

ORIGINAL ARTICLE

Exagamglogene Autotemcel for Transfusion-Dependent β -Thalassemia

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ABSTRACT

BACKGROUND

Exagamglogene autotemcel (exa-cel) is a nonviral cell therapy designed to reactivate fetal hemoglobin synthesis through ex vivo clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9 gene editing of the erythroid-specific enhancer region of *BCL11A* in autologous CD34+ hematopoietic stem and progenitor cells (HSPCs).

METHODS

We conducted an open-label, single-group, phase 3 study of exa-cel in patients 12 to 35 years of age with transfusion-dependent β -thalassemia and a β^0/β^0 , $\beta^0/\beta^{\text{low}}$ -like, or non- $\beta^0/\beta^{\text{low}}$ -like genotype. CD34+ HSPCs were edited by means of CRISPR-Cas9 with a guide mRNA. Before the exa-cel infusion, patients underwent myeloablative conditioning with pharmacokinetically dose-adjusted busulfan. The primary end point was transfusion independence, defined as a weighted average hemoglobin level of 9 g per deciliter or higher without red-cell transfusion for at least 12 consecutive months. Total and fetal hemoglobin concentrations and safety were also assessed.

RESULTS

A total of 52 patients with transfusion-dependent β -thalassemia received exa-cel and were included in this prespecified interim analysis; the median follow-up was 20.4 months (range, 2.1 to 48.1). Neutrophils and platelets engrafted in each patient. Among the 35 patients with sufficient follow-up data for evaluation, transfusion independence occurred in 32 (91%; 95% confidence interval, 77 to 98; $P < 0.001$ against the null hypothesis of a 50% response). During transfusion independence, the mean total hemoglobin level was 13.1 g per deciliter and the mean fetal hemoglobin level was 11.9 g per deciliter, and fetal hemoglobin had a pancellular distribution ($\geq 94\%$ of red cells). The safety profile of exa-cel was generally consistent with that of myeloablative busulfan conditioning and autologous HSPC transplantation. No deaths or cancers occurred.

CONCLUSIONS

Treatment with exa-cel, preceded by myeloablation, resulted in transfusion independence in 91% of patients with transfusion-dependent β -thalassemia. (Supported by Vertex Pharmaceuticals and CRISPR Therapeutics; CLIMB THAL-111 ClinicalTrials.gov number, NCT03655678.)

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*A list of the site investigators and coordinators from the CLIMB THAL-111 study group is provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.

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TRANSFUSION-DEPENDENT β -THALASSEMIA is a severe, monogenic, autosomal recessive disease caused by pathogenic variants in *HBB*, the gene encoding β -globin, that result in severely reduced or absent production of the β -chains in adult hemoglobin.¹ The imbalance between α -like and β -like globin chains results in ineffective erythropoiesis, chronic anemia, and dysregulated iron metabolism, which lead to impairment in quality of life, decreased life expectancy, and increased morbidity.²⁻⁷ Patients with transfusion-dependent β -thalassemia receive treatment throughout their lives with regular red-cell transfusions⁸ and regular iron-chelation therapy to control iron overload and minimize the risk of end-organ toxic effects and damage.^{9,10}

The established, potentially curative treatment option for patients with transfusion-dependent β -thalassemia is allogeneic hematopoietic stem-cell transplantation²; however, its use is greatly limited because of inadequate donor availability and the risk of transplantation-related complications, including graft-versus-host disease, graft rejection, and transplantation-related death.¹¹⁻¹³ Gene-addition therapy and gene-editing-based approaches that are designed to address the underlying pathophysiological mechanisms of transfusion-dependent β -thalassemia are being developed as potential therapeutic alternatives.¹⁴⁻¹⁸

Exagamglogene autotemcel (exa-cel) is a nonviral cell therapy designed to reactivate fetal hemoglobin synthesis through ex vivo clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9 gene editing of CD34+ autologous hematopoietic stem and progenitor cells (HSPCs) at the erythroid-specific enhancer region of *BCL11A*.^{15,19,20} (A study of the specificity of CRISPR-Cas9 gene editing of *BCL11A* in the engineering of exa-cel is now reported in the *Journal*.²¹) Elevated levels of fetal hemoglobin have been shown to reduce morbidity and mortality among patients with β -thalassemia and sickle cell disease.^{22,23} (A study of exa-cel for the treatment of sickle cell disease is also now reported in the *Journal*.²⁴) During the neonatal period, when high levels of fetal hemoglobin are present, patients with β -thalassemia are transfusion-free.²⁵ Patients who have

β -thalassemia and coinherit the condition of hereditary persistence of fetal hemoglobin, whereby increased levels of fetal hemoglobin persist throughout life, have little to no disease symptoms and do not require long-term treatment with transfusions.^{26,27} We hypothesized that similar increases in fetal hemoglobin and total hemoglobin, brought about by treatment with exa-cel, would eliminate the need for transfusions

METHODS

STUDY DESIGN, PATIENTS, AND OVERSIGHT

We are conducting this ongoing phase 3, open-label, single-dose study of exa-cel (CLIMB THAL-111) at 13 sites in Canada, Germany, Italy, the United Kingdom, and the United States. Patients 12 to 35 years of age were eligible if they had a confirmed diagnosis of transfusion-dependent β -thalassemia and a transfusion history of at least 100 ml of packed red cells per kilogram of body weight per year or 10 units of packed red cells per year for 2 years before screening. After completion of the 2-year study period, patients were offered enrollment in a 13-year long-term follow-up study (CLIMB-131; ClinicalTrials.gov number, NCT04208529).

Patients received a combination of granulocyte-colony stimulating factor (G-CSF) and plerixafor for HSPC mobilization followed by apheresis for up to 3 consecutive days to collect CD34+ HSPCs. Exa-cel was manufactured from CD34+ cells with the use of CRISPR-Cas9 and a single guide RNA molecule. Before the exa-cel infusion, patients received a myeloablative, pharmacokinetically adjusted busulfan conditioning regimen for 4 days. Exa-cel was infused intravenously through a central venous catheter at least 48 hours but no more than 7 days after completion of the busulfan infusion. Neutrophil engraftment was considered to have occurred on the first of 3 different days on which three consecutive measurements of the absolute neutrophil count were 500 per microliter or higher. Treatment with G-CSF was allowed in the study protocol (available with the full text of this article at NEJM.org) after day 21 at the discretion of the investigator. Platelet engraftment was considered to have occurred on the first of 3 dif-

ferent days on which three consecutive measurements of the unsupported platelet count (i.e., in the absence of platelet transfusions during the previous 7 days) were at least 20,000 per microliter. Additional details regarding eligibility, HSPC mobilization, the myeloablative busulfan conditioning regimen, and exa-cel manufacturing are provided in the Supplementary Appendix, available at NEJM.org.

The study was designed by Vertex Pharmaceuticals and CRISPR Therapeutics in collaboration with the steering committee. Each patient or patient's legal guardian provided written informed consent, with assent obtained when appropriate. Safety was monitored by an independent data monitoring committee. Data collection and analysis were performed by Vertex Pharmaceuticals in collaboration with the authors and the CLIMB THAL-111 Study Group. The authors had access to the study data after the data cutoff for the interim analysis, reviewed the submitted manuscript, and approved it for submission. The investigators vouch for the accuracy and completeness of data generated at their respective sites, and the investigators and Vertex Pharmaceuticals and CRISPR Therapeutics vouch for the fidelity of the study to the protocol.

END-POINT MEASURES

The primary end point was transfusion independence, defined as a weighted average hemoglobin level of at least 9 g per deciliter without red-cell transfusion for at least 12 consecutive months. The key secondary end point was a weighted average hemoglobin level of at least 9 g per deciliter without red-cell transfusion for at least 6 months. Evaluation of these two end points started 60 days after the last red-cell transfusion for post-transplantation support or management of transfusion-dependent β -thalassemia; 16 months or more of follow-up after exa-cel infusion were required for a patient to be evaluable for these end points. All red-cell transfusions after the exa-cel infusion were adjudicated by an independent end-point adjudication committee.

Other secondary end points included the duration of transfusion independence, total and fetal hemoglobin concentrations, reduction in red-cell transfusions, percentage of alleles with

intended genetic modification in peripheral-blood and bone marrow cells, change in iron-overload measures and measures of ineffective erythropoiesis, and change from baseline in patient-reported outcomes (EuroQol Visual Analogue Scale [EQ VAS], Functional Assessment of Cancer Therapy–General [FACT-G], and Bone Marrow Transplantation Subscale [BMTS]). Safety end points included assessments of neutrophil and platelet engraftment, adverse events, and mortality; clinical laboratory assessments; clinical evaluation of vital signs; electrocardiograms; and physical examinations.

STATISTICAL ANALYSIS

Analyses of efficacy and safety included all patients who received an infusion of exa-cel (the full analysis population). The primary and key secondary end points were evaluated in the primary efficacy population, which included all patients who received an infusion of exa-cel and were followed for at least 16 months after the infusion. Patients who completed follow-up were included in this primary efficacy population. A sample of 45 patients was determined to be sufficient to provide at least 95% statistical power to rule out a response of 50% when the true response is 80% for both the primary and key secondary end points with a one-sided alpha of 2.5%.

The protocol included three prespecified interim analyses with a prespecified boundary and a hierarchical testing procedure for the primary and key secondary end points to allow for early evaluation of efficacy. We describe data from the third prespecified interim analysis (data cutoff of January 16, 2023); the first and second interim analyses were optional and were not conducted. For the third interim analysis, the alpha level for the first and second interim analysis was recovered such that the results for the primary and key secondary end points were considered to be significant if the corresponding one-sided P value was less than 0.01692 against a response of 50% in the primary efficacy population. Although the statistical analysis plan specified that a one-sided P value would be used for hypothesis testing, test results are reported here with two-sided P values. The widths of confidence intervals were not adjusted for multiplicity

and therefore may not be used in place of hypothesis testing. Results for continuous variables were summarized with descriptive statistics, including the mean, median, standard deviation, and range. For categorical variables, results were summarized as counts and percentages. Baseline was defined as the most recent nonmissing measurement obtained before the start of HSPC mobilization.

After completion of the prespecified third interim analysis, we conducted an additional data cutoff, which was not prespecified, on April 16, 2023. Analyses of safety and efficacy outcomes at this data cutoff were consistent with those in the prespecified interim analysis. Data from this additional cutoff are provided in the Supplementary Appendix. Unless otherwise noted, all results described here are from the prespecified third interim analysis.

RESULTS

EVALUATION OF OFF-TARGET EDITING

Preclinically, the precision of CRISPR-Cas9 gene editing at the *BCL11A* locus was assessed with the use of orthogonal off-target evaluation methods. Yen et al.²¹ found no evidence of off-target editing in CD34+ HSPCs from eight healthy donors and three donors with transfusion-dependent β -thalassemia.

POPULATION AND ENGRAFTMENT CHARACTERISTICS

The first patient was enrolled on September 10, 2018, and enrollment has now been completed. As of January 16, 2023 (the date of the prespecified third interim analysis), 59 patients had started HSPC mobilization, 53 of whom started myeloablative busulfan conditioning and 52 of whom received exa-cel (full analysis population) (Fig. S1 in the Supplementary Appendix). β^0/β^0 or β^0/β^0 -like genotypes were present in 31 patients (60%) (β^0/β^0 in 19 [37%], $\beta^0/\text{IVS-I-110}$ in 9 [17%], and $\text{IVS-I-110}/\text{IVS-I-110}$ in 3 [6%]), and non- β^0/β^0 -like genotypes were present in 21 patients (40%) (β^+/β^0 in 12 [23%], β^+/β^+ in 4 [8%], and β^E/β^0 in 5 [10%]); 18 patients (35%) were between 12 and less than 18 years of age (Table 1). The representativeness of the patients is shown in Table S1.

All the patients had severe disease as indicated by dependence on red-cell transfusions.

The median annualized number of units of red cells transfused per year at baseline was 35.0 units (range, 11.0 to 71.0), and the median annualized volume of red cells transfused was 201.0 ml per kilogram (range, 48.3 to 330.9) in the 2-year period before screening (Table 1). The median number of mobilization cycles with G-CSF and plerixafor was 1 (range, 1 to 4), with most patients (81%) receiving one mobilization cycle (Table 1 and Table S2). The median dose of exa-cel was 7.5×10^6 CD34+ cells per kilogram (range, 3.0×10^6 to 19.7×10^6) (Table 1).

At the time of the prespecified interim analysis, the median duration of follow-up after exa-cel infusion was 20.4 months (range, 2.1 to 48.1); 14 patients completed the 2-year study period and are enrolled in the long-term follow-up study, CLIMB-131. After busulfan myeloablation and exa-cel infusion, all 52 patients in the full analysis population had engraftment of neutrophils and platelets. The median time to neutrophil and platelet engraftment was 29 days (range, 12 to 56) and 44 days (range, 20 to 200), respectively. Overall, 32 patients (62%) received G-CSF before neutrophil engraftment. Only one patient had neutrophil engraftment that occurred later than day 42; this patient had neutrophil engraftment on day 56 without the use of backup cells. Patients with a history of splenectomy had faster neutrophil and platelet recovery than patients with an intact spleen (Table S3).

A total of 35 patients had at least 16 months of follow-up and were eligible for analysis of the primary and key secondary end points. Of these, 20 patients (57%) had β^0/β^0 or β^0/β^0 -like genotypes (β^0/β^0 in 10 [29%], $\beta^0/\text{IVS-I-110}$ in 7 [20%], and $\text{IVS-I-110}/\text{IVS-I-110}$ in 3 [9%]) and 15 patients (43%) had non- β^0/β^0 -like genotypes (β^+/β^0 in 8 [23%], β^+/β^+ in 3 [9%], and β^E/β^0 in 4 [11%]). The demographic and baseline clinical characteristics of the patients in the analysis at the April 16, 2023, data cutoff are shown in Table S15.

PRIMARY END POINT AND KEY SECONDARY END POINT

After the infusion of exa-cel, among the 35 patients who were evaluable for the primary end point (primary efficacy population), transfusion independence occurred in 32 (91%; 95% confidence interval [CI], 77 to 98; $P < 0.001$ against the

Table 1. Demographic and Clinical Characteristics of the Patients at Baseline.*

Characteristic	Full Analysis Population (N = 52)	Primary Efficacy Population (N = 35)
Sex — no. (%)		
Male	27 (52)	18 (51)
Female	25 (48)	17 (49)
Age at screening		
Mean — yr	21.5±6.7	21.1±6.1
Distribution — no. (%)		
12 to <18 yr	18 (35)	11 (31)
≥18 to 35 yr	34 (65)	24 (69)
Race or ethnic group — no. (%)†		
White	18 (35)	15 (43)
Asian	22 (42)	13 (37)
Data not collected per local regulations	7 (13)	4 (11)
Other	2 (4)	0
Multiracial	3 (6)	3 (9)
Genotype — no. (%)		
β^0/β^0 -like	31 (60)	20 (57)
β^0/β^0	19 (37)	10 (29)
$\beta^0/\text{IVS-I-110}$	9 (17)	7 (20)
IVS-I-110/IVS-I-110	3 (6)	3 (9)
Non- β^0/β^0 -like	21 (40)	15 (43)
β^+/β^+	4 (8)	3 (9)
β^+/β^0	12 (23)	8 (23)
β^E/β^0	5 (10)	4 (11)
Annualized volume of red-cell transfusions — ml/kg		
Mean	196.8±63.0	202.0±57.1
Median (range)	201.0 (48.3–330.9)	205.2 (115.2–330.9)
Median annualized red-cell transfusions (range) — units	35.0 (11.0–71.0)	35.0 (20.5–71.0)
Total hemoglobin concentration — g/dl	10.4±2.0	10.4±1.9
Total fetal hemoglobin concentration — g/dl	0.6±1.0	0.5±0.6
Spleen intact — no. (%)	36 (69)	26 (74)
Iron status		
Median liver iron concentration (range) — mg/g	3.5 (1.2–14.0)	4.0 (1.4–14.0)
Median cardiac iron content by T2*-weighted MRI (range) — msec	34.0 (12.4–61.1)	34.8 (19.6–61.1)
Median serum ferritin concentration (range) — pmol/liter	2891.9 (584.2–10,837.3)	2653.7 (674.1–10,740.7)
Median no. of mobilization cycles (range)	1 (1–4)	1 (1–2)
Median exa-cel dose (range) — CD34+ cells/kg	7.5×10 ⁶ (3.0×10 ⁶ –19.7×10 ⁶)	6.4×10 ⁶ (3.0×10 ⁶ –19.7×10 ⁶)

* Plus-minus values are means ±SD. The full analysis population included all patients who received the exagamglogene autotemcel (exa-cel) infusion. The primary efficacy population included all patients who received the exa-cel infusion and had at least 16 months of follow-up after the infusion. To convert values for iron to micromoles per liter, multiply by 0.1791.

† Race and ethnic group were reported by the patient.

null hypothesis of a 50% response); the results were the same for the key secondary end point (weighted average hemoglobin level of at least 9 g per deciliter without red-cell transfusion for at least 6 months) (91%; 95% CI, 77 to 98; $P < 0.001$ against the null hypothesis of a 50% response) (Table 2 and Fig. 1). In these patients, red-cell transfusions were stopped at a mean ($\pm$ SD) of 35.2 ± 18.5 days after the exa-cel infusion. All the patients who reached transfusion independence remained transfusion-independent throughout the follow-up period, with a mean duration of 22.5 months (range, 13.3 to 45.1). The results of prespecified subgroup analyses of the primary end point according to age and genotype and post hoc analyses according to sex and race were consistent with those of the primary analysis (Table S4). Among the 52 patients who received exa-cel (the full analysis population), 48 (92%) stopped receiving red-cell transfusions and were transfusion-free for 0.3 to 45.1 months starting 60 days after the last red-cell transfusion; 3 of the 4 patients who did not stop receiving red-cell transfusions had less than 3 months of follow-up (Fig. 1).

One of the three patients who did not reach transfusion independence had a relative reduction from baseline of 84% in the annualized

red-cell transfusion volume. The other two patients did stop receiving red-cell transfusions — one at 14.5 months and the other at 12.2 months after the exa-cel infusion — and were transfusion-free for 7.3 months and 4.0 months, respectively, starting 60 days after the final transfusion. All three patients had intact spleens and β^0/β^0 -like genotypes (β^0 /IVS-I-110 in two and IVS-I-110/IVS-I-110 in one); systemic exposure to busulfan, the number of mobilization cycles, the cell dose, and allelic editing in nucleated peripheral-blood cells and in CD34+ cells in bone marrow in these three patients were similar to those in patients who met the criteria for the primary end point.

At the April 16, 2023, data cutoff, the results of the primary and key secondary analyses were consistent with those of the prespecified interim analyses (Fig. S7 and Table S16). In addition, the three patients who did not reach transfusion independence had stopped receiving red-cell transfusions; one of these patients was transfusion-free for 10.3 months, one for 7.0 months, and one for 2.8 months.

SECONDARY END POINTS

Total Hemoglobin and Fetal Hemoglobin

Early and sustained increases in total hemoglobin and fetal hemoglobin levels were seen after the exa-cel infusion, findings consistent with the observed transfusion independence. Among the patients overall, the mean total hemoglobin level was 11.4 ± 2.2 g per deciliter at month 3 and was maintained at 12 g per deciliter or higher from month 6 through the end of follow-up. The mean fetal hemoglobin level was 7.7 ± 2.9 g per deciliter at month 3 and remained at 10 g per deciliter or higher from month 6 to the end of follow-up, with pancellular distribution ($\geq 95\%$) (Fig. 2A and 2B and Fig. S2). Among the patients who reached transfusion independence, the mean total hemoglobin level was 13.1 ± 1.4 g per deciliter and the mean fetal hemoglobin level 11.9 ± 1.9 g per deciliter, with a pancellular distribution of at least 94% of red cells expressing fetal hemoglobin from month 6 onward. Increases in total hemoglobin and fetal hemoglobin levels were similar in the full analysis population and the primary efficacy population. Increases in fetal hemoglobin and

Table 2. Primary and Key Secondary Efficacy Results.

End Point	Primary Efficacy Population (N=35)
Primary end point: weighted average hemoglobin level of ≥ 9 g/dl without red-cell transfusion for ≥ 12 consecutive months	
No. of patients	32
Percentage of patients (95% CI)*	91 (77–98)
P value	<0.001
Key secondary end point: weighted average hemoglobin level of ≥ 9 g/dl without red-cell transfusion for ≥ 6 consecutive months	
No. of patients	32
Percentage of patients (95% CI)*	91 (77–98)
P value	<0.001

* Confidence interval widths were not adjusted for multiplicity and may not be used in place of hypothesis testing. Both P values are two-sided against a null hypothesis of a 50% response.

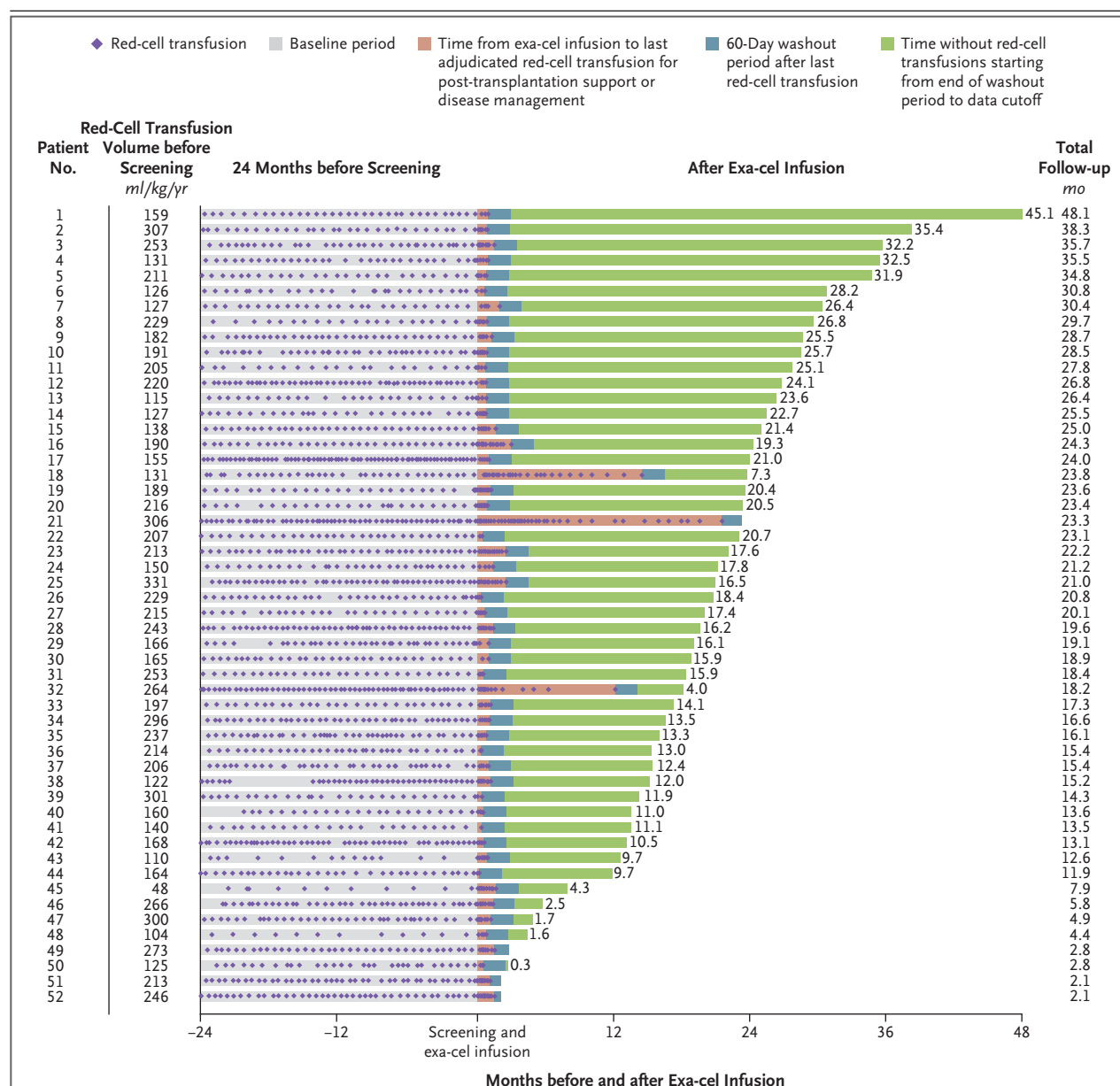
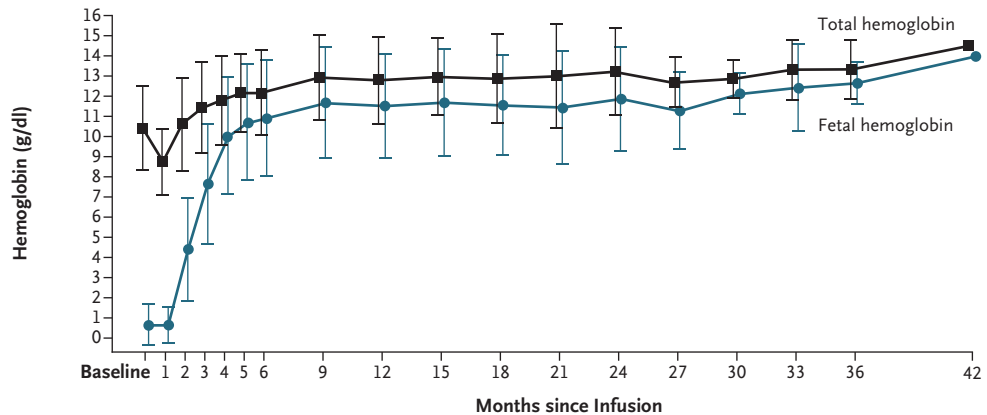


Figure 1. Transfusion Status after Exa-cel Infusion (Full Analysis Population).

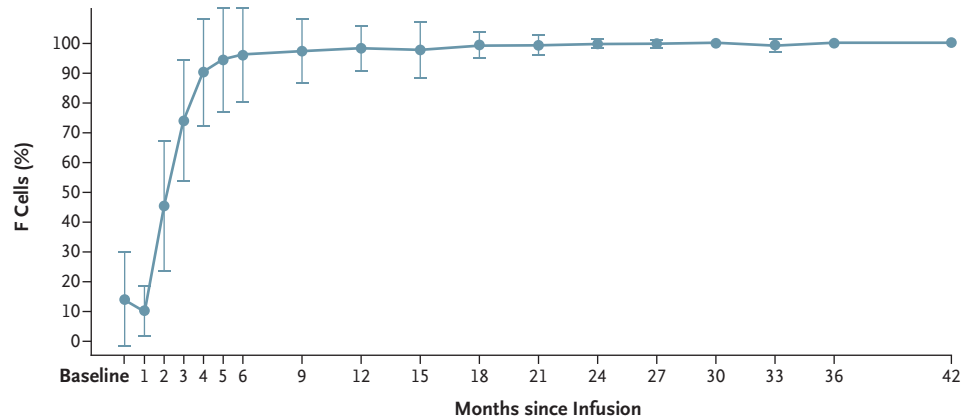
Data from the 52 patients in the full analysis population (all patients who received an infusion of exa-cel) are shown. Patients 1 through 35 had sufficient follow-up to be evaluable for the primary and key secondary end points. Transfusion history in the 2 years before screening is indicated to the left of each lane, and the total follow-up time after the exa-cel infusion is indicated to the right of each lane. All red-cell transfusions that occurred after the exa-cel infusion were adjudicated by the independent end-point adjudication committee. Among the 35 evaluable patients, transfusion independence (weighted average hemoglobin level of ≥ 9 g per deciliter without red-cell transfusion for at least 12 consecutive months) occurred in all except Patients 18, 21, and 32.

total hemoglobin levels were consistent across subgroups based on age, genotype, sex, and race (Tables S5 and S6). The three patients who

did not reach transfusion independence had increases in fetal hemoglobin and total hemoglobin levels that were slower and lower than

A Mean Total Hemoglobin and Fetal Hemoglobin Levels**No. of Patients**

Total hemoglobin	51	52	50	47	48	45	45	44	43	38	30	24	15	10	6	4	2	1
Fetal hemoglobin	51	52	49	46	48	43	45	43	42	35	27	23	15	10	6	4	2	1

B Mean Percentages of F Cells

No. of Patients	52	52	52	47	48	45	45	44	43	38	31	24	15	10	6	4	2	1
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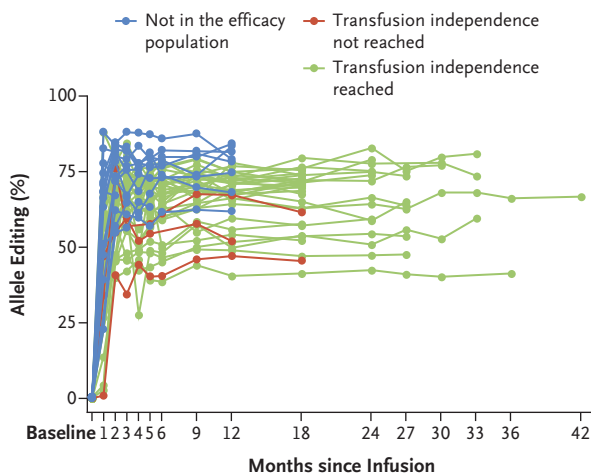
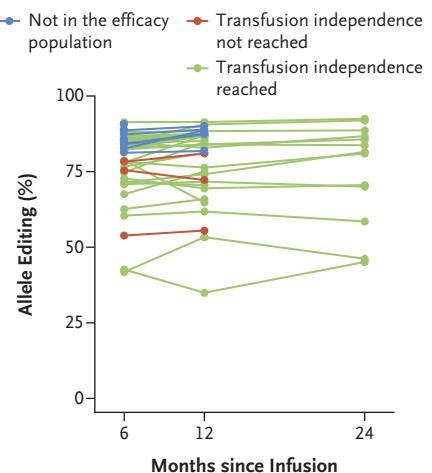
C Individual Percentages of Edited Alleles in Peripheral-Blood Nucleated Cells**D Individual Percentages of Edited Alleles in Bone Marrow CD34+ Cells**

Figure 2 (facing page). Changes in Total and Fetal Hemoglobin Levels and Percentages of Edited *BCL11A* Alleles after Exa-cel Infusion (Full Analysis Population).

Panel A shows the mean total hemoglobin and fetal hemoglobin levels at each study visit over the follow-up period. Panel B shows the percentages of F cells (red cells that express fetal hemoglobin) at each study visit over the follow-up period. In Panels A and B, data are shown as means, and I bars indicate standard deviations. The number of patients evaluated at each time point is shown below the x axis. Panel C shows individual percentages of edited *BCL11A* alleles in peripheral-blood nucleated cells at each study visit. Panel D shows individual percentages of edited *BCL11A* alleles in bone marrow CD34+ cells at each study visit. Per protocol, assessments of allelic editing in bone marrow were performed at months 6, 12, and 24.

those who did (Fig. S3). At the April 16, 2023, data cutoff, with 3 additional months of follow-up, these three patients continued to have stable to improving total and fetal hemoglobin levels (Fig. S4 and S5).

BCL11A Edits in Peripheral Blood and Bone Marrow
Clinically, allelic editing at the *BCL11A* locus was detectable in peripheral red cells within 1 month after the exa-cel infusion. In all patients, the mean percentage of edited *BCL11A* alleles was stable and continued to be generally maintained at or above 64% from month 2 through the end of follow-up (Fig. 2C). Of the *BCL11A* alleles in CD34+ cells of the bone marrow, a mean of $78.0 \pm 11.6\%$ were edited at month 6 (the first month assessed), a percentage that remained stable throughout follow-up (Fig. 2D). Percentages of edited alleles were similar among the patients in the primary efficacy population and were maintained over time. The three patients who did not reach transfusion independence had allelic editing in peripheral blood and bone marrow similar to those who did reach transfusion independence (Fig. 2C and 2D).

Iron Overload, Erythropoiesis, and Patient-Reported Outcomes

Patients in the primary efficacy population had elevated baseline serum ferritin levels (mean, 3508.9 ± 2735.3 pmol per liter) (Table S7). Initial increases in serum ferritin were observed after exa-cel infusion, as expected as a result of the

myeloablative conditioning and red-cell transfusions administered during the peritransplantation period, when chelation therapy was withheld because of its potential interference with the transplantation procedure.²⁸ Serum ferritin levels subsequently decreased to below baseline levels at month 24 (mean, 2295.1 ± 1930.9 pmol per liter [based on measurements in 15 patients]). Similarly, the mean liver iron level as assessed by R2 magnetic resonance imaging (MRI) initially increased after the exa-cel infusion, then gradually decreased (Table S8). T2*-weighted MRI measurements of cardiac iron content remained consistent over time, with all values higher than 20 msec (Table S9).

At baseline, all 35 patients in the primary efficacy population were receiving iron-chelation therapy. After the exa-cel infusion, 28 of the 35 patients restarted iron-removal therapy with chelation, phlebotomy, or both. Of these 28 patients, 10 were able to subsequently stop iron-removal measures.

Erythropoiesis, measured as the myeloid-to-erythroid cell ratio (M:E ratio), improved after the exa-cel infusion. Among the 15 patients with available data from baseline and 24 months, the mean M:E ratio in the bone marrow increased from a mean of 0.62 ± 0.45 at baseline to 0.81 ± 0.42 at month 24. Patients with transfusion-dependent β -thalassemia generally have an M:E ratio of less than 0.1 without transfusion, which increases toward a normal M:E ratio of 1.2 to 5 during receipt of long-term transfusion support.²⁹ Laboratory values associated with erythropoiesis were stable after month 6 (Table S10). The increased M:E ratio at month 24 after the exa-cel infusion, combined with transfusion independence, is indicative of a reduction in ineffective erythropoiesis.

Among adults in the primary efficacy population, patient-reported outcomes (EQ VAS, FACT-G, and BMTS scores) were consistent with improved quality of life by month 24 (Table S11). Improvements in secondary end-point measures (i.e., total hemoglobin level, fetal hemoglobin level, percentage of F cells, allelic editing in peripheral-blood nucleated cells, and allelic editing in bone marrow) that were assessed in the prespecified interim analysis were maintained at the April 16, 2023, data cutoff (Figs. S4, S5, and S6).

SAFETY

All the patients had at least one adverse event after the exa-cel infusion, most of which were of grade 1 or 2 in severity. A total of 46 patients (88%) also had adverse events of grade 3 or 4 in

severity (Table 3). Most adverse events occurred within the first 6 months after the exa-cel infusion, and the frequency decreased thereafter (Tables S13 and S14).

Serious adverse events occurred in 17 patients (33%) (Table S12). The most common serious adverse event was veno-occlusive liver disease (in 5 patients), which was attributed to the busulfan conditioning regimen; it occurred between day 13 and day 32, was of grade 3 or lower in severity, and resolved after defibrotide treatment without any patient receiving ventilatory support or dialysis. Three of the patients who had this serious adverse event were between 12 and 17 years of age, and two were between 18 and 35 years of age. Before the development of veno-occlusive liver disease, all the patients had previously received or were receiving prophylaxis (with ursodeoxycholic acid, defibrotide, or both) from the time of busulfan conditioning. Liver iron content, ferritin, and busulfan exposure (cumulative area under the curve) in patients who had veno-occlusive liver disease were similar to those in patients who did not.

Two patients had serious adverse events that were considered by the investigators to be related to exa-cel. In one of the patients, these serious adverse events were headache, acute respiratory distress syndrome, and idiopathic pneumonia syndrome (the last of which was also considered to be related to busulfan), all of which occurred in the context of hemophagocytic lymphohistiocytosis, which started within 32 days after exa-cel infusion and resolved. In the second patient, the serious adverse events were delayed engraftment and thrombocytopenia (both also considered by the investigators to be related to busulfan), which resolved. This adolescent patient had engraftment of neutrophils on day 56, without the use of backup cells because of the treating physician's choice, which was based on the increasing count of neutrophils; the patient also had platelet engraftment on day 199 with no serious infections or bleeding events. One patient had serious adverse events (cerebellar hemorrhage and subarachnoid hemorrhage) that were considered by the investigators to be related to busulfan and not related to exa-cel. These events

Table 3. Grade 3 or 4 Adverse Events after Exa-Cel Infusion.

Adverse Event	Full Analysis Population (N=52)
	no. of patients (%)
Any grade 3 or 4 event	46 (88)
Grade 3 or 4 events occurring in $\geq 5\%$ of patients*	
Febrile neutropenia	28 (54)
Stomatitis	21 (40)
Anemia	20 (38)
Platelet count decrease	18 (35)
Thrombocytopenia	18 (35)
Mucosal inflammation	17 (33)
Neutrophil count decrease	14 (27)
Decrease in appetite	12 (23)
Epistaxis	7 (13)
Neutropenia	7 (13)
White-cell count decrease	7 (13)
Veno-occlusive liver disease	5 (10)
Blood bilirubin increase	4 (8)
Hypokalemia	4 (8)
Hypophosphatemia	4 (8)
Iron overload	4 (8)
Nausea	4 (8)
Vomiting	4 (8)
CD4 lymphocyte count decrease	3 (6)
Hematuria	3 (6)
Headache	3 (6)
Hypoxia	3 (6)

* Terms for adverse events are adapted from the *Medical Dictionary for Regulatory Activities*, version 25.1, preferred terms.

occurred on day 21 before platelet engraftment and during the early period after myeloablative conditioning. Both events resolved, and the patient recovered. No deaths, discontinuations, or cancers occurred after the exa-cel infusion. The adverse events that were reported at the April 16, 2023, data cutoff were generally consistent with those reported at the third interim analysis (Table S17).

DISCUSSION

In this phase 3 study of exa-cel, we enrolled patients with transfusion-dependent β -thalassemia who had a high burden of red-cell transfusions and impaired quality of life. The study met the primary end point: 91% of the patients had transfusion independence and mean total hemoglobin levels within the normal range. Patients stopped transfusions approximately 1 month after the exa-cel infusion, and transfusion independence was durable, with a mean duration of 22.5 months (range, 13.3 to 45.1). The majority of hemoglobin was fetal hemoglobin, which had a pancellular distribution. These results confirm that CRISPR-Cas9–edited erythroid-specific enhancer region of *BCL11A* in the exa-cel product effectively reactivates fetal hemoglobin production to levels that are known to be protective in persons with hereditary persistence of fetal hemoglobin and that are observed during the neonatal period, which confers a transformational benefit in patients with transfusion-dependent β -thalassemia.²⁵ The increases in total and fetal hemoglobin levels were consistent among patients with various genotypes, including those with the most severe β^0/β^0 genotype (who produce no endogenous hemoglobin A), a finding consistent with a normalization of hemoglobin levels by exa-cel independent of genotype.

In addition, the treatment benefit in adolescents was consistent with that in adults. Taken together, these results show that exa-cel can lead to early and clinically meaningful increases in both total and fetal hemoglobin levels that are sufficient for transfusion independence in both adolescent and adult patients with transfusion-dependent β -thalassemia, regardless of the disease genotype. Improvements in iron-overload

measures, including decreases in serum ferritin levels and liver iron content, were also observed after the exa-cel infusion in conjunction with patients being able to discontinue transfusions, which ultimately led to cessation of iron-removal therapy, including chelation. Improvements in bone marrow biomarkers of erythropoiesis were also observed, findings consistent with reductions in ineffective erythropoiesis. It should be noted that the reduction in iron-overload measures leading to cessation of iron-removal therapy occurs slowly over time and has been shown to take years after successful allogeneic hematopoietic stem-cell transplantation (HSCT) in patients with transfusion-dependent β -thalassemia, which is consistent with the known slow rate of homeostatic processes for iron metabolism and removal.^{30,31}

Of the three patients in the primary efficacy population who did not reach transfusion independence, one had a reduction in the annual transfusion volume of red cells (by 84%) starting at month 10 after receiving exa-cel and the other two stopped red-cell transfusions, although at a later time than other patients in the study. Increases in fetal hemoglobin levels were slower in these three patients than in other patients. The percentage of allelic editing at the intended *BCL11A* locus in peripheral red cells and bone marrow in these three patients was similar to that in patients who reached transfusion independence. The three patients who did not reach transfusion independence may still do so, depending on whether fetal hemoglobin levels continue to increase with additional follow-up. Recent findings have been consistent with this possibility: on additional follow-up (April 16, 2023, data cutoff), all three patients had stopped receiving red-cell transfusions and had been transfusion-free for 10.3, 7.0, and 2.8 months, with continued stable to increasing total hemoglobin and fetal hemoglobin levels.

Frequent red-cell transfusion and iron-chelation therapy, along with the chronic anemia associated with transfusion-dependent β -thalassemia, have been shown to have a negative effect on quality of life.³² We assessed changes in several patient-reported quality-of-life measures after the exa-cel infusion. For all measures evaluated, scores for patient-reported outcomes improved substan-

tially after the exa-cel infusion and were sustained throughout the follow-up period, with increases at month 24 exceeding the established minimal clinically important differences for each measure. These results indicate that patients had improvements in both their general well-being and their overall quality of life after the exa-cel infusion.

Although follow-up in this study is limited (median, 20.4 months), the durability of the benefit of exa-cel treatment is supported by the stable percentages of *BCL11A* alleles maintained in both nucleated peripheral-blood cells and bone marrow CD34+ cells. Allelic editing in peripheral blood was detected within 1 month after the exa-cel infusion, and the mean percentage of alleles with the intended genetic modification generally remained stable after month 2. The mean percentage of allelic editing in bone marrow was 78.0% at month 6, which was the first time point assessed, and was maintained at 75% or higher throughout follow-up. The slightly lower percentage of editing in peripheral blood than in bone marrow is expected, since nucleated peripheral-blood cells include lymphocytes, which are not derived from the CD34+ stem cells and may not be depleted when single-agent busulfan conditioning is used.

The CRISPR-Cas9 editing process was precise, with no evidence of off-target editing on the basis of preclinical evaluations in samples from six patients (three patients with transfusion-dependent β -thalassemia and three patients with sickle cell disease) and no evidence of chromosomal abnormalities, as reported by Yen et al.²¹ Stable trilineage hematopoiesis was recovered in all patients, and no cancers were reported in the exa-cel clinical development program. We did not assess for clonal hematopoiesis, which, if found, would have been of unclear clinical significance. However, additional stud-

ies are under way to test for the effect of sequence variation on the risk of off-targeting editing.³³

All the patients had engraftment of neutrophils and platelets, and the median time to engraftment was generally consistent with other genetic therapies.¹⁴ Overall, the adverse event profile of exa-cel was consistent with anticipated events after myeloablative busulfan conditioning and autologous stem-cell transplantation, with the majority of the adverse events occurring within the first 6 months after the exa-cel infusion. Veno-occlusive liver disease is a known risk with busulfan treatment, and its incidence in this study was similar to that previously reported after busulfan myeloablative conditioning and gene-addition therapy or HSCT in patients with transfusion-dependent β -thalassemia.^{14,34-36} No cases of myelodysplasia or other hematologic cancer occurred.

The data from this prespecified interim analysis show that a one-time infusion of exa-cel provides early and sustained increases in both total hemoglobin and fetal hemoglobin levels that result in durable transfusion independence and improved quality of life for patients with β -thalassemia.

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APPENDIX

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