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Exagamglogene Autotemcel for Severe Sickle Cell Disease

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ABSTRACT

BACKGROUND

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

METHODS

We conducted a phase 3, single-group, open-label study of exa-cel in patients 12 to 35 years of age with sickle cell disease who had had at least two severe vaso-occlusive crises in each of the 2 years before screening. CD34+ HSPCs were edited with the use of CRISPR-Cas9. Before the exa-cel infusion, patients underwent myeloablative conditioning with pharmacokinetically dose-adjusted busulfan. The primary end point was freedom from severe vaso-occlusive crises for at least 12 consecutive months. A key secondary end point was freedom from inpatient hospitalization for severe vaso-occlusive crises for at least 12 consecutive months. The safety of exa-cel was also assessed.

RESULTS

A total of 44 patients received exa-cel, and the median follow-up was 19.3 months (range, 0.8 to 48.1). Neutrophils and platelets engrafted in each patient. Of the 30 patients who had sufficient follow-up to be evaluated, 29 (97%; 95% confidence interval [CI], 83 to 100) were free from vaso-occlusive crises for at least 12 consecutive months, and all 30 (100%; 95% CI, 88 to 100) were free from hospitalizations for vaso-occlusive crises for at least 12 consecutive months ($P<0.001$ for both comparisons against the null hypothesis of a 50% response). The safety profile of exa-cel was generally consistent with that of myeloablative busulfan conditioning and autologous HSPC transplantation. No cancers occurred.

CONCLUSIONS

Treatment with exa-cel eliminated vaso-occlusive crises in 97% of patients with sickle cell disease for a period of 12 months or more. (CLIMB SCD-121; ClinicalTrials.gov number, NCT03745287.)

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*A list of the site investigators and coordinators in the CLIMB SCD-121 Study Group is provided in the Supplementary Appendix, available at NEJM.org.

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CME

Sickle cell disease is an autosomal recessive disorder caused by a single point variant in *HBB*, encoding β-globin, that results in the production of hemoglobin S.[1,2] Clinically, sickle cell disease is characterized by recurrent vaso-occlusive crises, progressive vasculopathy, and chronic hemolytic anemia, which lead to end-organ damage and early death.[2-4]

Current treatments for sickle cell disease consist primarily of disease-modifying therapies that reduce disease severity but do not correct the underlying cause.[5,6] Allogeneic hematopoietic stem-cell transplantation (HSCT) from an HLA-matched sibling donor is a potentially curative option[7]; however, its use is limited because less than 20% of patients have an HLA-matched sibling donor,[8,9] and there are risks of graft-versus-host disease (GVHD) and complications associated with immunosuppression and graft rejection, which can be fatal.[10,11] HSCT from an unrelated donor has been associated with an unacceptably high risk of acute and chronic GVHD.[12] HSCT from a haploidentical donor is another strategy being explored in clinical trials, but the risk of immunologic complications is higher than with HLA-matched sibling donors.[13-15]

Exagamglogene autotemcel (exa-cel) is a nonviral, autologous cell therapy that is designed to reactivate fetal hemoglobin production by means of ex vivo clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9 gene editing at the erythroid enhancer region of *BCL11A* in a patient's own hematopoietic stem and progenitor cells (HSPCs). (The results of a study of the specificity of CRISPR-Cas9 gene editing of *BCL11A* in the engineering of exa-cel are now reported in the *Journal* by Yen et al.[16]) BCL11A is a transcription factor that represses the expression of fetal hemoglobin in erythroid cells after birth.[17,18] Elevated levels of fetal hemoglobin are associated with reduced morbidity and mortality from sickle cell disease and β-thalassemia, another disease caused by variant *HBB*. (The results of a study of exa-cel to treat transfusion-dependent β-thalassemia are now reported in the *Journal* by Locatelli et al.[19]) Persons with sickle cell disease who coinherit the condition of hereditary persistence of fetal hemoglobin and have increased levels of fetal hemoglobin that persist throughout life have few or no symptoms of the disease.[20-22] We therefore hypothesized that an increase in levels of both fetal hemoglobin and total hemoglobin, brought about by treatment with exa-cel, would eliminate vaso-occlusive crises in patients with sickle cell disease.

METHODS

PATIENTS, STUDY DESIGN, AND OVERSIGHT

We are conducting this ongoing phase 3, open-label, single-dose, 2-year study of exa-cel (CLIMB SCD-121) at 16 sites in Belgium, Canada, France, Germany, Italy, the United Kingdom, and the United States. Patients 12 to 35 years of age with a confirmed diagnosis of severe sickle cell disease and a history of at least two vaso-occlusive episodes per year during the 2 years before screening were eligible. After completion of this study, patients were encouraged to enroll in the 13-year long-term follow-up study (CLIMB-131; ClinicalTrials.gov number, NCT04208529).

Autologous CD34+ HSPCs were obtained after plerixafor mobilization of HSPCs, followed by apheresis for up to 3 consecutive days for each episode in which the cells were obtained. Exa-cel was manufactured from these CD34+ cells with the use of CRISPR-Cas9, with a single-guide RNA molecule selectively targeting the *BCL11A* erythroid-specific enhancer.[23] Before the exa-cel infusion, patients received myeloablative conditioning with pharmacokinetically adjusted single-agent busulfan for 4 days.

Exa-cel was administered intravenously through a central venous catheter at least 48 hours, but no more than 7 days, after the 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. According to the study protocol (available with the full text of this article at NEJM.org), treatment with granulocyte colony-stimulating factor (G-CSF) was allowed after day 21 at the discretion of the investigator. Platelet engraftment was considered to have occurred on the first of 3 different days on which three consecutive measurements of the unsupported platelet count (i.e., no platelet

transfusions for the previous 7 days) was 50,000 per microliter or higher. Additional details about study eligibility, mobilization of HSPCs, 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 the patient's legal guardian provided written informed consent, with assent obtained when appropriate. An independent data monitoring committee is reviewing safety data throughout the study. Data collection and analysis were performed by Vertex Pharmaceuticals in collaboration with the authors and the CLIMB SCD-121 Study Group. All the authors had access to the study data after the data-cutoff date for the interim analysis, reviewed the manuscript, and approved it for submission. The investigators vouch for the accuracy and completeness of the data generated at their respective sites, and the investigators, Vertex Pharmaceuticals, and CRISPR Therapeutics vouch for the fidelity of the study to the protocol.

END-POINT MEASURES

The primary end point was freedom from any severe vaso-occlusive crises for at least 12 consecutive months. A severe vaso-occlusive crisis was defined as an event of acute pain that led to a visit to a medical facility and the administration of pain medications (opioids or intravenous nonsteroidal antiinflammatory drugs) or red-cell transfusion, acute chest syndrome, priapism that lasted for more than 2 hours and led to a visit to a medical facility, or splenic sequestration. The two key secondary efficacy end points were freedom from inpatient hospitalization for severe vaso-occlusive crises for at least 12 consecutive months and freedom from severe vaso-occlusive crises for at least 9 consecutive months. Evaluation of the primary end point and the two key secondary end points started 60 days after the last red-cell transfusion for post-transplantation support or for the management of sickle cell disease. All the vaso-occlusive crises that were reported in the 2 years before screening and after exa-cel infusion were adjudicated by an independent end-point adjudication committee.

Other efficacy end points included the duration of time free from severe vaso-occlusive crises, total and fetal hemoglobin concentrations, the percentage of red cells with fetal hemoglobin, the percentage of alleles with intended genetic modification in the nucleated peripheral-blood cells and CD34+ cells of the bone marrow, the change in hemolysis markers (absolute reticulocyte count and indirect bilirubin, lactate dehydrogenase, and haptoglobin levels), and the change from baseline in patient-reported outcomes (the Adult Sickle Cell Quality of Life Measurement Information System [ASCQ-Me; a validated quality of life measure that is used specifically for patients with sickle cell disease], the EuroQol Visual Analogue Scale [EQ VAS], the Bone Marrow Transplantation Subscale, and the Pain Numeric Rating System). Scores on the subscales of the ASCQ-Me range from 0 to 100; for all subscales except pain-related domains, higher scores indicate improvement (minimal clinically important difference, 5 points), and for pain-related domains, lower scores indicate less pain (minimal clinically important difference, −5 points). Scores on the EQ VAS, a patient-rated quantitative measure of health, range from 0 to 100, with higher scores indicating improvement (minimal clinically important difference, 7 to 10 points). Scores on the Bone Marrow Transplantation Subscale, a patient-reported questionnaire for patients who are undergoing bone marrow transplantation, range from 0 to 40, with higher scores indicating improvement (minimal clinically important difference, 2 to 3 points). Scores on the Pain Numeric Rating System range from 0 to 10, with lower scores indicating less pain (minimal clinically important difference, −1 point). The analyses of safety included assessment of neutrophil and platelet engraftment, adverse events, and mortality; clinical laboratory assessments; clinical evaluation of vital signs; electrocardiograms; and physical examinations.

STATISTICAL ANALYSIS

Patients who could be evaluated for the primary end point and the first key secondary end point (freedom from hospitalization for severe vaso-

occlusive crises for ≥12 consecutive months), defined as the primary efficacy population, included all the patients who received an infusion of exa-cel and were followed for at least 16 months after the infusion. Patients who were followed for at least 12 months after the exa-cel infusion, defined as the early efficacy population, could be evaluated for the second key secondary end point (freedom from severe vaso-occlusive crises for ≥9 consecutive months). We determined that a sample size of 45 would be sufficient to provide the study with at least 95% power, with a one-sided alpha of 2.5%, to rule out a response of 50% if a response occurred in 80% for both the primary and the first key secondary end points.

The study protocol included up to three prespecified interim analyses with a prespecified boundary to allow for the early evaluation of efficacy and with a hierarchical testing procedure for the primary and key secondary end points to control for the type I error. The protocol-specified second interim analysis (which included 20 patients) was the first of the interim analyses to be performed (data-cutoff date in February 2023); the first interim analysis was not performed. On the basis of the second interim analysis, the results for all the primary and key secondary end points were significant ($P<0.001$). Another data cutoff was conducted in June 2023, when the sample included 30 patients; the sample size at this data cutoff was similar to that of the prespecified third interim analysis. Because the first interim analysis was not conducted and the statistical boundary was crossed at the second interim analysis, the alpha from the first and second interim analyses was recovered. The alpha for the June data cutoff was 0.0198 (i.e., 0.01074+0.00366+0.00540), and so the results for the primary end point and the first key secondary end point would be considered to be significant if the corresponding one-sided P values were less than 0.0198 for the comparison with a response in 50% of the patients for the primary efficacy population. Although the statistical analysis plan specified that one-sided P values would be used for hypothesis testing, we report the results with two-sided P values, in accordance with *Journal* policy.

Secondary and other end points were assessed as either continuous or categorical variables. The widths of the confidence intervals were not adjusted for multiplicity and therefore may not be used in place of hypothesis testing. For continuous variables, results were summarized with the use of descriptive statistics, including means, medians, and ranges. For categorical variables, results were summarized with the use of counts and percentages. Baseline was defined as the most recent nonmissing measurement that was obtained before the start of mobilization. If multiple measurements of hemolysis markers were available before mobilization, the most recent measurement before the start of exchange transfusions was used. The safety analysis was conducted in the full analysis population, which included all the patients who received an infusion of exa-cel.

RESULTS

EVALUATION OF OFF-TARGET EDITING

Preclinically, the precision of CRISPR-Cas9 gene editing at the *BCL11A* locus was assessed by means of orthogonal off-target evaluation methods. Yen et al. found no evidence of off-target editing in CD34+ HSPCs of eight healthy donors and three donors with sickle cell disease.[16]

POPULATION AND ENGRAFTMENT CHARACTERISTICS

Enrollment has now been completed, with 63 patients enrolled. The first patient was enrolled on November 27, 2018. As of June 14, 2023, a total of 58 patients had started mobilization, 44 of whom had completed myeloablative busulfan conditioning and received exa-cel (full analysis population) (Fig. S1). A total of 40 patients (91%) had the β^S/β^S genotype, 3 (7%) had the β^S/β^0 genotype, and 1 (2%) had the β^S/β^+ genotype. Twelve patients (27%) were 12 to 17 years of age (Table 1). The representativeness of the study population is shown in Table S1.

A total of 15 patients (34%) had a single α-globin gene deletion, and 2 patients (5%) had two α-globin gene deletions (Table S2). The mean historical annualized number of vaso-occlusive crises was 4.1 (range, 2.0 to 18.5), with 26 patients (59%) having had 3 or more vaso-occlusive crises per year for the previous 2 years at baseline (Table 1). The mean annualized rate of in-

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

Characteristic	Full Analysis Population (N=44)	Primary Efficacy Population (N=30)
Sex — no. (%)		
Male	24 (55)	16 (53)
Female	20 (45)	14 (47)
Age at screening		
Mean — yr	21.2±6.1	22.1±6.0
Distribution — no. (%)		
12 to <18 yr	12 (27)	6 (20)
18 to 35 yr	32 (73)	24 (80)
Race — no. (%)†		
White	3 (7)	1 (3)
Black	38 (86)	26 (87)
Other	3 (7)	3 (10)
Genotype — no. (%)		
β^S/β^S	40 (91)	29 (97)
Non-β^S/β^S		
β^S/β^0	3 (7)	1 (3)
β^S/β^+	1 (2)	0
Annualized rate of severe vaso-occlusive crises‡		
No. of severe vaso-occlusive crises/yr	4.1±3.0	3.9±2.1
Distribution — no. (%)		
≥3 vaso-occlusive crises/yr	26 (59)	17 (57)
<3 vaso-occlusive crises/yr	18 (41)	13 (43)
Total hemoglobin — g/dl§	9.1±1.6	9.0±1.6
Total fetal hemoglobin — %§	5.4±3.9	5.2±3.8
Median no. of mobilization cycles (range)	2 (1–6)	2 (1–5)
Median exa-cel dose (range) — CD34+ cells/kg	4.0×10^6 (2.9×10^6–14.4×10^6)	4.0×10^6 (2.9×10^6–14.4×10^6)

* Plus–minus values are means ±SD. The full analysis population included all the patients who received an infusion of exa-cel, and the primary efficacy population included all those who received an exa-cel infusion and were followed for at least 16 months after the infusion. Baseline was defined as the visit when the most recent nonmissing measurement (scheduled or unscheduled) was obtained before the start of mobilization.
† Race was reported by the patient.
‡ Baseline data on severe vaso-occlusive crises were based on the 2 years before the most recent screening. Only severe vaso-occlusive crises that were adjudicated by an end-point adjudication committee as meeting the protocol definition of severe vaso-occlusive crises were included. The annualized rate was calculated as the total number of events divided by the number of years, with 1 year being equivalent to 365.25 days.
§ Hemoglobin measurements were from central laboratories.

Table 2. Primary and Key Secondary Efficacy Results in Patients in the Primary Efficacy Population and the Early Efficacy Population.*

End Point	Value
Primary end point	
Freedom from severe vaso-occlusive crises for ≥12 mo	
No. of patients who met end-point criteria/total no.	29/30
Percentage of patients (95% CI)	97 (83–100)
P value	<0.001
Key secondary efficacy end points	
Freedom from inpatient hospitalization for severe vaso-occlusive crises for ≥12 mo	
No. of patients who met end-point criteria/total no.	30/30
Percentage of patients (95% CI)	100 (88–100)
P value	<0.001
Freedom from vaso-occlusive crises for ≥9 mo	
No. of patients who met end-point criteria/total no.	31/32
Percentage of patients (95% CI)	97 (84–100)
P value	<0.001

* The primary efficacy population included all the patients who received an infusion of exa-cel and were followed for at least 16 months after the infusion; this population was evaluable for the primary end point (freedom from severe vaso-occlusive crises for ≥12 consecutive months) and the first key secondary end point (freedom from hospitalization for severe vaso-occlusive crises for ≥12 consecutive months). The early efficacy population included all the patients who were followed for at least 12 months after the exa-cel infusion; this population was evaluable for the second key secondary end point (freedom from severe vaso-occlusive crises for ≥9 consecutive months). The widths of the confidence intervals were not adjusted for multiplicity and therefore may not be used in place of hypothesis testing. All P values are two-sided against a null hypothesis of a 50% response.

patient hospitalizations for severe vaso-occlusive crises was 2.7 (range, 0.5 to 9.5) per year, with an annualized duration of inpatient hospitalizations for severe vaso-occlusive crises of 19.7 days (range, 2.0 to 136.5).

Before mobilization, patients received transfusion support for a minimum of 8 weeks. A total of 26 patients (59%) received only exchange transfusions, 17 (39%) received both exchange and simple transfusions, and 1 (2%) received only received simple transfusions. During this time, 4 patients (9%) had a hemoglobin S concentration below 30% at every assessment, and 32 patients (73%) had a total hemoglobin level of less than 11 g per deciliter at every assessment. The median number of mobilization cycles with plerixafor was two (range, one to six). A total of 30 patients (68%) underwent one or two mobilization cycles, and 14 patients (32%) underwent three or more cycles (Table S3). The median dose of exa-cel was 4.0×10^6 CD34+ cells per kilogram of body weight (range, 2.9×10^6 to 14.4×10^6) (Table 1).

At the time of this interim analysis, the median follow-up after the exa-cel infusion was 19.3 months (range, 0.8 to 48.1). A total of 17 patients (39%) completed the 2-year study and enrolled in the long-term follow-up study, CLIMB-131. After myeloablation with busulfan and the exa-cel infusion, neutrophil engraftment was observed in all patients (at a median of 27 days; range, 15 to 40) as was platelet engraftment (at a median of 35 days; range, 23 to 126). Overall, 19 patients (43%) received G-CSF after the exa-cel infusion.

PRIMARY AND KEY SECONDARY END POINTS

Of the 30 patients who could be evaluated for the primary end point after the infusion of exa-cel (primary efficacy population), 29 (97%; 95% confidence interval [CI], 83 to 100) were free from vaso-occlusive crises for at least 12 consecutive months (P<0.001 against the null hypothesis of a 50% response) (Table 2 and Fig. 1A). Among these 29 patients, the mean duration of freedom from vaso-occlusive crises was 22.4 months (range, 14.8 to 45.5), and 28 patients have remained free from vaso-occlusive crises as of the data-cutoff date. All 30 patients (100%; 95% CI, 88 to 100) met the criteria for the first key secondary end point (P<0.001 against the null hypothesis of a 50% response) (Table 2).

Results of subgroup analyses of the primary and first key secondary end points according to age group (≥12 and <18 years or ≥18 and ≤35 years), sex (male or female), and annualized rate of vaso-occlusive crises for the previous 2 years at baseline (<3 or ≥3 per year) were consistent with those of the primary analysis (Tables S4 and S5). A total of 31 of 32 patients (97%; 95% CI, 84 to 100) in the early efficacy population met the criteria for second key secondary end point of freedom from severe vaso-occlusive crises for at least 9 consecutive months (P<0.001 against the null hypothesis of a 50% response). One patient with a medical history of chronic pain from sickle cell disease did not meet the

criteria for either the primary end point or the second key secondary end point but did meet the criteria for the first key secondary end point (i.e., no hospitalization for a severe vaso-occlusive crisis for ≥12 months).

After meeting the criteria for the primary and two key secondary end points, one patient had an adjudicated severe vaso-occlusive crisis in the context of parvovirus B19 infection at approximately 22.8 months after the infusion of exa-cel. This patient, who had a reduction in the hemoglobin level from 14.1 g per deciliter to 9.7 g per deciliter, a finding that is consistent with parvovirus infection, recovered fully from the infection, had recovery of the hemoglobin level to normal levels without transfusions, and was free from vaso-occlusive crises for the remainder of the follow-up period (Fig. 1A). Before the parvovirus B19 infection, the patient's reticulocyte count (163.3×10^9 per liter at month 1) was similar to those of other patients. After the vaso-occlusive crisis, the patient's reticulocyte counts were numerically higher (273.1×10^9 per liter at month 24) than those of other patients; however, this result is consistent with recovery from parvovirus B19 infection and normal functioning of the exa-cel graft.[24]

SECONDARY END POINTS

Vaso-occlusive Events in the Full Analysis Population

Of the 44 patients who received an infusion of exa-cel, 43 had at least 60 days of follow-up after their last red-cell transfusion for post-transplantation support or for management of sickle cell disease. (This 60-day period is referred to as the transfusion washout period.) Of these 43 patients, 37 were free from severe vaso-occlusive crises for 0.6 to 45.5 months (Fig. 1A), and 40 patients were free from inpatient hospitalization for severe vaso-occlusive crises after the transfusion washout period for 0.6 to 45.5 months (Fig. 1B). The 6 patients who had a severe vaso-occlusive crisis after the exa-cel infusion had total hemoglobin and fetal hemoglobin levels, percentages of F cells, reticulocyte counts, and allelic editing consistent with those in patients who were free from vaso-occlusive crises (Table S6). Owing to insufficient follow-up, 4 of these 6 patients were not included in the primary efficacy population.

Total Hemoglobin and Fetal Hemoglobin

Early and sustained increases in the total hemoglobin and fetal hemoglobin levels were observed after exa-cel infusion. Patients stopped red-cell transfusions at a median of 20.0 days (standard deviation, 15.1) after the exa-cel infusion. Among all the patients, the mean (±SD) total hemoglobin level was 11.9±1.5 g per deciliter at month 3 and 12.5±1.8 g per deciliter at month 6; thereafter, the total hemoglobin values were maintained at normal or near-normal levels (normal range, 12.1 to 17.2 g per deciliter) (Fig. 2A and Fig. S2A). Individual patient hemoglobin levels were stable over the duration of follow-up to 48.1 months (Fig. S3). The mean percentage of fetal hemoglobin was 36.9±9.0% at month 3, increased to 43.9±8.6% at month 6, and was at least 40% during follow-up (Fig. 2B and Fig. S2B). In a post hoc analysis, the levels of hemoglobin and hemoglobin F levels were similar in patients with α-globin gene deletions and in those without α-globin gene deletions. The mean percentage of F cells (i.e., the percentage of red cells expressing fetal hemoglobin) was 70.1±13.8% at 3 months of follow-up and was greater than 93% by 6 months of follow-up (a result consistent with pancellular distribution) (Fig. 2C and Fig. S2C).

BCL11A *Edits in Peripheral Blood and Bone Marrow*

In clinical samples, allelic editing at the *BCL11A* locus was detected in nucleated peripheral-blood cells within 1 month after the exa-cel infusion. Among all the patients, the mean percentage of edited *BCL11A* alleles was 53.5% at month 1 and was at least 70% from month 2 through the end of follow-up (Fig. 2D). Of the *BCL11A* alleles in CD34+ cells of the bone marrow, the mean percentage of edited cells was 86.1±7.5% at month 6 (which was the first month assessed), a value that remained stable through follow-up (Fig. 2E).

Markers of Hemolysis and Assessment of Patient-Reported Outcomes

Improvements in hemolysis measures were observed over time in all 30 patients in the primary efficacy population. At baseline, the mean lactate dehydrogenase level was 474.3±200.3 U per liter among 29 patients who could be evalu-

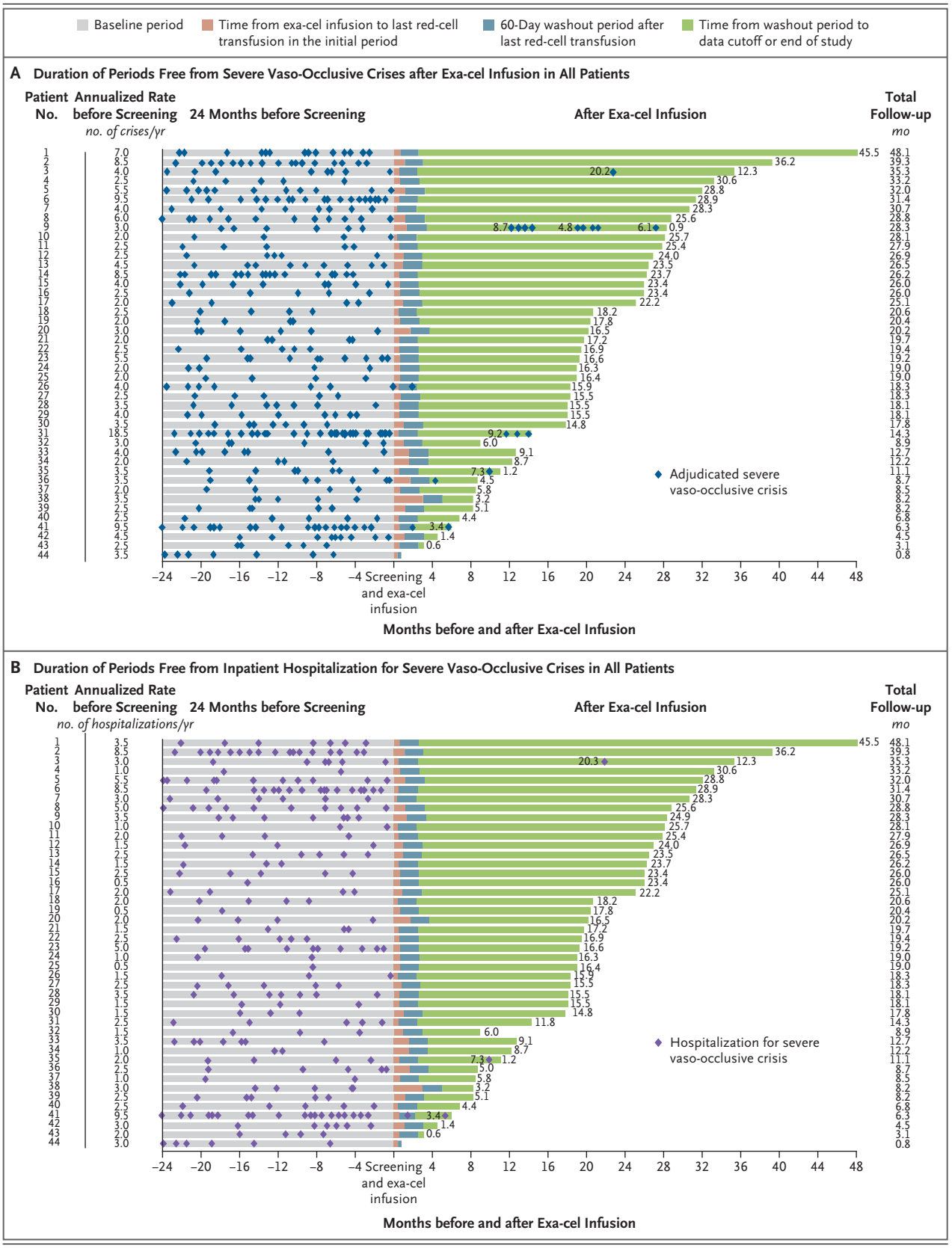

Baseline period
Time from exa-cel infusion to last red-cell transfusion in the initial period
60-Day washout period after last red-cell transfusion
Time from washout period to data cutoff or end of study
A Duration of Periods Free from Severe Vaso-Occlusive Crises after Exa-cel Infusion in All Patients
Patient No.
Annualized Rate before Screening
no. of crises/yr
24 Months before Screening
After Exa-cel Infusion
Total Follow-up
mo
Adjudicated severe vaso-occlusive crisis
Screening and exa-cel infusion
Months before and after Exa-cel Infusion
B Duration of Periods Free from Inpatient Hospitalization for Severe Vaso-Occlusive Crises in All Patients
Patient No.
Annualized Rate before Screening
no. of hospitalizations/yr
24 Months before Screening
After Exa-cel Infusion
Total Follow-up
mo
Hospitalization for severe vaso-occlusive crisis
Screening and exa-cel infusion
Months before and after Exa-cel Infusion

Figure 1 (facing page). Duration of Freedom from Severe Vaso-Occlusive Crises and Freedom from Inpatient Hospitalization for Severe Vaso-Occlusive Crises after Exa-cel Infusion.

Panel A shows the duration of freedom from severe vaso-occlusive crises for all 44 patients in the full analysis population after exa-cel infusion. Patients in the primary efficacy population were evaluable for the primary and key secondary end points. Panel B shows the duration of freedom from inpatient hospitalizations for severe vaso-occlusive crises (a key secondary end point) for all 44 patients in the full analysis population. In both panels, numeric values that appear before diamonds indicate the duration of freedom from the event before its occurrence. In both analyses, Patients 1 through 30 had sufficient follow-up to be included in the primary efficacy population. The history of severe vaso-occlusive crises or hospitalization due to severe vaso-occlusive crises (as an annual rate) in the 2 years before screening is indicated to the left of each lane, with total follow-up time after exa-cel infusion shown on to the right of each lane. In the primary efficacy population (Patients 1 through 30), all the patients except Patient 9 met the criteria for the primary end point; all the patients in this population met the criteria for the secondary end point of freedom from hospitalization for severe vaso-occlusive crises. Patient 32 died from respiratory failure caused by severe acute respiratory syndrome coronavirus 2 infection.

ated. After exa-cel treatment, the mean lactate dehydrogenase level normalized by month 9 (239.2±145.3 U per liter) and was generally maintained within the normal range over time (Fig. S4).

At baseline, 22 of 29 patients (76%) had detectable haptoglobin, with a mean haptoglobin level of 0.08±0.09 g per liter. The mean haptoglobin level increased by month 3 and was maintained: haptoglobin was detectable in 27 of 29 patients (93%) at month 3, in 29 of 29 patients at month 12, and in 17 of 17 patients at month 24, among patients who had visits at those time points (Table S7). The absolute reticulocyte counts and indirect bilirubin levels decreased from baseline and were generally maintained (Table S8). Patient-reported outcomes (i.e., the ASCQ-Me, EQ VAS, Bone Marrow Transplantation Subscale, and Pain Numeric Rating System scores) supported an improved quality of life at month 24 (Tables S9 and S10). The mean changes at month 24 in the EQ VAS score (an increase of 26.9 points) and in the Bone Marrow Transplantation Subscale score (an increase of 3.9 points) exceeded the established minimal clinically important differences for each of these measures (7 to 10 points and 2 to 3 points, respectively). All the subscores (including the pain frequency and severity subscores) on the ASCQ-Me improved from month 6 to month 24, and a decrease in the Pain Numeric Rating System score (–1.7 points) was seen at the month 24 assessment.

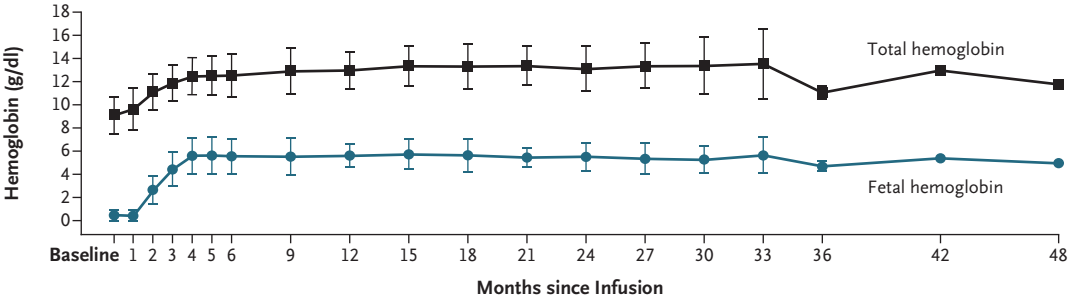
SAFETY

All 44 patients had at least one adverse event after the exa-cel infusion, most of which were of grade 1 or grade 2 in severity. A total of 42 patients (95%) also had adverse events of grade 3 or 4, the most common of which were stomatitis (in 55% of the patients), febrile neutropenia (in 48%), a decreased platelet count (in 48%), and decreased appetite (in 41%) (Table 3). Most adverse events occurred within 6 months after the infusion (Table S12). Graft failure or cancer did not develop in any patient.

A total of 20 patients (45%) had serious adverse events, none of which were considered by the investigators to be related to exa-cel therapy (Tables S11 and S13). One patient (2%) had veno-occlusive liver disease that did not constitute a serious adverse event; the event started on day 11 and was not considered by the investigators to be related to exa-cel therapy. The patient received defibrotide (on days 13 to 19), and the event resolved within 12 days, without the use of ventilatory support, dialysis, or paracentesis.

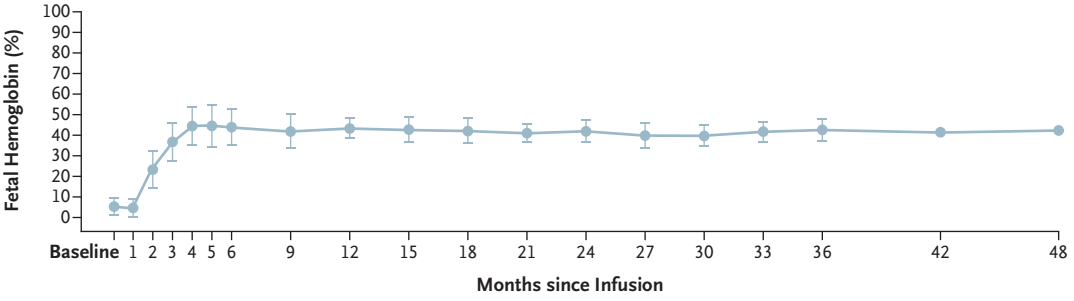
One death from respiratory failure due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection occurred in an adult patient (who also had busulfan-associated lung injury and preexisting lung disease) 268 days after the exa-cel infusion. After the exa-cel infusion, this patient had an uneventful course, with neutrophil and platelet engraftment observed at times consistent with other patients in the study. The patient received a diagnosis of symptomatic SARS-CoV-2 infection on day 71 and was subsequently hospitalized (from day 112 to day 268). During this time, the patient had serious adverse events (pneumonia, hypoxia, and respiratory failure), which were all assessed by the investigator as being unrelated to exa-cel therapy and as being related to SARS-CoV-2 infection and busulfan therapy.

A Mean Total Hemoglobin and Fetal Hemoglobin Levels



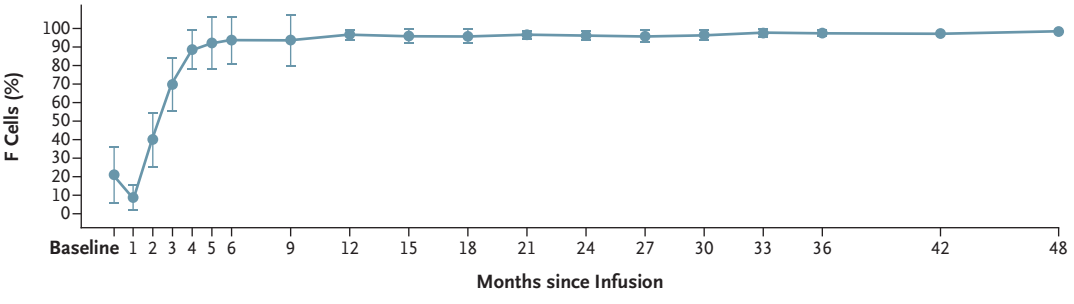
No. of Patients																	
Total hemoglobin	43	42	43	43	41	41	38	34	31	29	27	16	17	10	7	4	2	1	1
Fetal hemoglobin	43	42	43	43	41	40	38	34	31	29	27	16	17	10	7	4	2	1	1

B Mean Fetal Hemoglobin as Percentage of Total Hemoglobin



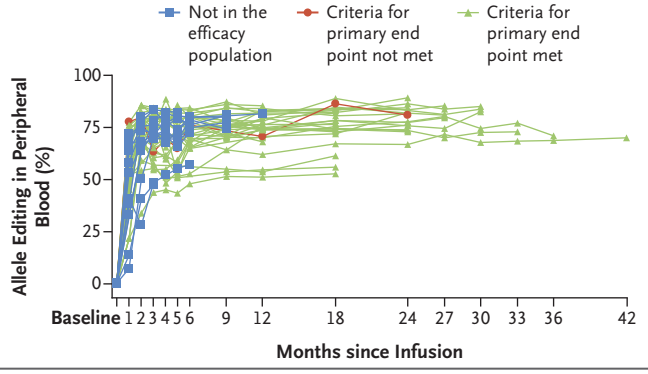
No. of Patients																	
	44	42	43	43	41	40	38	34	32	29	27	16	17	10	7	4	2	1	1

C Mean Percentages of F Cells



No. of Patients																	
	44	43	41	43	41	41	39	34	32	29	27	17	17	10	7	4	2	1	1

D Individual Percentages of Edited Alleles in Peripheral-Blood Nucleated Cells



E Individual Percentages of Edited Alleles in Bone Marrow CD34+ Cells

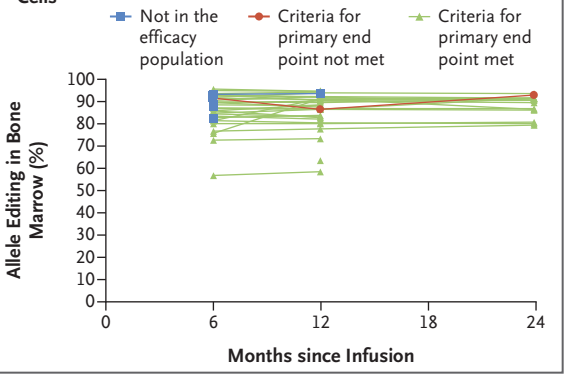


Figure 2 (facing page). Changes in Total and Fetal Hemoglobin Levels, the Percentage of F Cells, and the Proportion of Edited *BCL11A* Alleles in Blood and Bone Marrow after Exa-cel Infusion.

Shown are data for the 44 patients in the 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 mean fetal hemoglobin level as a percentage of total hemoglobin at each visit. Hemoglobin measurements were obtained from central laboratories. Panel C shows the mean total percentage of F cells at each visit. I bars in Panels A, B, and C indicate the standard deviation. Panel D shows the individual percent allelic editing in the peripheral blood at each visit, and Panel E the individual percent allelic editing in bone marrow at each visit; one patient had data at month 12 but not at month 6. Panels D and E show results in patients who were not in the primary efficacy population and in those who met or did not meet the criteria for the primary end point, freedom from vaso-occlusive crises for at least 12 consecutive months.

DISCUSSION

This phase 3, single-group study of exa-cel met the primary end point and both key secondary end points: 97% of patients with sickle cell disease were free from vaso-occlusive crises for at least 12 months, 100% were free from inpatient hospitalization for severe vaso-occlusive crises for at least 12 months, and 97% were free from vaso-occlusive crises for at least 9 months. Patients were free from vaso-occlusive crises for a mean duration of 22.4 months (range, 14.8 to 45.5). Patients had early and sustained increases in total and fetal hemoglobin levels, with a mean total hemoglobin level of 11.9±1.5 g per deciliter at month 3 and 12.5±1.8 g per deciliter at month 6, and normal or near-normal levels (normal range, 12.1 to 17.2 g per deciliter) were maintained thereafter. Improvements were seen in all markers of hemolysis evaluated, including normalization of lactate dehydrogenase and detectable haptoglobin levels, findings that indicated resolution of intravascular hemolysis. Patients also had clinically meaningful improvements in quality of life. These results show that a one-time treatment with nonviral ex vivo CRISPR-Cas9 editing of the erythroid-specific enhancer region of *BCL11A* reactivated fetal hemoglobin production in erythrocytes to levels previously shown to be protective in persons with hereditary persistence of fetal hemoglobin and sickle cell disease (>20% fetal hemoglobin in pancellular distribution) and resulted in a clinical benefit.

The effect of exa-cel on *BCL11A* editing remained stable. At month 2, the mean percentage of edited *BCL11A* alleles was at least 70% in peripheral blood and at least 86% in bone marrow. These percentages remained stable over the course of the study. The slightly lower percentage of editing in peripheral blood than in bone marrow is expected, since nucleated peripheral-blood

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

Event	Full Analysis Population (N=44)
	no. of patients (%)
Grade 3 or 4 adverse event	42 (95)
Grade 3 or 4 adverse event occurring in ≥5% of patients*	
Stomatitis	24 (55)
Febrile neutropenia	21 (48)
Platelet count decrease	21 (48)
Appetite decrease	18 (41)
Neutrophil count decrease	17 (39)
Mucosal inflammation	14 (32)
Anemia	11 (25)
Thrombocytopenia	11 (25)
Neutropenia	10 (23)
White-cell count decrease	6 (14)
Abdominal pain	5 (11)
CD4 lymphocyte count decrease	5 (11)
Cholelithiasis	5 (11)
Pruritus	5 (11)
Constipation	4 (9)
Headache	4 (9)
Nausea	4 (9)
Noncardiac chest pain	4 (9)
Pneumonia	4 (9)
Upper abdominal pain	3 (7)
Arthralgia	3 (7)
Back pain	3 (7)
Deep-vein thrombosis	3 (7)
Oropharyngeal pain	3 (7)
Pain	3 (7)
Weight decreased	3 (7)

* Adverse events are adapted from the *Medical Dictionary of Regulatory Activities*, version 26.0, preferred terms.

cells include lymphocytes, which are not derived from the CD34+ stem cells and may not be depleted when single-agent busulfan conditioning is used. In the study of specificity of CRISPR-Cas9 gene editing of *BCL11A*, no off-target editing was identified.[16] Additional studies to assess the effect of sequence variation are being performed to gain information about the risk of off-target editing.[25]

Patient-reported outcome measures supported clinically meaningful improvements in pain, health and disease status, and general well-being. The mean changes at month 24 in the EQ VAS score and the Bone Marrow Transplantation Subscale score exceeded the established minimal clinically important differences for each of these measures. All the subscores (including the pain frequency and severity subscores) on the ASCQ-Me improved from month 6 to month 24. The ASCQ-Me tool was developed specifically for patients with sickle cell disease and is not intended for comparisons with populations without sickle cell disease. A decrease of –1.7 points in the Pain Numeric Rating System score was seen at the month 24 assessment, which further supports decreases in the occurrence and severity of pain among patients who received exa-cel as compared with the general population of adults with sickle cell disease.

Vaso-occlusive crises are regarded as a subjective end point and so were adjudicated by an independent adjudication committee. It is important to note that there is no consensus definition for vaso-occlusive episodes, so definitions of these episodes vary across clinical trials. We used a broad definition: any episode that led to evaluation and treatment at a health care facility (in an emergency department, outpatient clinic, or hospital for any duration), including acute pain events, events of priapism, acute chest syndrome, and splenic sequestration.

Two patients in the primary efficacy population had vaso-occlusive crises starting 60 days after the last transfusion. Elevated levels of fetal hemoglobin and stable allelic editing levels were maintained in these patients over time, with one patient meeting the primary and first key secondary end points and the other meeting the first key secondary end point. One of these patients had a vaso-occlusive crisis in the context of parvovirus B19 infection, which is a cause of vaso-occlusive crises in patients with sickle cell disease[24,26]; this patient had a milder-than-expected course. With regard to the other patient, pain events manifesting as vaso-occlusive crises are known to occur in some patients with sickle cell disease after allogeneic HSCT, including pain related to preexisting sickle cell disease–related end-organ damage (e.g., avascular necrosis and pain hypersensitivity or hyperalgesia syndromes).

Although all the patients had adverse events, in most patients these events were considered by the investigators to be related to the busulfan-based conditioning regimen and occurred within the first 6 months after the exa-cel infusion. Overall, the safety profile of exa-cel was generally consistent with myeloablative busulfan-based conditioning and autologous transplantation.[27] No cases of GVHD or myelodysplasia or other hematologic cancer occurred. The efficacy of the *BCL11A* gene-editing approach is similar to that of the best results reported with allogeneic HSCT from an HLA-identical donor without GVHD or graft failure.[7,28,29]

A long-term follow-up study is continuing to monitor total and fetal hemoglobin levels and safety, including (but not limited to) the potential for secondary cancers, vaso-occlusive events, hospitalization for vaso-occlusive events, patient-reported outcome measures, and markers of end-organ damage in patients who have completed the current study (CLIMB-131; NCT04208529); other studies are being conducted to assess the risk of secondary cancers and off-target effects after genome editing.[25] Markers of end-organ damage include levels of N-terminal prohormone of brain natriuretic peptide, urinary albumin-to-creatine ratios, and estimated glomerular filtration rates.

The findings of this study showed that an increase in fetal hemoglobin and total hemoglobin levels that was brought about by exa-cel eliminated vaso-occlusive crises for 12 consecutive months or more in nearly all patients (29 of 30) at the time of the data cutoff. Exa-cel treatment was also accompanied by reductions in hemolysis. Data from the follow-up studies may provide insight into the proportion of patients who continue to have vaso-occlusive crises

after treatment and the long-term safety of the treatment.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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APPENDIX

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