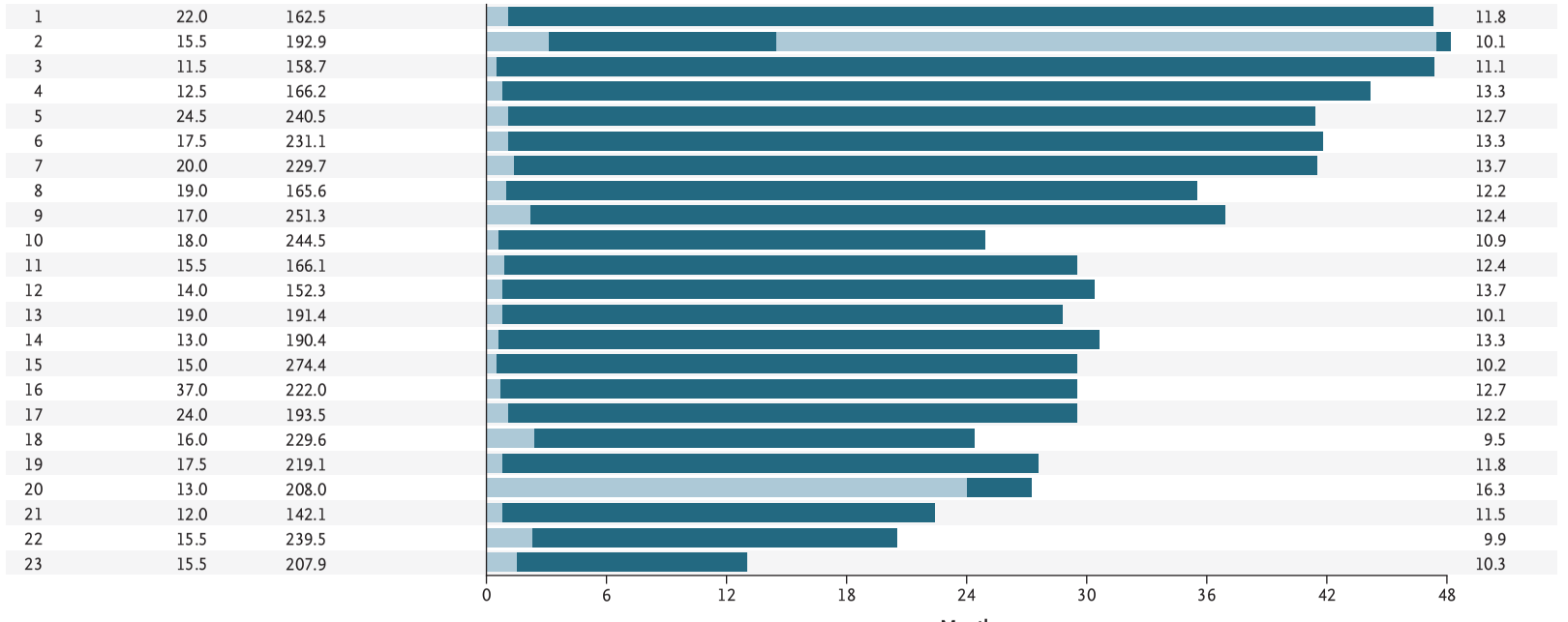
INEFFECTIVE ERYTHROPOIESIS AND IRON STUDIES

Ineffective erythropoiesis improved after transfusion independence (Fig. S4 and Table S7). In patients who had transfusion independence, the median ratio of myeloid to erythroid cells increased from 1:2.4 (range, 1:10 to 1:0.24 [normal range, 1:0.33 to 1:0.25][23]) in 20 patients at baseline to 1:1.2 (range, 1:3.3 to 1:0.53) in 16 patients at 24 months. These ratios improved but remained outside the normal reference range, which suggests the persistence of a small proportion of cells with residual ineffective erythropoiesis. In a comparison of baseline values with those at 24 months, serum levels of soluble transferrin receptor decreased from 118.2 nmol per liter (range, 21.2 to 235.3) in 20 patients to 59.4 nmol per liter (range, 17.7 to 105.9) in 18 patients, and serum levels of erythropoietin decreased from 31.1 U per liter (range, 10.2 to 169.6) in 17 patients to 15.2 U per liter (range, 7.1 to 90.0) in 17 patients who had transfusion independence. Serum levels of soluble transferrin receptor increased from 136.5 nmol per liter at baseline to 208.2 nmol per liter at 24 months in 1 of the patients who did not have transfusion independence, and they decreased from 115.3 nmol per liter at baseline to 61.2 nmol per liter at month 24 in the other patient. Figure S5 shows the results of analyses of correlations between markers of ineffective erythropoiesis and vector copy number in PBMCs and hemoglobin levels that were not supported by transfusion in patients who had transfusion independence.

Of the 20 patients who had transfusion independence, 11 restarted iron chelation after beti-cel infusion at a median of 7.2 months (range, 1.2 to 15.2). Of these patients, 4 later discontinued chelation; the liver iron concentrations at approximately the time of discontinuation of chelation were 1.5, 2.0, 5.8, and 6.1 mg per gram of dry weight. Nine patients never restarted iron chelation after infusion. Seven patients underwent phlebotomy to reduce iron levels. Three patients did not restart iron chelation or undergo phlebotomy after beti-cel infusion.

T2*-weighted MRI measurements of myocardial iron in patients who had transfusion independence are shown in Figure 2; the median value did not change significantly over time, and measurements of myocardial iron were maintained within the normal range in all the patients except for 1 patient whose baseline value of 21 msec decreased to 15 msec at 12 and 24 months. At baseline, the median liver iron concentration in 20 patients was 5.1 mg per gram of dry weight (range, 1.0 to 41.0), and at 24 months, the median liver iron concentration in 17 patients was 4.9 mg per gram of dry weight (range, 1.4 to 20.3). In the 3 patients with 36 months of follow-up, the median liver iron concentration was within the normal range.

Among the patients who had transfusion independence, the median hepcidin level was within the normal range (1 to 53 μg per liter) at 12 months (30.5 μg per liter; range, 13 to 65) and



Median liver iron concentration (range) — mg/g of dry weight (reference, <1.8)	5.1 (1.0–41.0); N=20	NA	6.7 (2.0–19.2); N=19	NA	4.9 (1.4–20.3); N=17	NA	2.0 (1.4–2.2); N=3
Correlation with VCN in PBMCs at 12 mo — copies/diploid genome			0.17; P=0.49		0.17; P=0.50		
Correlation with total hemoglobin at 12 mo — g/dl			0.16; P=0.52		0.40; P=0.12		
Median T2*-weighted MRI measurement of myocardial iron (range) — msec (reference, >20)	36.5 (21–57); N=20	NA	36.0 (15–43); N=17	NA	35.1 (15–47); N=17	NA	33.5 (29–41); N=7
Correlation with VCN in PBMCs at 12 mo — copies/diploid genome			0.07; P=0.78		0.08; P=0.75		
Correlation with total hemoglobin at 12 mo — g/dl			0.34; P=0.20		0.15; P=0.58		
Median serum ferritin (range) — ng/ml (reference range, 30–400)	1826 (349–5978); N=20	2048 (840–9635); N=20	1293 (527–7780); N=19	1141 (437–7453); N=20	862 (94–8443); N=18	NA	698 (126–2134); N=8
Correlation with VCN in PBMCs at 12 mo — copies/diploid genome			0.36; P=0.13		0.30; P=0.23		
Correlation with total hemoglobin at 12 mo — g/dl			0.25; P=0.32		0.30; P=0.24		

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Figure 2 (facing page). Transfusion Status and Change in Iron Levels in Patients Who Had Transfusion Independence.

Patients 1, 3 through 19, and 21 and 22 had transfusion independence, which was defined as a weighted average hemoglobin level of 9 g per deciliter or greater in patients who did not receive packed red-cell transfusions for at least 12 months at any time after beti-cel infusion. The weighted average hemoglobin level was defined as the weighted average of the hemoglobin values during the transfusion-independent period. All correlations are reported as Pearson coefficients. Data on transfusions before enrollment were annualized to the 2 years before enrollment. In Patients 2 and 20, the hemoglobin level was supported by transfusions. After planned orthopedic surgery, Patient 11 had blood loss and received one packed red-cell transfusion 22.2 months after beti-cel infusion. Unsupported total hemoglobin values were used for correlations with the total hemoglobin level at 12 months. The reference value for liver iron concentration was described by Cappellini et al.,[6] and the reference value for T2*-weighted magnetic resonance imaging (MRI) measurements of myocardial iron was described by Anderson et al.[22] NA denotes not available, PBMC peripheral-blood mononuclear cell, and VCN vector copy number.

Table 2. Summary of Adverse Events and Serious Adverse Events after Beti-cel Infusion.*

Event	All Patients (N = 23)
	no. (%)
Grade ≥3 adverse events occurring in ≥2 patients through 2 yr of follow-up	
Thrombocytopenia	22 (96)
Neutropenia	18 (78)
Anemia	14 (61)
Stomatitis	14 (61)
Leukopenia	13 (57)
Febrile neutropenia	8 (35)
Epistaxis	5 (22)
Pyrexia	4 (17)
Decreased appetite	3 (13)
Hepatic veno-occlusive disease	3 (13)
Increased alanine aminotransferase level	2 (9)
Increased blood bilirubin level	2 (9)
Hypoxia	2 (9)
Lymphopenia	2 (9)
Neutropenic sepsis	2 (9)
Pharyngeal inflammation	2 (9)
Serious adverse events reported in ≥2 patients through last follow-up	
Hepatic veno-occlusive disease	3 (13)
Thrombocytopenia	2 (9)
Pyrexia	2 (9)

* Four patients had at least one adverse event that was considered by the investigator to be related or possibly related to beti-cel, according to the study protocol. These events included grade 1 abdominal pain (in 1 patient) and grade 1 tachycardia (in 1 patient) on the day of infusion, and grade 3 thrombocytopenia (in 2 patients) and grade 1 pain in an extremity (in 1 patient) after infusion. All events were nonserious except for one event of thrombocytopenia.

at 24 months (21.0 μg per liter; range, 4 to 53). The median plasma ratio of hepcidin to ferritin in these patients was 0.02 (range, 0.002 to 0.04) at 12 months and 0.019 (range, 0.002 to 0.06) at 24 months. These ratios were below the mean (±SD) values reported in healthy persons (0.094±0.036).[24] In the two patients who did not have transfusion independence, the hepcidin levels were 38 and 48 μg per liter at 12 months and 57 and 43 μg per liter at 24 months, respectively; the ratios of hepcidin to ferritin were 0.004 and 0.01 at 12 months and 0.01 and 0.02 at 24 months, respectively.

SAFETY

All the patients had at least one adverse event during or after beti-cel infusion, and all were alive at the last follow-up. Adverse events of grade 3 or higher and serious adverse events after the infusion of beti-cel are summarized in Table 2.

The adverse events observed after beti-cel infusion were consistent with those that are typical of busulfan-based myeloablation. Three patients had grade 4 serious hepatic veno-occlusive disease, and one patient had grade 2 nonserious hepatic veno-occlusive disease (Table S8). These events were attributed to conditioning and resolved after defibrotide treatment. The liver iron concentration and systemic exposure to busulfan were similar in patients who had hepatic veno-occlusive disease and those who did not. Two patients had events of grade 3 thrombocytopenia that were considered by the investigators to be possibly related to beti-cel, and one of these events was considered to be serious; neither patient had serious bleeding events after infusion of beti-cel. Replication-competent lentivirus, clonal predominance, and insertional oncogenesis were not detected. An analysis of integration sites showed that no single vector insertion

Table 3. Characteristics of Beti-cel and Characteristics of Patients with a Non–β^0/β^0 Genotype after Beti-cel Infusion in the HGB-204, HGB-205, and HGB-207 Studies.*

Characteristic	Phase 1–2 Studies		Phase 3 Study
	HGB-204 (N=18)	HGB-205 (N=4)†	HGB-207 (N=23)
Non–β^0/β^0 genotype — no. of patients	10	3‡	23
Drug product			
Median vector copy no. (range) — copies/diploid genome	0.7 (0.3–1.5)§	1.3 (0.8–2.1)§	3.3 (1.9–5.6)
Median percentage of cells positive for lentiviral vector (range)	32 (17–58)§	ND	79.3 (34.0–90.0)
Median dose of CD34+ cells (range) — millions/kg of body weight	7.1 (5.2–13.0)	12.0 (8.9–13.6)	8.1 (5.0–19.9)
Characteristics of patients after infusion			
Transfusion independence — no./total no. (%)¶	8/10 (80)	3/3 (100)	20/22 (91)‖
Median duration of transfusion independence (range) — mo	38.0 (21.2–45.3)	56.3 (38.2–57.6)	20.4 (15.7–21.6)
Weighted average hemoglobin level during transfusion independence (range) — g/dl	10.3 (9.1–13.2)	11.4 (10.5–13.0)	11.7 (9.5–12.8)
Median HbA^{T87Q} level (range) — g/dl**	6.3 (0.4–9.5)	Patient 4: 9.0 at 40.5 mo; Patient 1: 7.6 at 58.6 mo; Patient 2: 11.2 at 60.6 mo	8.7 (1.1–10.6) at 6 mo; 8.6 (0.9–10.6) at 12 mo
Median unsupported endogenous hemoglobin level (range) — g/dl**	NR	NR	2.7 (1.0–5.0) at 6 mo; 3.0 (0.9–5.0) at 12 mo
Median vector copy no. in PBMCs (range) — copies/diploid genome**	0.3 (0.1–0.9) at 15 mo‡	2.0 (0.3–4.2) at 15 mo‡	1.4 (0.2–4.5) at 6 mo; 1.4 (0.2–5.0) at 12 mo

* Data are from Thompson et al.,[19] Kwiatkowski et al.,[20] and Magrin et al.[21] HbA^{T87Q} denotes adult hemoglobin (HbA) with a T87Q amino acid substitution, ND not determined, NR not reported, and PBMC peripheral-blood mononuclear cell.

† Manufacturing of beti-cel for HGB-205 was completed on site.

‡ In HGB-205, 3 patients had β^E/β^0 genotypes and 1 patient had a $\beta^{+IVS\text{-}I\text{-}110}/\beta^{+IVS\text{-}I\text{-}110}$ genotype; however, this patient was excluded here because HGB-207 excluded patients who were homozygous for an IVS-I-110 mutation.

§ Data were pooled for each study and were not separated according to genotype.

¶ Transfusion independence was defined as a weighted average hemoglobin level of at least 9 g per deciliter in patients who had not received any packed red-cell transfusions for at least 12 months.

‖ A total of 22 evaluable patients were assessed for transfusion independence, and 20 had transfusion independence at any time. Among these patients, those who had transfusion independence completed the month 24 visit. Lack of transfusion independence was defined according to the following two criteria: if the patient received long-term transfusions after 324 days (750 days − 14 × 30 days) of follow-up or if the hemoglobin level never reached t_0 (hemoglobin level ≥9 g per deciliter with no transfusions in the preceding 60 days) before 385 days (750 days − 365 days) of follow-up.

** The data shown for HGB-204 and HGB-205 are from the last follow-up.

event accounted for more than 10% of vector insertions in any of the patients (Table S9).

DISCUSSION

This interim analysis, in which most patients in both age cohorts met the success criteria for the trial with respect to transfusion independence, showed that beti-cel gene therapy resulted in production of functional, gene therapy–derived HbA^{T87Q}, which by approximately 6 months after infusion led to near-normal hemoglobin levels. This therapy enabled 91% of the patients, including 6 of 7 patients who were younger than 12 years of age, to become transfusion independent, with an average hemoglobin level of 11.7 g per deciliter. The patients in this study had a broad spectrum of non–β^0/β^0 genotypes, and some had minimal endogenous hemoglobin production. After the infusion of beti-cel, assessments of bone marrow and blood biomarkers during transfusion independence showed improvement in erythropoiesis. The liver iron concentration also decreased over time in patients who had transfusion independence.

The two patients who did not have transfusion independence received beti-cel that satisfied targets for vector copy numbers and vector-positive CD34+ cells; however, both patients had lower circulating vector copy numbers than those in patients who had transfusion independence. It is possible that the long-term hematopoietic stem cells infused were not adequately transduced, since the population is heterogeneous and transduction is not uniform. Although progenitor cells are more easily transduced by the lentiviral vector than true hematopoietic stem cells, transduction of long-term hematopoietic stem cells predicts clinical outcomes.[25-27]

In the two phase 1–2 studies of beti-cel, 11 of 13 patients with either hemoglobin E–β-thalassemia or β^+/β^+ genotypes had transfusion independence; in HGB-204, the average total hemoglobin level during transfusion independence was 10.3 g per deciliter (Table 3).[19-21,28] The median vector copy number in beti-cel was higher in the current HGB-207 study than in the phase 1–2 studies, and it resulted in higher vector copy numbers in PBMCs, higher hemoglobin levels, and a higher incidence of transfusion independence than in the previous studies.[19] Similarly, the median percentage of lentiviral vector–positive CD34+ cells per patient was higher in the current study than in HGB-204. Refined manufacturing maximized HbA^{T87Q} production and mitigated the influence of transduction variability and endogenous hemoglobin production on outcomes. Although patients in HGB-205 had HbA^{T87Q} levels that were similar to those in patients in HGB-207, meaningful conclusions cannot be drawn given the small sample of 4 patients in HGB-205.

Studies of gene therapy involving gamma retroviral vectors identified the risks of insertional oncogenesis and revealed a need to improve the safety profile of viral vectors.[29-31] Lentiviral vectors are currently the vectors of choice for most hematopoietic stem-cell gene therapies, and third-generation lentiviral vectors such as BB305 have been modified to be replication-incompetent and self-inactivating, further reducing the risk of insertional oncogenesis.[18,32-36] Furthermore, lentiviral vector–related safety can be monitored with integration site analysis and testing to detect replication-competent lentivirus.[18,37,38] Two cases of leukemia have been reported in patients with sickle cell disease after unsuccessful treatment with BB305-based gene therapy. Nonetheless, vector-mediated events, insertional oncogenesis, evidence of clonal predominance, and cases of cancer have not been reported in patients with β-thalassemia who have received beti-cel.[39,40]

The limitations of our study include a small patient population and a short follow-up period. The phase 3 HGB-212 (Northstar-3) study (NCT03207009) is under way to evaluate patients with the β^0/β^0, $\beta^0/\beta^{+IVS\text{-}I\text{-}110}$, and $\beta^{+IVS\text{-}I\text{-}110}/\beta^{+IVS\text{-}I\text{-}110}$ genotypes. Additional follow-up is needed to fully characterize the long-term efficacy and safety of beti-cel.

Improvement in hematopoietic stem-cell transduction resulted in higher HbA^{T87Q} levels in the current study than in the previous phase 1–2 studies.[19-21] These data suggest that in most patients with transfusion-dependent β-thalassemia and a non–β^0/β^0 genotype, one-time infusion of beti-cel is potentially curative through transfusion independence and the attainment of near-normal hemoglobin levels.

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APPENDIX

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