ORIGINAL ARTICLE

Biologic and Clinical Efficacy of LentiGlobin for Sickle Cell Disease

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ABSTRACT

BACKGROUND

Sickle cell disease is characterized by the painful recurrence of vaso-occlusive events. Gene therapy with the use of LentiGlobin for sickle cell disease (bb1111; lovotibeglogene autotemcel) consists of autologous transplantation of hematopoietic stem and progenitor cells transduced with the BB305 lentiviral vector encoding a modified β-globin gene, which produces an antisickling hemoglobin, HbA^{T87Q}.

METHODS

In this ongoing phase 1–2 study, we optimized the treatment process in the initial 7 patients in Group A and 2 patients in Group B with sickle cell disease. Group C was established for the pivotal evaluation of LentiGlobin for sickle cell disease, and we adopted a more stringent inclusion criterion that required a minimum of four severe vaso-occlusive events in the 24 months before enrollment. In this unprespecified interim analysis, we evaluated the safety and efficacy of LentiGlobin in 35 patients enrolled in Group C. Included in this analysis was the number of severe vaso-occlusive events after LentiGlobin infusion among patients with at least four vaso-occlusive events in the 24 months before enrollment and with at least 6 months of follow-up.

RESULTS

As of February 2021, cell collection had been initiated in 43 patients in Group C; 35 received a LentiGlobin infusion, with a median follow-up of 17.3 months (range, 3.7 to 37.6). Engraftment occurred in all 35 patients. The median total hemoglobin level increased from 8.5 g per deciliter at baseline to 11 g or more per deciliter from 6 months through 36 months after infusion. HbA^{T87Q} contributed at least 40% of total hemoglobin and was distributed across a mean (±SD) of 85±8% of red cells. Hemolysis markers were reduced. Among the 25 patients who could be evaluated, all had resolution of severe vaso-occlusive events, as compared with a median of 3.5 events per year (range, 2.0 to 13.5) in the 24 months before enrollment. Three patients had a nonserious adverse event related or possibly related to LentiGlobin that resolved within 1 week after onset. No cases of hematologic cancer were observed during up to 37.6 months of follow-up.

CONCLUSIONS

One-time treatment with LentiGlobin resulted in sustained production of HbA^{T87Q} in most red cells, leading to reduced hemolysis and complete resolution of severe vaso-occlusive events. (Funded by Bluebird Bio; HGB-206 ClinicalTrials.gov number, NCT02140554.)

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Sickle cell disease is caused by a single point mutation in the gene encoding β-globin (*HBB*), which leads to the production of sickle hemoglobin and impaired red-cell function.[1,2] Patients with sickle cell disease often have vaso-occlusive events, progressive vasculopathy, and chronic hemolytic anemia, which are associated with complications and an increased risk of early death.[1-3] With current supportive treatment options, sickle cell disease is managed without halting disease progression.[4,5] HLA-matched sibling allogeneic hematopoietic stem-cell transplantation is a potentially curative treatment option[3,6-8] but is recommended mainly for younger patients and is limited by the fact that only 14 to 20% of patients have HLA-matched donors, the risk of graft-versus-host disease and graft rejection, and the risk of transplantation-related death.[1,6,7,9,10] Gene therapies that use autologous stem cells may overcome these hurdles and are advancing into clinical trials.[11-13]

Gene therapy with LentiGlobin for sickle cell disease (bb1111; lovotibeglogene autotemcel, Bluebird Bio) consists of the autologous transplantation of hematopoietic stem and progenitor cells (HSPCs) transduced with the BB305 lentiviral vector encoding a modified β-globin gene, which results in the production of an antisickling hemoglobin, HbA^{T87Q}.[14,15] HbA^{T87Q} is a modified adult hemoglobin with an amino acid substitution (threonine to glutamine at position 87) designed to sterically inhibit polymerization of sickle hemoglobin.[16] HbA^{T87Q} maintains 99.9% identity to adult hemoglobin, has a similar oxygen-binding affinity and oxygen–hemoglobin dissociation curve,[14] and can be differentiated from transfused adult hemoglobin. The long terminal repeat sequences that flank the transgene in the lentiviral vector allow for identification of the insertion sites during safety monitoring. The use of autologous HSPCs eliminates the risk of graft-versus-host disease associated with potentially curative allogeneic hematopoietic stem-cell transplantation.

In the ongoing phase 1–2 HGB-206 study to evaluate the efficacy and safety of LentiGlobin, patients are being followed for 24 months, after which they enter a 13-year follow-up study (LTF-307; ClinicalTrials.gov number, NCT04628585). In the HGB-206 study, we optimized the treatment process in the initial cohorts, which consist of 7 patients in Group A and 2 patients in Group B.[17] Group C was established for the pivotal evaluation of LentiGlobin for sickle cell disease, and we adopted a more stringent inclusion criterion that required a minimum of four severe vaso-occlusive events in the 24 months before enrollment. Here, we report data from an unprespecified interim analysis involving 35 patients who received LentiGlobin treatment in Group C.

METHODS

TRIAL DESIGN AND OVERSIGHT

This nonrandomized, open-label, single-dose clinical trial is being conducted at 11 sites across the United States in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines of the International Council for Harmonisation. The trial protocol (available with the full text of this article at NEJM.org) was amended for Group C and approved by the institutional review board at each clinical site. Patients or their legal guardians provided written informed consent, with minors providing assent where applicable.

The first two authors and the last five authors were involved in designing the trial in collaboration with the sponsor, Bluebird Bio. All the authors were involved in the collection and interpretation of the data. An independent data monitoring committee is reviewing safety data throughout the trial, and an adjudication committee is assessing reported vaso-occlusive events. All the authors had access to the clinical data, reviewed the manuscript, and approved its submission for publication. They also attest that the trial was conducted in accordance with the protocol and vouch for the completeness and accuracy of the data. The manuscript was developed with medical writing support funded by the sponsor.

ENTRY CRITERIA AND PATIENT POPULATIONS

Eligible patients were between 12 and 50 years of age, had received a diagnosis of sickle cell disease with a β^S/β^S, β^S/β^0, or β^S/β^+ genotype, and had a clinically stable Karnofsky performance status of at least 60 (for patients ≥16 years of age) or a Lansky performance status of at least 60 (for those <16 years of age). (Both scales range from 0 to 100, with higher scores indicating better functioning.) Patients were also required to have had a failure of hydroxyurea treatment (stan-

dard care) in the past, a 24-month history of active treatment of sickle cell disease before enrollment, and no opportunity for matched HLA-identical hematopoietic-cell donation.

The initial eligibility criteria for Group C permitted a history of overt stroke and required a less stringent definition for severe vaso-occlusive events before enrollment. During the course of the study, we amended the protocol to require a minimum of four severe vaso-occlusive events in the 24 months before enrollment, a revision that allowed us to evaluate the number of patients who had a complete resolution of severe vaso-occlusive events as an efficacy end point. (Details regarding this amendment are provided in the Methods section in the Supplementary Appendix, available at NEJM.org.)

The intention-to-treat population included all the patients who had undergone any study procedures, beginning with stem-cell collection (mobilization and apheresis). Protocol updates generated two study populations within Group C for analysis: the transplant population (which included all the patients who had received a LentiGlobin infusion) and the transplant population with vaso-occlusive events (TPVOE) (which included patients who had met the updated inclusion requirement of four severe vaso-occlusive events in the 24 months before enrollment). Six patients in the transplant population did not meet the TPVOE criterion.

TRIAL PROCEDURES

Treatment with LentiGlobin in the first 7 patients (Group A) who were enrolled in the HGB-206 study resulted in durable but suboptimal expression of HbA^{T87Q} (Table S1 in the Supplementary Appendix). We therefore refined both the treatment process (including the transfusion regimen before HSPC collection and busulfan administration) and the LentiGlobin manufacturing process to improve transduction efficiency. After these changes, we observed improvements in biologic and clinical outcomes in the 2 patients enrolled in Group B. For Group C, we made additional changes to the treatment protocol, which included HSPC collection by plerixafor mobilization and apheresis (rather than by bone marrow harvesting)[17] and an increased cell dose. Details regarding these initial protocol changes will be reported separately.

The study design and LentiGlobin gene therapy process are shown in Figure S1 and Figure S2, respectively. Patients were screened to determine eligibility and subsequently underwent a transfusion regimen for at least 60 days before stem-cell harvesting to reduce stress erythropoiesis and stabilize the bone marrow before mobilization.[18] The treatment process in Group C involved the following steps: precollection preparation and collection through plerixafor mobilization and apheresis,[16,17,19] a refined LentiGlobin manufacturing process through the ex vivo transduction of enriched autologous HSPCs with the BB305 lentiviral vector encoding the $\beta^{A\text{-}T87Q}$ transgene,[16,17] myeloablative conditioning (target area under the curve, 5000 μmol × min),[16,19] LentiGlobin infusion (day 1),[16,20] engraftment of HSPCs leading to the production of HbA^{T87Q}, and follow-up[20,21] (Fig. S2). Data from both the 24-month HGB-206 study and longitudinal follow-up study are presented.

END POINTS

The primary efficacy end point of the HGB-206 study was the complete resolution of severe vaso-occlusive events, which was measured between 6 months and 18 months after the LentiGlobin infusion. Here, however, we report all vaso-occlusive events and severe vaso-occlusive events that were assessed in accordance with the protocol in the TPVOE group from the time of infusion through the last visit. A vaso-occlusive event was defined as an episode of acute pain with no medically determined cause other than a vaso-occlusion and included acute episodes of pain, acute chest syndrome, acute hepatic sequestration, acute splenic sequestration, and acute priapism. A severe vaso-occlusive event was defined as such an event that resulted in a visit to a hospital or emergency department that exceeded 24 hours, at least two visits to a day unit or emergency department during a 72-hour period (with both visits requiring intravenous treatment), or a priapism episode lasting more than 2 hours and leading to a medical-facility visit.

All other efficacy end points were analyzed in the transplant population. Safety end points were assessed in the intention-to-treat population for adverse events that were attributed to cell collection and in the transplant population for adverse events that were attributed to conditioning or events that occurred after LentiGlobin infusion. Secondary end points were the change

in the hemoglobin level from baseline, the absolute total hemoglobin level, and the change in markers of hemolysis. Pharmacodynamic end points included the vector copy number over time and expression of $\beta^{A\text{-}T87Q}$ globin, β^{S} globin, and other β-like globins over time. We performed an exploratory single-cell Western analysis to determine the presence of β^{S} and $\beta^{A\text{-}T87Q}$ within individual red cells.

Safety assessments involved the reporting of all adverse events during the LentiGlobin treatment process, including those attributed to plerixafor mobilization and apheresis or conditioning. We monitored clonal predominance or insertional oncogenesis after infusion. A complete list of end points is provided in the Methods section in the Supplementary Appendix.

STATISTICAL ANALYSIS

Data that were obtained through February 17, 2021, were analyzed descriptively and are presented as medians with minimum and maximum values (range) unless otherwise specified. We performed this unprespecified interim analysis for the purposes of updating the scientific community. We determined that the enrollment of 35 patients would provide more than 99% power to reject the null hypothesis of complete resolution of severe vaso-occlusive events in 40% of the patients, at a two-sided alpha level of 0.05, assuming that 85% of the TPVOE patients in Group C would meet the primary efficacy end point.

RESULTS

PATIENTS

From December 2016 through January 2020, 51 patients were screened and 43 were enrolled in the study and underwent HSPC collection (intention-to-treat population) (Fig. S3). On February 17, 2021, a total of 35 patients had been treated with LentiGlobin (transplant population) and were subsequently followed for a median of 17.3 months (range, 3.7 to 37.6) for a total of 54.8 patient-years. A total of 29 patients met the criteria for inclusion in the TPVOE group; of these patients, 25 met the minimum 6-month follow-up required for assessment of vaso-occlusive events.

The demographic characteristics of the study patients were representative of the wider population with sickle cell disease (Table S2). In the transplant population, the median age of study participants was 24 years; 23% were adolescents, 37% were female, and all had the β^{S}/β^{S} genotype (Table 1).[22,23]

In the 24 months before enrollment, the median rate of severe vaso-occlusive events was 3.0 events per year (range, 0 to 13.5), and 5 patients (14%) had a history of overt stroke. During the LentiGlobin treatment process, patients received a median of 2 mobilization cycles (range, 1 to 4). The median total dose of CD34+ cells was 6.9×10^{6} cells per kilogram of body weight (range, 3.0×10^{6} to 25.0×10^{6}), with a median LentiGlobin vector copy number of 3.7 copies (range, 2.3 to 5.7) per diploid genome. A median of 80% of CD34+ cells (range, 63 to 93) were positive for BB305 lentiviral vector.

BIOLOGIC AND CLINICAL EFFICACY

The vector copy number in peripheral blood remained stable in all the patients during the period from 6 months after infusion until the last study visit (for up to 36 months), which indicated the persistence of vector-positive, long-term repopulating HSPCs capable of sustaining erythroid-cell generation and the production of HbA^{T87Q}. The median vector copy number in peripheral blood was at least 1.1 copies per diploid genome and the median HbA^{T87Q} level was at least 5.1 g per deciliter starting from 6 months through 36 months (Fig. 1A and B).

Without additional infusions of packed red cells, the median total hemoglobin value increased from 8.5 g per deciliter at baseline to 11.0 g or more per deciliter at 6 months and was sustained through 36 months, with HbA^{T87Q} contributing to at least 40% of total hemoglobin. Sickle hemoglobin levels were approximately 50% from 6 to 36 months after infusion, as compared with levels of 30 to 40% in persons with sickle trait (β^{S}/β^{A}).[24] Fetal hemoglobin production was minimal after LentiGlobin infusion (Fig. 1C). At the last visit among the 8 adolescent patients with at least 6 months of follow-up, the median total hemoglobin level was 13.4 g per deciliter and the median HbA^{T87Q} level was 5.9 g per deciliter.

On the basis of exploratory assay data, HbA^{T87Q} expression was observed at least 6 months after infusion, with a mean (±SD) of 85±8% of red cells estimated to contain $\beta^{A\text{-}T87Q}$ by 24 months in 10

Table 1. Characteristics of the Patients at Baseline and LentiGlobin Treatment.*

Characteristic	Value (N=35)
Patients	
Age	
Median (range) — yr	24 (12–38)
Distribution — no. (%)	
18–50 yr	27 (77)
12–17 yr	8 (23)
Female sex — no. (%)	13 (37)
Race — no. (%)†	
Black	34 (97)
Not provided	1 (3)
β^S/β^S genotype — no. (%)	35 (100)
History of sickle cell disease	
Annualized incidence of severe vaso-occlusive events in 24 mo before enrollment — median (range)‡	3.0 (0–13.5)
History of stroke — no. (%)§	5 (14)
History of tricuspid regurgitant jet velocity of ≥2.5 m per sec — no. (%)¶	6 (17)
Hydroxyurea treatment ≤3 mo before study enrollment — no. (%)	23 (66)
LentiGlobin treatment process	
Median no. of plerixafor mobilization cycles (range)‖	2 (1–4)
Median no. of CD34+ cells collected per mobilization cycle (range) — million/kg‖	10.5 (1.6–55.4)
Median estimated average AUC for busulfan (range) — μmol × min	4829 (2937–7322)
Use of granulocyte colony-stimulating factor after infusion — no. of patients	0
Median no. of days until neutrophil engraftment (range)**	20 (12–35)
Median no. of days until platelet engraftment (range)††	36 (18–136)
Median no. of days of hospitalization from conditioning to discharge (range)	35 (26–65)
LentiGlobin drug product	
Cell-collection process	Plerixafor mobilization followed by apheresis
Median vector copy number (range) — copies/diploid genome	3.7 (2.3–5.7)
Median percent of CD34+ cells transduced (range)	80 (63–93)
Median total dose of CD34+ cells (range) — million/kg‡‡	6.9 (3.0–25.0)
Mean no. of long-term CD34^{bright} HSPCs (range) — million/kg§§	5.7 (2.7–12.1)

* Data are reported for all 35 patients in the transplant population. AUC denotes area under the curve.
† Race was reported by the patients.
‡ The frequency of severe vaso-occlusive events was evaluated during the 24 months before study enrollment. Included in this category are 6 patients who were enrolled before the adoption of the more stringent updated eligibility criterion for the definition of a severe vaso-occlusive event, which was introduced to evaluate vaso-occlusive events as key efficacy end points.
§ During the enrollment period, the inclusion criteria were updated to remove a history of overt stroke, at which point 6 patients with a history of overt stroke had already been enrolled; of these patients, 5 received a LentiGlobin infusion and were assessed as part of the transplant population.
¶ A tricuspid regurgitant jet velocity of 2.5 m per second or more confers an increased risk of death.[22,23]
‖ This number is reported in the intention-to-treat population of 43 patients and included mobilization cycles that were for rescue cells only.
** Neutrophil engraftment was defined as the first absolute neutrophil count of at least 0.5×10^9 per liter for three consecutive measurements on different days without receiving backup cells at any time during the neutropenic phase.
†† Platelet engraftment was defined as the first of three consecutive platelet measurements of at least 50×10^9 per liter on different days without platelet transfusions for 7 days immediately preceding and during the evaluation period.
‡‡ The total dose of CD34+ cells was evaluated in 33 patients after the exclusion of data for 2 patients who had received cell doses that could not be accurately calculated, although the dose was above the lower limit required according to the protocol.
§§ Long-term hematopoietic stem and progenitor cells (HSPCs) were evaluated in 28 patients with available data.

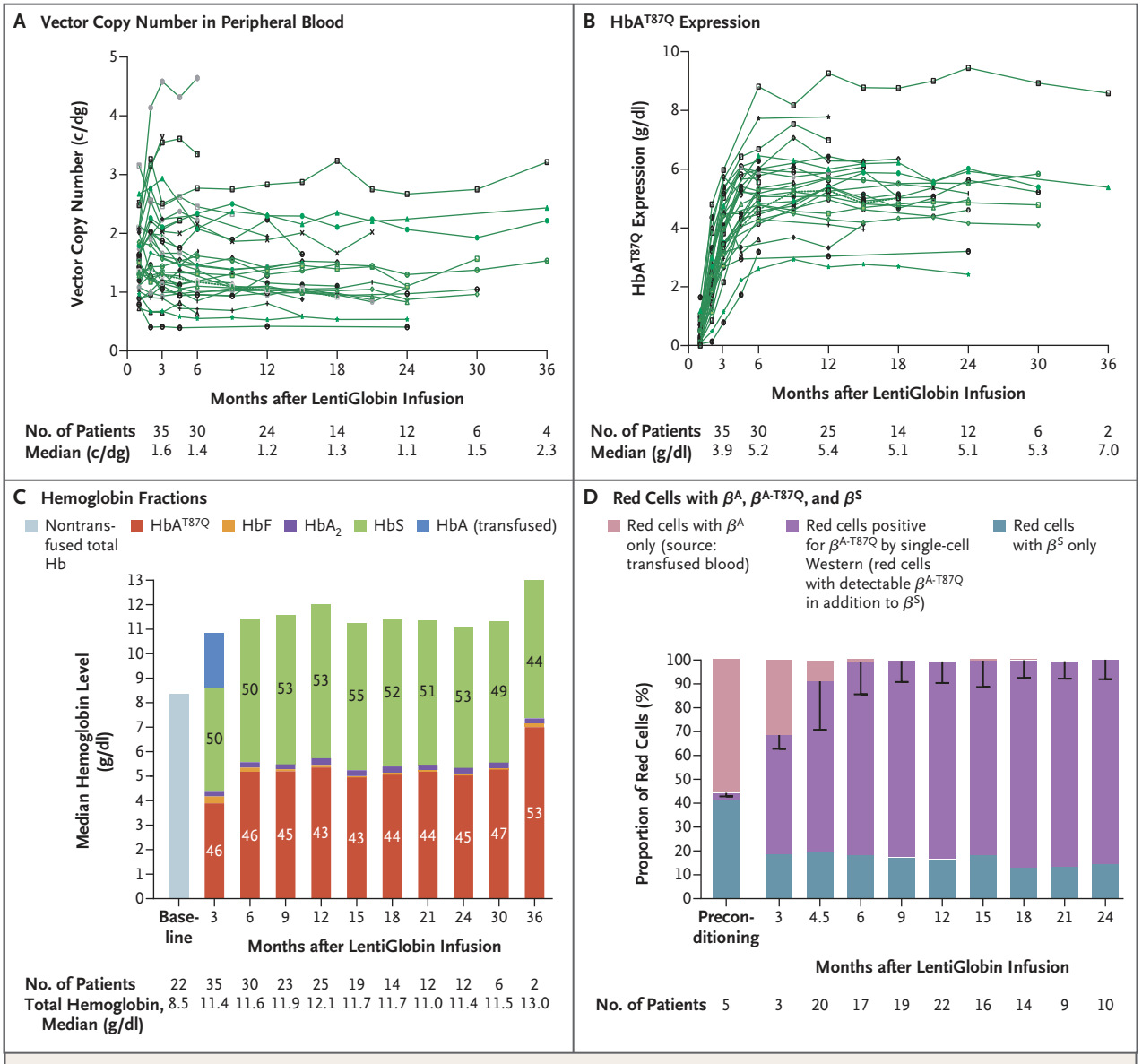

Figure 1. Measures of Biologic and Clinical Efficacy.

Shown are data obtained from 35 patients in the transplant population during a period of up to 36 months regarding the vector copy number in peripheral blood, as measured in copies per diploid genome (c/dg) (Panel A); the expression of hemoglobin with the modified β-globin gene ($\beta^{A\text{-}T87Q}$) (Panel B); the hemoglobin fractions, with percentages indicated on the bars and the median hemoglobin level shown on the y axis (Panel C); and the percentage of red cells containing β^{A}, $\beta^{A\text{-}T87Q}$, and β^{S} (Panel D). Data are missing at some time points in Panels A, B, and C according to the length of follow-up at the time of the data cutoff and were omitted for one patient at month 36 in Panels B and C because samples were obtained within 3 days after the patient had received an exchange transfusion for gallstone-induced pancreatitis. In Panel C, the percentages represent the median hemoglobin fraction as a percentage of nontransfused total hemoglobin; the baseline was an average of two qualified nontransfused total hemoglobin values (measured in grams per deciliter) during the 24 months before study enrollment. Panel D presents exploratory single-cell Western analysis in patients with available data shown as means; the truncated I bars indicate the standard deviation for $\beta^{A\text{-}T87Q}$. Preconditioning samples were collected before busulfan administration, and the observed $\beta^{A\text{-}T87Q}$ signals in these samples are due to the rate at which multiple cells were loaded into a single well and to antibody recognition of both $\beta^{A\text{-}T87Q}$ and β^{A}. HbA denotes adult hemoglobin, HbF fetal hemoglobin, and HbS sickle hemoglobin.

patients with available data (Fig. 1D). Approximately 15% of red cells did not contain $\beta^{A\text{-}T87Q}$ and remained at risk for sickling. Within HbA^{T87Q}-containing red cells, the median HbA^{T87Q} was estimated to be 15.3 pg per red cell (range, 11.7 to 22.7), a finding that was similar to the range of 13 to 18 pg per cell of adult hemoglobin reported in persons with sickle cell trait.[25]

MARKERS OF HEMOLYSIS

From 6 months after infusion through the last visit, lactate dehydrogenase and indirect bilirubin levels were similar to normal levels.[26,27] Starting at 6 months, reticulocyte counts were lower than those at baseline but were higher than normal levels (Fig. 2). Haptoglobin levels were at least 0.1 g per liter in all the patients in the transplant population at the last visit (Table S3).

VASO-OCCLUSIVE EVENTS

No severe vaso-occlusive events were reported in the 25 patients in the TPVOE group with at least 6 months of follow-up after LentiGlobin infusion, as compared with a median rate of 3.5 per year (range, 2.0 to 13.5) in the 24 months before enrollment (Fig. 3A). Three patients (12%) had vaso-occlusive events after infusion; of these patients, 2 had vaso-occlusive events between engraftment and the last visit, with an overall median rate of 0 per year (range, 0 to 5.9) (Fig. 3B).

SAFETY

All adverse events that occurred during the LentiGlobin treatment process, including those attributed to cell collection and conditioning, are summarized in Table 2, along with adverse events of grade 3 or higher. Serious adverse events are reported in Table S4.

The median time until neutrophil engraftment (absolute neutrophil count, $\geq 0.5\times10^9$ per liter for 3 days) was 20 days (range, 12 to 35), and the median time until platelet engraftment (platelet count, $\geq 50\times10^9$ per liter for 3 days without platelet transfusion) was 36 days (range, 18 to 136) (Table 1). After LentiGlobin infusion, 12 patients (34%) had at least one serious adverse event; the most frequently reported were abdominal pain, drug withdrawal syndrome (opiate), nausea, and vomiting (6% each). Overall, 3 patients had adverse events that were attributed by the investigator to LentiGlobin infusion, including

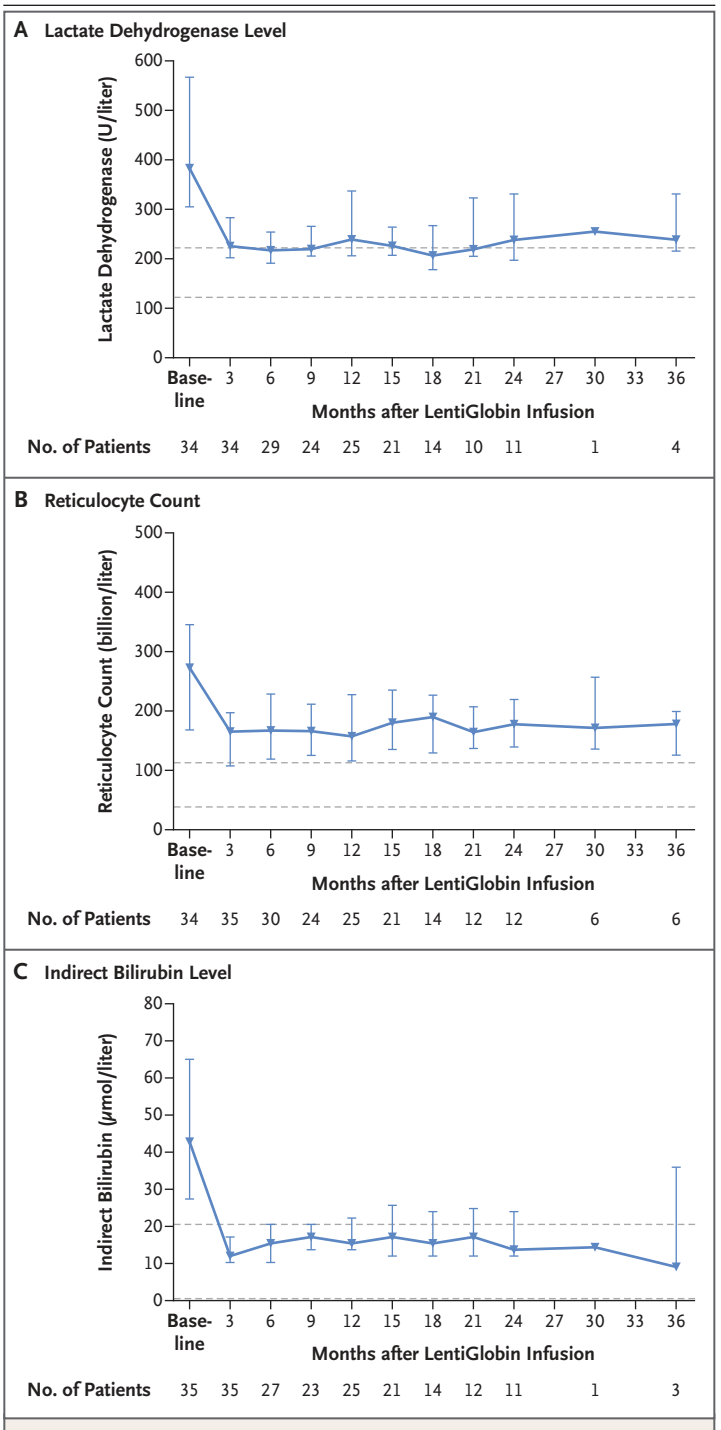

Figure 2. Hemolysis Markers.

Shown are values for the lactate dehydrogenase level (Panel A), the reticulocyte count (Panel B), and the indirect bilirubin level (Panel C) in samples obtained from the 35 patients in the transplant population with available data. Data points indicate medians, and I bars indicate interquartile ranges. The dashed horizontal lines indicate the lower and upper limits of the reference range in a healthy population.[26,27]

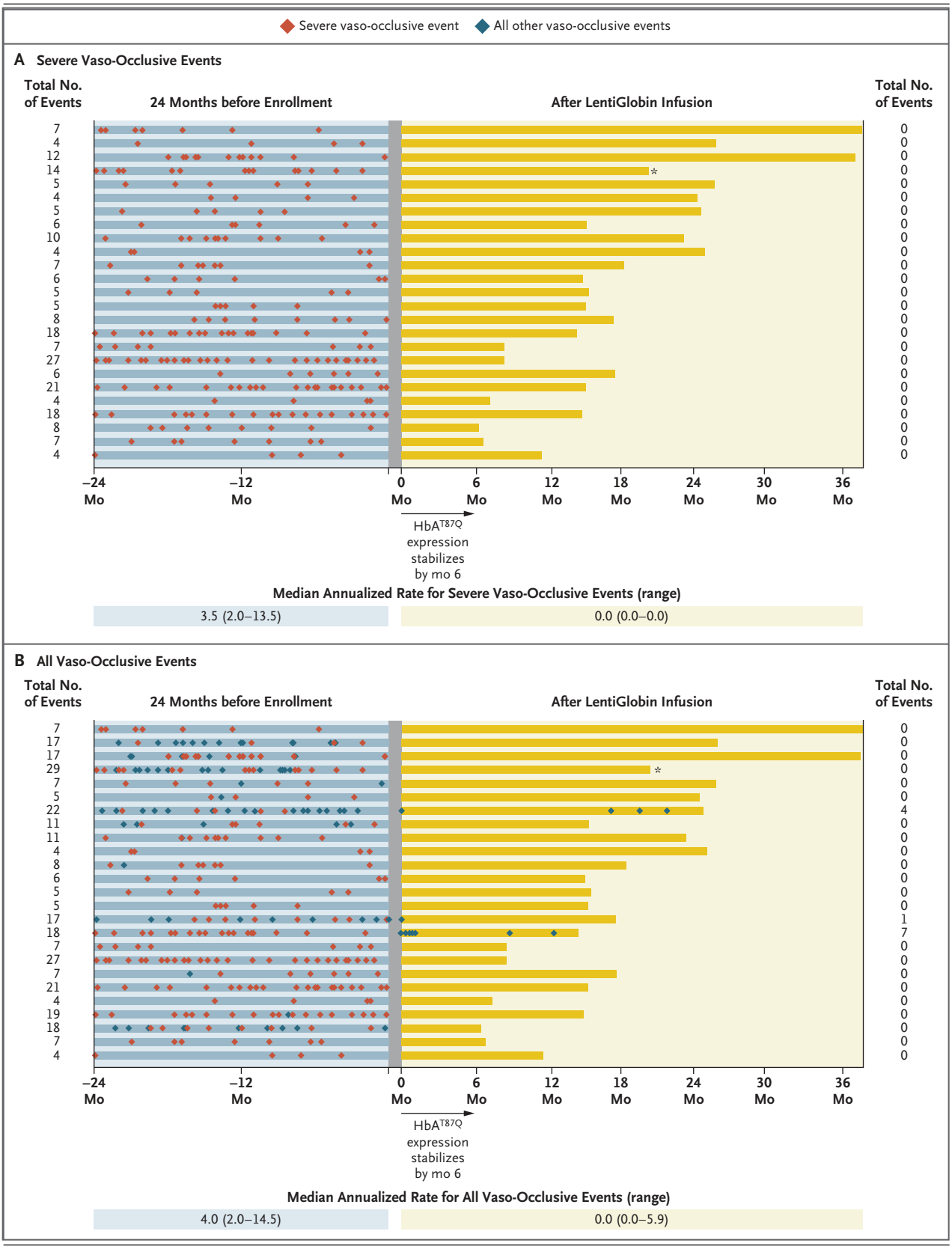

Figure 3 (facing page). Changes in the Rate of Vaso-Occlusive Events before and after LentiGlobin Infusion.

Severe vaso-occlusive events (Panel A) and all vaso-occlusive events (Panel B) were assessed in 25 patients in the transplant population with vaso-occlusive events who had met the criterion of a minimum of four severe vaso-occlusive events during the 24 months before study enrollment and also met the minimum follow-up of 6 months after the LentiGlobin infusion required for analysis. The gray shaded area represents the period during which the patients were receiving preharvest transfusions. A vaso-occlusive event was defined as an episode of acute pain with no medically determined cause other than a vaso-occlusion, including acute episodes of pain, acute chest syndrome, acute hepatic sequestration, acute splenic sequestration, and acute priapism. A severe vaso-occlusive event was defined as such an event leading to a visit to a hospital or emergency department lasting for at least 24 hours, at least two visits to a day unit or emergency department during a 72-hour period (with both visits requiring intravenous treatment), or an episode of priapism lasting more than 2 hours and resulting in a visit to a medical facility. In Panels A and B, the asterisk indicates the death of a patient from sickle cell–related cardiopulmonary disease that was deemed to be unrelated to the LentiGlobin infusion. An adjudication committee is currently assessing reported vaso-occlusive events.

2 events that were deemed to be possibly related (grade 2 leukopenia and grade 1 decreased diastolic blood pressure) and 1 event that was deemed to be definitely related (grade 2 febrile neutropenia); all 3 adverse events resolved within 1 week after onset.

One death occurred 20 months after infusion in a patient with cardiopulmonary disease related to sickle cell disease at baseline. The 27-year-old patient, who had severe sickle cell disease (29 vaso-occlusive events in the 2 years before enrollment) and a history of pulmonary hypertension and venous thrombosis, died after cardiac arrest. No vaso-occlusive events were reported after infusion in this patient, and the total hemoglobin level was at least 12.6 g per deciliter and the HbA^{T87Q} level was at least 4.6 g per deciliter from 6 months through the last visit. An autopsy showed cardiac biventricular dilation with concentric left ventricular hypertrophy and moderate cardiac interstitial fibrosis, with no evidence of pulmonary embolism or stroke. The investigator reported that the sudden death was associated with cardiac fibrosis and other chronic cardiopulmonary organ injury.

No cases of veno-occlusive liver disease, graft failure, circulating replication-competent lentivirus, or vector-mediated insertional oncogenesis were observed in Group C. Genes that had previously been associated with proto-oncogenesis (*MECOM* and *LMO2*)[28] were not in the top 10 genes harboring an insertion site in any patient. No unique insertion site was present as more than 3.8% of all unique insertion sites at any time point in the 30 patients with available data from the transplant population. (Details are provided in the Results section and in Figure S4 in the Supplementary Appendix.)

Since February 17, 2021, two patients presented with similar constellations of clinical and pathological findings, including anemia, bone marrow showing abnormal erythroid precursors (binucleated or trinucleated cells), and trisomy 8 observed by fluorescence in situ hybridization analysis. One of these patients had serious adverse events of pain, whereas the other patient did not. These were the only two patients who were enrolled in the study with two α-globin deletions ($-\alpha 3.7/-\alpha 3.7$). Both patients were initially suspected to have myelodysplastic syndrome; subsequently, both diagnoses were amended to anemia, and further investigations, including next-generation sequencing, determined that there were no predisposing oncogenic mutations associated with hematologic cancers in either patient. In addition, data from integration site analysis pointed to highly polyclonal reconstitution, and the hematologic abnormalities remained restricted to the erythroid lineage. One patient had negative results on a direct Coombs' test and for parvovirus B19. Additional investigations into the root cause of the anemia in these two patients are ongoing.

DISCUSSION

In Group C of the phase 1–2 HGB-206 study, the 25 patients in the TPVOE group with at least 6 months of follow-up who were treated with LentiGlobin had complete resolution of severe vaso-occlusive events. One-time treatment with LentiGlobin led to stable, durable production of HbA^{T87Q}, with expression of HbA^{T87Q} in approximately 85% of red cells, a reduction in levels of sickle hemoglobin and in key hemolysis markers, and normalization of total hemoglobin during 54.8 patient-years of follow-up.

Sickle cell disease is a lifelong, genetic disease,[1,2] and the effect of one-time gene therapy

Table 2. Adverse Events.*

Event	No. of Patients (%)
During treatment	
Attributed to plerixafor for mobilization or apheresis	
No. of patients in analysis†	43 (100)
Any adverse event	22 (51)
Grade ≥3 adverse event	11 (26)
Serious adverse event	5 (12)
Attributed to conditioning	
No. of patients in analysis‡	35 (100)
Any adverse event	35 (100)
Grade ≥3 adverse event	32 (91)
Serious adverse event	5 (14)
After LentiGlobin infusion until last visit§	
No. of patients in analysis‡	35 (100)
Any adverse event	35 (100)
Any adverse event attributed by the investigator to LentiGlobin¶	3 (9)
Grade ≥3 adverse event	34 (97)
Serious adverse event‖	12 (34)
Grade ≥3 adverse events after LentiGlobin infusion in ≥2 patients§	
No. of patients in analysis‡	35 (100)
Stomatitis	24 (69)
Thrombocytopenia	23 (66)
Neutropenia	19 (54)
Febrile neutropenia	15 (43)
Anemia	13 (37)
Leukopenia	11 (31)
Increase in aspartate aminotransferase	6 (17)
Increase in γ-glutamyltransferase	5 (14)
Nausea	4 (11)
Increase in alanine aminotransferase	3 (9)
Decrease in appetite	3 (9)
Abdominal pain	2 (6)
Upper abdominal pain	2 (6)
Increase in blood bilirubin	2 (6)
Lymphopenia	2 (6)
Pharyngeal inflammation	2 (6)
Premature menopause	2 (6)

* All adverse events were coded on the basis of definitions used in the *Medical Dictionary for Regulatory Activities*.
† This analysis was performed in the intention-to-treat population.
‡ This analysis was performed in the transplant population.
§ After the infusion, patients were followed for 24 months in the HGB-206 study before transitioning into the long-term follow-up LTF-307 study.
¶ Two adverse events were reported by the investigator as possibly related to the LentiGlobin infusion (grade 2 leukopenia and grade 1 decreased diastolic blood pressure); another adverse event was deemed to be definitely related to the LentiGlobin infusion (grade 2 febrile neutropenia). All 3 adverse events resolved within 1 week after onset.
‖ Details regarding serious adverse events are reported in Table S4 in the Supplementary Appendix.

requires long-term evaluation. Additional exploration over time is needed to evaluate the effect of biologic changes to fundamental sickle cell physiology, reduction of hemolysis, and amelioration of vaso-occlusive events on the multiorgan complications of sickle cell disease and premature death.[1,2] It remains to be determined whether the effects of LentiGlobin gene therapy are lifelong, as anticipated on the basis of durability in clinical[13] and preclinical studies[14,15,29,30] and in studies regarding allogeneic hematopoietic stem-cell transplantation.[31] A prediction of long-term durability is supported by data from Groups A and B of the HGB-206 study (Table S1), in whom hematopoiesis, vector copy numbers in peripheral blood, and HbA^{T87Q} levels stabilized at 6 months and persisted through the last visit to date (5.5 years), and by results from studies in which investigators used the BB305 lentiviral vector to treat transfusion-dependent β-thalassemia.[18,28]

The LentiGlobin treatment process that was used in Group C underwent substantial optimization as compared with the process used in Group A, including the administration of at least 60 days of packed red-cell transfusions before collection of HSPCs to reduce stress erythropoiesis, the collection of HSPCs by plerixafor mobilization and apheresis (rather than bone marrow harvesting) to improve the safety and yield of cell collection,[17] refined manufacturing to improve transduction efficiency, and higher cell doses to improve engraftment and polyclonal repopulation of the bone marrow. In comparison with patients in Groups A and B, patients in Group C had minimal fetal hemoglobin production after LentiGlobin infusion.[32] After a median follow-up of 5.1 years (range, 4.6 to 5.5) in Group A, two cases of acute myeloid leukemia were reported 3 years and 5.5 years after LentiGlobin infusion; however, no evidence of insertional oncogenesis was identified in either case.[33,34] Optimization of the treatment process in Group C was implemented to improve clinical benefit and reduce the risk of post-transplantation hematologic cancers, including the increased transduction of CD34+ cells, which resulted in increased production of HbA^{T87Q}. Although researchers generally consider that increased vector copy numbers are a risk factor for potential insertional oncogenesis, the data from documented cases involving integrating vectors have identified clones containing few insertion

sites.[35-37] Although our study follow-up is limited (median, 17.3 months; range, 3.7 to 37.6), no cancers have been reported in Group C patients to date.

There were no reported strokes after LentiGlobin infusion, including in patients with a history of stroke. The LentiGlobin regimen for the treatment of sickle cell disease has a safety profile that generally remains consistent with the known risks of autologous stem-cell transplantation, myeloablative single-agent busulfan conditioning, and underlying sickle cell disease.

The demographic characteristics of the study patients are broadly representative of the wider population with sickle cell disease in the United States (Table S2). Limitations of the study design include the relatively small number of patients, a limited duration of follow-up, and the lack of a control group. A limitation of this report is that it describes an unprespecified interim analysis of the study. Continued follow-up is needed to understand the long-term efficacy and safety of LentiGlobin in the treatment of sickle cell disease.

These interim results have shown that one-time treatment with LentiGlobin had an effect on the pathophysiological features of sickle cell disease through sustained production of antisickling hemoglobin HbA^{T87Q}, which led to the complete resolution of severe vaso-occlusive events.

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APPENDIX

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