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Pipe, Steven W.; Leebeek, Frank W.G.; Recht, Michael; Key, Nigel S.; Castaman, Giancarlo; Miesbach, Wolfgang; Lattimore, Susan; Peerlinck, Kathelijne; Van Der Valk, Paul; Coppens, Michiel

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ORIGINAL ARTICLE

Gene Therapy with Etranacogene Dezaparvovec for Hemophilia B

S.W. Pipe, F.W.G. Leebeek, M. Recht, N.S. Key, G. Castaman, W. Miesbach, S. Lattimore, K. Peerlinck, P. Van der Valk, M. Coppens, P. Kampmann, K. Meijer, N. O'Connell, K.J. Pasi, D.P. Hart, R. Kazmi, J. Astermark, C.R.J.R. Hermans, R. Klamroth, R. Lemons, N. Visweshwar, A. von Drygalski, G. Young, S.E. Crary, M. Escobar, E. Gomez, R. Kruse-Jarres, D.V. Quon, E. Symington, M. Wang, A.P. Wheeler, R. Gut, Y.P. Liu, R.E. Dolmetsch, D.L. Cooper, Y. Li, B. Goldstein, and P.E. Monahan

ABSTRACT

BACKGROUND

Moderate-to-severe hemophilia B is treated with lifelong, continuous coagulation factor IX replacement to prevent bleeding. Gene therapy for hemophilia B aims to establish sustained factor IX activity, thereby protecting against bleeding without burdensome factor IX replacement.

METHODS

In this open-label, phase 3 study, after a lead-in period (≥6 months) of factor IX prophylaxis, we administered one infusion of adeno-associated virus 5 (AAV5) vector expressing the Padua factor IX variant (etranacogene dezaparvovec; 2×10^{13} genome copies per kilogram of body weight) to 54 men with hemophilia B (factor IX activity ≤2% of the normal value) regardless of preexisting AAV5 neutralizing antibodies. The primary end point was the annualized bleeding rate, evaluated in a noninferiority analysis comparing the rate during months 7 through 18 after etranacogene dezaparvovec treatment with the rate during the lead-in period. Noninferiority of etranacogene dezaparvovec was defined as an upper limit of the two-sided 95% Wald confidence interval of the annualized bleeding rate ratio that was less than the noninferiority margin of 1.8. Superiority, additional efficacy measures, and safety were also assessed.

RESULTS

The annualized bleeding rate decreased from 4.19 (95% confidence interval [CI], 3.22 to 5.45) during the lead-in period to 1.51 (95% CI, 0.81 to 2.82) during months 7 through 18 after treatment, for a rate ratio of 0.36 (95% Wald CI, 0.20 to 0.64; P<0.001), demonstrating noninferiority and superiority of etranacogene dezaparvovec as compared with factor IX prophylaxis. Factor IX activity had increased from baseline by a least-squares mean of 36.2 percentage points (95% CI, 31.4 to 41.0) at 6 months and 34.3 percentage points (95% CI, 29.5 to 39.1) at 18 months after treatment, and usage of factor IX concentrate decreased by a mean of 248,825 IU per year per participant in the post-treatment period (P<0.001 for all three comparisons). Benefits and safety were observed in participants with predose AAV5 neutralizing antibody titers of less than 700. No treatment-related serious adverse events occurred.

CONCLUSIONS

Etranacogene dezaparvovec gene therapy was superior to prophylactic factor IX with respect to the annualized bleeding rate, and it had a favorable safety profile. (Funded by uniQure and CSL Behring; HOPE-B ClinicalTrials.gov number, NCT03569891.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Dr. Pipe can be contacted at ummdswp@med.umich.edu or at the Departments of Pediatrics and Pathology, University of Michigan, 1500 E. Medical Center Dr., Ann Arbor, MI 48109-5718.

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Hemophilia B is an X-linked bleeding disorder caused by partial or complete deficiency of circulating factor IX activity due to mutations in the gene *F9*.[1] Worldwide, approximately 33,000 persons have hemophilia B[2]; two thirds of these persons have hemophilia classified on the basis of plasma factor IX activity as moderate (activity of 1 to 5% of the normal value) or severe (activity of <1%).[3] Severe hemophilia B causes spontaneous bleeding into the joints and muscles, which leads to synovitis and arthropathy.[4] Some patients with moderately deficient factor IX levels also have a clinically severe bleeding phenotype and musculoskeletal complications.[5-7] Prophylactic intravenous factor IX replacement therapy is the current standard of care for such patients.[5,8] However, arthropathy and other long-term complications persist because of poor access and adherence to treatment,[9,10] individual dose variations, and low trough factor IX activity.[4,8,11]

Since 2011, several early-phase trials of adeno-associated virus (AAV)–based factor IX gene therapies for hemophilia B showed increased factor IX activity, reduced bleeding episodes, and a decreased need for factor IX replacement.[12-18] Patients with preexisting AAV neutralizing antibodies have been excluded from most studies of AAV-based gene therapies.[12,14,16,17,19-24]

Preclinical and phase 1–2 trial data indicated that preexisting AAV5 neutralizing antibodies may not preclude transduction with AAV5-based gene therapy for hemophilia B.[25,26] A single infusion of AMT-060 (5×10^{12} or 2×10^{13} genome copies [gc] per kilogram of body weight), an AAV5 vector expressing a wild-type human factor IX gene-expression cassette, increased factor IX activity and reduced the annualized bleeding rate and usage of factor IX replacement over a period longer than 5 years, even in patients with hemophilia B who (in hindsight) had detectable AAV5 neutralizing antibody titers.[13,15,18,25] Transient increases in alanine aminotransferase (ALT) levels in 3 of 10 patients, treated with prednisone, were not associated with a loss of factor IX activity.

Etranacogene dezaparvovec is a successor to AMT-060, with the same recombinant AAV5 capsid containing the identical codon-optimized gene-expression cassette, generated by a 2-nucleotide change to the wild-type human factor IX sequence to encode the naturally occurring human factor IX Padua (R338L) variant. Factor IX Padua protein has factor IX–specific activity that is 6 to 8 times as high as that seen with wild-type factor IX.[27,28] In a phase 2b study involving three participants, treatment with etranacogene dezaparvovec resulted in sustained mean factor IX activity of 44.2% at 2 years, irrespective of whether AAV5 neutralizing antibodies were detectable at baseline.[29]

Here, we report the safety and efficacy of etranacogene dezaparvovec from Health Outcomes with Padua Gene; Evaluation in Hemophilia B (HOPE-B), a phase 3 study of gene therapy for the treatment of hemophilia B regardless of preexisting AAV5 neutralizing antibody status.

A Quick Take is available at NEJM.org

METHODS

STUDY DESIGN

HOPE-B is a phase 3, open-label, single-dose, multicenter study conducted at 33 sites (17 in the United States, 13 in the European Union, and 3 in the United Kingdom). The study is conducted in accordance with International Council for Harmonisation Good Clinical Practice guidelines and ethical principles originating in the Declaration of Helsinki. The study began on June 27, 2018, and the 18-month registrational analysis includes data up to October 18, 2021; additional safety and efficacy data collection is ongoing for 5 years. The protocol, available with the full text of this article at NEJM.org, was approved by institutional review boards and independent ethics committees at each study site. All the participants provided written informed consent. The data were collected and analyzed by CSL Behring; the academic authors had full access to the data. Medical writers (funded by CSL Behring) wrote the manuscript that was submitted, with input from the authors, and the authors provided approval of the manuscript before submission. The authors vouch for the accuracy and completeness of the data and for the fidelity of the study to the protocol.

POPULATION

The participants are men who are at least 18 years of age and have inherited hemophilia B classified as severe (plasma factor IX activity of <1%) or moderately severe (plasma factor IX activity of 1 to 2%) with a severe bleeding phenotype.[1,30] Participants received stable continuous factor IX prophylaxis, with the dose and factor IX product determined by their physician. Participants with a history of factor IX inhibitor

use, uncontrolled human immunodeficiency virus infection, or advanced liver fibrosis were excluded. Details of the inclusion and exclusion criteria are provided in the Supplementary Appendix, available at NEJM.org.

PROCEDURES

The study design is shown in Figure S1 in the Supplementary Appendix. After a lead-in period of at least 6 months during which participants received continuous factor IX prophylactic therapy and recorded all bleeding events, participants received a single intravenous dose of etranacogene dezaparvovec (2×10^{13} gc per kilogram; the vector and production are described in the Supplementary Appendix). Participants were initially followed for 18 months after treatment for evaluation of efficacy and safety; they then entered a follow-up phase that extends through 5 years after dosing. The methods used for data collection and assays are described in the Supplementary Appendix.

END POINTS

The primary end point was the annualized bleeding rate (based on all bleeding episodes), evaluated in a noninferiority analysis comparing the rate during the 52 weeks after stable factor IX expression (defined uniformly as months 7 through 18 after treatment) with the rate during the lead-in period. Factor IX expression was considered stable and the primary end-point analysis began after all participants who received glucocorticoids discontinued this concomitant medication (i.e., 6 months after receipt of etranacogene dezaparvovec).

Key secondary end points were chosen to assess the efficacy of etranacogene dezaparvovec as compared with the standard of care, including endogenous factor IX activity at 6, 12, and 18 months after treatment and 12 months after stable factor IX expression. These end points were the number of infusions and annualized consumption of factor IX replacement therapy, the percentage of participants with trough factor IX activity lower than 12%, the superiority of etranacogene dezaparvovec (based on the annualized bleeding rate for all bleeding episodes), the annualized bleeding rate for episodes of spontaneous bleeding and joint bleeding, the percentage of participants who discontinued routine prophylaxis, and the correlation between pretreatment AAV5 neutralizing antibody titers (measured with a sensitive luciferase-based AAV transduction inhibition assay) and post-treatment annualized bleeding rate and factor IX activity (see the Supplementary Appendix). Scores on two disease-nonspecific patient-reported outcome (PRO) instruments — the International Physical Activity Questionnaire (iPAQ)[31] and the EuroQol 5-Dimension 5-Level questionnaire (EQ-5D-5L)[32] visual analogue scale (VAS) — were additional secondary end points. Scores obtained with hemophilia-specific PRO instruments were included among the exploratory end points. A full list of exploratory end points is provided in Table S1.

Principal safety outcomes included adverse events that occurred or worsened during or after treatment, liver-function abnormalities, vector shedding, and an immune response directed at the AAV5 vector or the transgene product (factor IX).

STATISTICAL ANALYSIS

The sample size was constrained by the noninferiority analysis of the primary end point, the annualized bleeding rate. Through simulation of the annualized bleeding rate under a negative binomial distribution with a yearly rate of 2.4 events for the lead-in period and 1.9 events for the post-treatment period, with a Pearson correlation of 0.05 for the number of events between the two periods, and with a common negative binomial dispersion parameter of 1.5, we calculated that a sample of 54 participants would be needed in order to demonstrate noninferiority with a noninferiority margin of 1.8 and at least 82% power.

The primary population for statistical analysis was the full analysis population, which included all participants who were enrolled, entered the lead-in phase, received etranacogene dezaparvovec, and had at least one efficacy end-point assessment after receipt of etranacogene dezaparvovec. The safety population included all participants who were enrolled and received etranacogene dezaparvovec. Details of the study populations, general statistical principles, and analytical methods used for all secondary end points and subgroup analyses are provided in the Supplementary Appendix. For the analysis of the primary end point, the number of reported bleeding episodes was analyzed with a negative binomial regression model involving repeated-measures generalized estimating equations, with

the paired design and the different collection periods accounted for in the model. The estimated rate ratio, two-sided 95% Wald confidence interval, and corresponding P value were determined. Noninferiority of etranacogene dezaparvovec was claimed if the upper limit of the two-sided 95% Wald confidence interval was less than the noninferiority margin of 1.8.

Formal statistical testing of the efficacy end points was performed with the use of a closed testing principle for type I error control for multiple testing. End points were tested for superiority at a two-sided alpha level of 0.05. Fixed sequential testing was performed with the use of a hierarchical approach (see the Supplementary Appendix). Sensitivity and exploratory analyses that were not included in the type I error-control procedure are presented with point estimates and confidence intervals only, and the confidence intervals cannot be used for hypothesis testing. A sensitivity analysis was performed in which the main analysis for the annualized bleeding rate was repeated with consideration of only bleeds treated with exogenous factor IX.

RESULTS

STUDY POPULATION

A total of 54 participants received etranacogene dezaparvovec (Fig. S2); one participant prematurely discontinued treatment after an adverse event of hypersensitivity that occurred after a partial dose (approximately 10% of the full dose) had been received; the participant did not have a response to etranacogene dezaparvovec treatment but continued to participate in the study. The 18-month post-treatment follow-up was completed by 53 participants. The demographic and clinical characteristics of the participants are shown in Table 1. The racial and ethnic distribution of the enrolled participants reflects the distribution in the countries that participated in the study. A summary of the representativeness of the study participants is shown in Table S2.

PRIMARY END POINT

An overview of the key results is provided in Table 2. The annualized bleeding rate for all bleeding episodes decreased after treatment, from 4.19 (95% confidence interval [CI], 3.22 to 5.45) during the lead-in period to 1.51 (95% CI, 0.81 to 2.82) in the post-treatment period (Table 3). The observed annualized bleeding rate ratio for the post-treatment period as compared with the lead-in period was 0.36 (95% Wald CI, 0.20 to 0.64; two-sided P<0.001) (Table 2). Because the upper boundary of the 95% confidence interval (0.64) was less than the noninferiority margin of 1.8 and also lower than 1, both noninferiority and superiority (a secondary end point) to factor IX prophylaxis during the lead-in period were declared. The results for the analyzed subgroups were consistent with the overall results (Fig. S3). The annualized bleeding rate for factor IX–treated bleeding episodes also decreased after treatment (annualized bleeding rate ratio, 0.23; 95% Wald CI, 0.12 to 0.46) (Table 2). Fourteen participants (26%) had no bleeding episodes during the lead-in period, and this number increased to 34 participants (63%) after treatment.

SECONDARY END POINTS

Endogenous factor IX activity during the 18 months after treatment is shown in Figure 1. At diagnosis, most participants (44; 81%) had factor IX activity of less than 1%. Increases in endogenous factor IX activity were apparent from 3 weeks after treatment (mean [±SD] factor IX activity, 26.8±12.7%; range, 4.9 to 56.7). At 6 months after treatment, factor IX activity increased to 39.0±18.7% (range, 8.2 to 97.1), with a least-squares mean increase from baseline of 36.2 percentage points (95% CI, 31.4 to 41.0; P<0.001). Increases in factor IX activity were sustained through month 12 (least-squares mean increase from baseline, 38.8 percentage points; 95% CI, 34.0 to 43.6; P<0.001) and month 18 (least-squares mean increase from baseline, 34.3 percentage points; 95% CI, 29.5 to 39.1; P<0.001).

Factor IX activity was lower when measured with the chromogenic assay than when measured with the one-stage (activated partial-thromboplastin time–based) assay (Fig. S4). The mean factor IX activity as measured by chromogenic assay was 16.5±8.8%, 17.9±10.1%, and 19.7±11.7% at months 6, 12, and 18, respectively, after treatment.

A total of 52 participants (96%) discontinued factor IX prophylaxis during the period from day 21 through month 18 after treatment. Of the remaining 2 participants, whose factor IX activity was less than 5% at month 18 and who did not discontinue factor IX prophylaxis, one received

Table 1. Demographic and Clinical Characteristics of the Participants (Safety Population).*

Characteristic	Value (N = 54)
Age — yr	
Mean	41.5±15.8
Range	19–75
Male sex — no. (%)	54 (100)
Race or ethnic group — no. (%)†	
White	40 (74)
Other	6 (11)
Middle Eastern	3 (6)
Spanish or Hispanic	2 (4)
East Indian	1 (2)
Missing data	5 (9)
Asian	2 (4)
Black	1 (2)
Hispanic or Latino ethnic group — no. (%)†	
Not Hispanic or Latino	45 (83)
Missing data	5 (9)
Hispanic or Latino	4 (7)
Geographic location — no. (%)	
European Union or United Kingdom	34 (63)
United States	20 (37)
BMI‡	
Mean	27.2±5.1
Range	21–51
BMI category — no. (%)‡	
<35	52 (96)
35 to <40	1 (2)
≥40	1 (2)
Severity of hemophilia B at time of diagnosis — no. (%)§	
Severe	44 (81)
Moderately severe	10 (19)
Any bleeding episodes in year before screening — no. (%)	44 (81)
History of infection — no. (%)	
HIV-positive	3 (6)
Previous HBV infection	9 (17)
Previous HCV infection	28 (52)
HCV-positive at screening	0
Factor IX replacement therapy type — no. (%)¶	
Prophylactic	54 (100)
On demand	4 (7)
Most recent prescreening factor IX therapy category — no. (%)	
Extended half-life	31 (57)
Standard half-life	23 (43)

Table 1. (Continued.)

Characteristic	Value (N = 54)
Detectable neutralizing antibodies to AAV5 at baseline — no. (%)	21 (39)
Maximum titer	3212.3
No bleeds in lead-in period — no. (%)	14 (26)
Unadjusted mean annualized exogenous factor IX consumption during lead-in period — IU/yr	257,339±149,013
Adjusted annualized factor IX replacement therapy infusion rate during lead-in period — infusions/yr	72.49

* Plus–minus values are means ±SD. The safety population included all participants who were enrolled and received etranacogene dezaparvovec. AAV denotes adeno-associated virus, HBV hepatitis B virus, HCV hepatitis C virus, and HIV human immunodeficiency virus.
† Race and ethnic group were reported by the investigator. Participants with missing data on race and ethnic group came from two centers in a single Northern European country in which collection and reporting of these data were not permitted by the ethics committee because of the European Union General Data Protection Regulation at the time of enrollment.
‡ Body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters.
§ Severe hemophilia B was defined as plasma factor IX activity of less than 1%, and moderately severe hemophilia B was defined as plasma factor IX activity of 1 to 2%.
¶ Some participants received both prophylactic and on-demand factor IX replacement therapy during the lead-in period.

only a partial etranacogene dezaparvovec dose (approximately 10% of the dose), and the other had the highest day-of-dosing AAV5 neutralizing antibody titer in the study (3212).

During the lead-in period, participants used a mean of 257,338±149,013 IU of factor IX per year (range, 83,541 to 755,892). The mean use of factor IX per participant decreased by 248,825 IU per year from the lead-in period to the post-treatment period (P<0.001) (Table 2). The annualized factor IX infusion rate per participant decreased from 72.5 infusions (95% CI, 63.6 to 82.7) during the lead-in period to 2.5 infusions (95% CI, 0.92 to 6.96) after treatment (adjusted rate ratio, 0.03; P<0.001) (Table 2). During months 7 through 18 after treatment, 15 participants received a total of 134 factor IX infusions.

The annualized rates of spontaneous bleeding episodes and all joint bleeding episodes decreased significantly after treatment (Table 3). The rate ratios for the lead-in period as compared with the post-treatment period were 0.29 for spontaneous bleeding (i.e., a 71% reduction) and 0.22 for joint bleeding (i.e., a 78% reduction) (Table 2).

In the analysis of quality-of-life measures, no significant differences were observed in the iPAQ total physical activity scores and EQ-5D-5L VAS scores between the lead-in period and month 12 after treatment (Table 2). However, at month 12 after treatment, the mean total score on the Hemophilia Quality of Life Questionnaire for Adults (Hem-A-QoL, an exploratory end point; scores range from 0 to 100, with lower scores indicating better hemophilia-related quality of life) had decreased by 21.5% as compared with the lead-in period (least-squares mean change in score, −5.5; 95% CI, −7.4 to −3.6), from a least-squares mean score of 25.6 at lead-in to 20.1 at month 12 (Table S3).

Before treatment, 21 participants (38.9%) had detectable neutralizing antibodies against AAV5 (i.e., a titer that was at or above the limit of detection of 7). The relationship between 18-month factor IX activity and pretreatment AAV5 neutralizing antibody titer is shown in Figure S5. The mean factor IX activity was 31.1% and 39.9% for participants with and without preexisting anti-AAV5 neutralizing antibodies, respectively, at 18 months after treatment. Up to a titer of 678, no correlation was seen between a participant's preexisting AAV5 neutralizing antibody titer and the participant's factor IX activity at 18 months after treatment.

In prespecified subgroup analyses performed to provide potential insight into the benefits and risks of the drug, no reduction in the annualized bleeding rate was observed in the subgroup of participants with preexisting AAV5 neutralizing antibody titers at baseline (rate ratio, 1.77). In addition, non-White race and the presence of joint disease at screening appeared to favor

Table 2. Primary and Secondary End Points (Controlled for Type I Error; Full Analysis Population).*

End Point	Estimate (95% CI)	Two-Sided P Value
Primary efficacy		
Adjusted ABR ratio for noninferiority assessment, months 7–18 after treatment vs. lead-in period†	0.36 (0.20 to 0.64)	NA
Secondary efficacy		
Change from baseline in one-stage aPTT-based factor IX activity 6 mo after treatment — percentage points‡	36.18 (31.41 to 40.95)	<0.001
Change from baseline in one-stage aPTT-based factor IX activity 12 mo after treatment — percentage points‡	38.81 (34.01 to 43.60)	<0.001
Change from baseline in one-stage aPTT-based factor IX activity 18 mo after treatment — percentage points‡	34.31 (29.52 to 39.11)	<0.001
Adjusted mean difference in annualized consumption of factor IX replacement therapy, months 7–18 after treatment vs. lead-in period — IU/yr§	−248,825.0 (−291,149.9 to −206,500.1)	<0.001
Adjusted ratio for annualized infusion rate of factor IX replacement therapy, months 7–18 after treatment vs. lead-in period¶	0.03 (0.01 to 0.10)	<0.001
Odds ratio for one-stage aPTT-based factor IX activity <12% of normal, months 6–18 after treatment vs. lead-in period‖	0.036 (0.014 to 0.093)	<0.001
Adjusted ABR ratio for superiority, months 7–18 after treatment vs. lead-in period	0.36 (0.20 to 0.64)	<0.001
Sensitivity analysis: adjusted annualized factor IX–treated bleeding rate ratio, months 7–18 after treatment vs. lead-in period	0.23 (0.12 to 0.46)**	NA
Adjusted ABR ratio for spontaneous bleeding episodes, months 7–18 after treatment vs. lead-in period	0.29 (0.12 to 0.71)	0.007
Sensitivity analysis: adjusted ABR ratio for factor IX–treated spontaneous bleeding episodes, months 7–18 after treatment vs. lead-in period	0.34 (0.11 to 1.00)**	NA
Adjusted ABR ratio for joint bleeding episodes, months 7–18 after treatment vs. lead-in period	0.22 (0.10 to 0.46)	<0.001
Sensitivity analysis: adjusted ABR ratio for factor IX–treated joint bleeding episodes, months 7–18 after treatment vs. lead-in period	0.20 (0.09 to 0.45)**	NA
Least-squares mean difference in iPAQ total physical activity score, 12 mo after treatment vs. lead-in period††	−721.2 (−1770.6 to 328.3)**	NA
Least-squares mean difference in EQ-5D-5L VAS score, 12 mo after treatment vs. lead-in period‡‡	0.1 (−3.5 to 3.8)**	NA

* The full analysis population included all participants who were enrolled, entered the lead-in phase, received etranacogene dezaparvovec, and had at least one efficacy end-point assessment after receipt of etranacogene dezaparvovec. End points are listed in the order of the testing hierarchy (not including the end points used for sensitivity analyses). ABR denotes annualized bleeding rate, aPTT activated partial-thromboplastin time, CI confidence interval, and NA not applicable.

† The upper limit of the confidence interval of the ABR ratio was compared with the noninferiority margin of 1.8. If the upper limit was less than 1.8, then noninferiority was declared.

‡ The value is the least-squares mean from a repeated-measures linear mixed model with visit as a categorical covariate. A two-sided P value of less than or equal to 0.05 for the comparison of the post-treatment value with the baseline value was considered to indicate statistical significance.

§ The P value was calculated with the use of a paired t-test comparing the post-treatment period with the lead-in period. A two-sided P value of less than or equal to 0.05 for the difference between the post-treatment period and the lead-in period was considered to indicate statistical significance.

¶ Rate ratios were calculated as the value for the post-treatment period divided by the value in the lead-in period. A two-sided P value of less than or equal to 0.05 was considered to indicate statistical significance.

‖ The odds ratio is from a generalized linear mixed logistic regression model with visit as a categorical covariate. A two-sided P value of less than or equal to 0.05 for the post-treatment period as compared with the lead-in period was considered to indicate statistical significance.

** The width of the confidence interval has not been adjusted for multiplicity and may not be used in place of hypothesis testing.

†† International Physical Activity Questionnaire (iPAQ) total physical activity scores range from 0 (no activity) to 80,640 metabolic equivalent task–minutes per week.

‡‡ EuroQol 5-Dimension 5-Level (EQ-5D-5L) visual analogue scale (VAS) scores range from 0 to 100, with higher scores indicating better health.

Table 3. Annualized Bleeding Rates (Full Analysis Population).

Type of Episode	All Episodes		Factor IX–Treated Episodes	
	Lead-in Period (N=54)*	Months 7–18 (N=54)†	Lead-in Period (N=54)*	Months 7–18 (N=54)†
Bleeding episodes				
Participants with an episode — no. (%)	40 (74)	20 (37)	37 (69)	15 (28)
Cumulative no. of episodes	136	54	118	30
Annualized bleeding rate (95% CI)‡	4.19 (3.22–5.45)	1.51 (0.81–2.82)	3.65 (2.82–4.74)§	0.84 (0.41–1.73)§
Spontaneous bleeding episodes				
Participants with an episode — no. (%)	24 (44)	9 (17)	22 (41)	6 (11)
Cumulative no. of episodes	50	14	44	11
Annualized bleeding rate (95% CI)‡	1.52 (1.01–2.30)	0.44 (0.17–1.12)	1.34 (0.87–2.06)§	0.45 (0.15–1.39)§
Joint bleeding episodes				
Participants with an episode — no. (%)	32 (59)	11 (20)	31 (57)	9 (17)
Cumulative no. of episodes	77	19	70	16
Annualized bleeding rate (95% CI)‡	2.35 (1.74–3.16)	0.51 (0.23–1.12)	2.13 (1.58–2.88)§	0.44 (0.19–1.00)§
Traumatic bleeding episodes				
Participants with an episode — no. (%)	29 (54)	12 (22)	26 (48)	9 (17)
Cumulative no. of episodes	70	30	58	11
Annualized bleeding rate (95% CI)‡	2.09 (1.42–3.08)§	0.62 (0.31–1.23)§	1.74 (1.21–2.49)§	0.22 (0.11–0.45)§

* The lead-in period equated to 33.1 person-years.
† Months 7–18 equated to 49.8 person-years.
‡ The annualized bleeding rate and comparison of the annualized bleeding rate between the lead-in period and the post-treatment period were estimated from a negative binomial regression model involving repeated-measures generalized estimating equations; the paired design of the study was accounted for in the model with an offset parameter to account for the differential collection periods. Treatment period was included as a categorical covariate.
§ The width of the confidence interval has not been adjusted for multiplicity and may not be used in place of hypothesis testing.

prophylactic factor IX over etranacogene dezaparvovec treatment (Fig. S3A). However, these observations were driven by the participant who had a predose AAV5 neutralizing antibody titer of 3212 on the day of dosing and a statistical anomaly resulting from prespecified rules, as detailed in Figure S3B (post hoc analysis). When this participant was excluded from the post hoc analysis to prevent the statistically anomalous result from obscuring the analysis in the remainder of the study cohort, the adjusted rate ratio was 0.30 (95% CI, 0.15 to 0.62) for participants with preexisting AAV5 neutralizing antibody titers at baseline, 0.28 (95% CI, 0.14 to 0.56) for non-White participants, and 0.30 (95% CI, 0.18 to 0.52) for participants with joint disease at screening (Fig. S3B).

SAFETY

All 54 participants had adverse events that occurred or worsened during or after treatment (465 adverse events in total; 364 mild, 87 moderate, and 14 severe). Adverse events affecting at least 10% of the participants are shown in Table 4. Thirteen participants (24%) had 14 unique serious adverse events; none were considered by the investigators to be treatment-related (Table S4).

Seven participants (13%) had infusion-related adverse events of special interest (Table S5). One participant died approximately 15 months after treatment from cardiogenic shock that was considered by the investigators to be unrelated to treatment (see the Supplementary Appendix). A serious adverse event of hepatocellular carcinoma occurred 12 months after treatment in a participant with multiple independent risk factors for hepatocellular carcinoma; the event was determined on the basis of independent molecular tumor characterization and vector-integration analysis to be unrelated to the AAV5 vector.[33] Adverse events were similar among participants with or without preexisting AAV5 neutralizing antibodies.

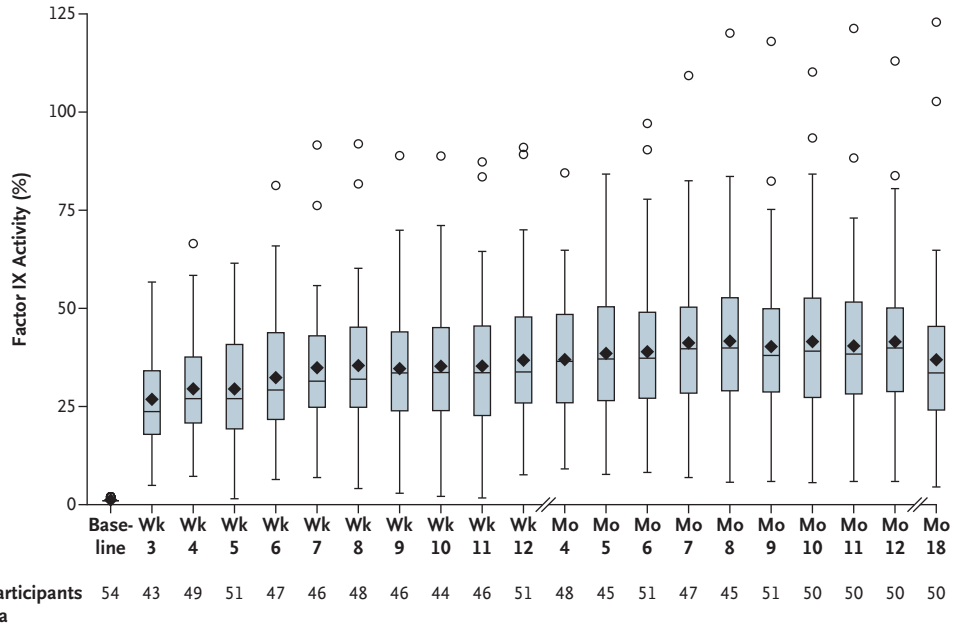

Figure 1. Endogenous Factor IX Activity over 18 Months after Treatment (Full Analysis Population).

Factor IX activity (from the one-stage assay based on activated partial-thromboplastin time) is shown only for blood sampling that did not occur within 5 half-lives of exogenous factor IX use (i.e., "uncontaminated" data). Factor IX activity beginning with the week 3 assessment was used in the analysis. Both the date and the time of the exogenous factor IX use (start) and the blood sampling were considered in determining contamination. Participants with no uncontaminated central laboratory post-treatment values had their postbaseline values set to equal their baseline value. The lower and upper edges of the box indicate the interquartile range, the line at the middle of the box indicates the median, and the I bars indicate the lowest and highest observation within 1.5 times the interquartile range of the bottom and top of the box, respectively. The diamond is the arithmetic mean. Any points outside the whiskers are plotted individually. Baseline factor IX activity was imputed on the basis of each participant's historical hemophilia B severity. If the participant had documented severe factor IX deficiency (plasma factor IX activity of <1%), the baseline factor IX activity was imputed as 1%. If the participant had documented moderately severe factor IX deficiency (plasma factor IX activity of 1 to 2%), the baseline factor IX activity was imputed as 2%. The mean and median are provided at baseline.

Eleven participants (20%) had elevations in ALT levels that were reported as adverse events; most were mild or moderate (Table S6). Nine participants (17%) received and subsequently discontinued glucocorticoid treatment for elevations in liver aminotransferase levels and for the preservation of *F9* Padua–expressing hepatocytes. The guidance supplied to investigators for the use of glucocorticoids is provided in the Supplementary Appendix. The mean duration of glucocorticoid use for elevated aminotransferase levels was 79.8±26.6 days (range, 51 to 130). All the participants discontinued immune suppression for elevations in aminotransferase levels between day 85 and day 170 after treatment, and no glucocorticoid-related adverse events were reported (Table S7). The mean factor IX activity (expressed as a percentage of the normal value) among 9 participants treated with glucocorticoids peaked at 22.2±10.1% before glucocorticoid treatment, was 17.1±8.1% before glucocorticoid treatment was started, and was 17.9±10.6% at 2 weeks after glucocorticoid treatment. The mean factor IX activity in the 11 participants with elevated ALT levels was 21.6±11.8%, 20.3±11.5%, and 18.1±9.1% at 6, 12, and 18 months after treatment, respectively. No participants resumed continuous factor IX prophylaxis.

At 18 months after treatment, clearance of vector DNA (i.e., absence of shedding) was confirmed in semen specimens obtained from 33 participants (61%) and in blood specimens obtained from 25 participants (46%) (Table S8). No clinically relevant changes in inflammatory bio-

Table 4. Adverse Events from Any Cause That Occurred or Worsened in at Least 10% of the Participants and Drug-Related Adverse Events in the Post-treatment Period (Safety Population).

Type of Event*	Adverse Events from Any Cause (N = 54)		Treatment-Related Adverse Events (N = 54)†	
	no. of participants (%)	*no. of events*	*no. of participants (%)*	*no. of events*
Any adverse event	54 (100)	465	37 (69)	92
Arthralgia	18 (33)	31	3 (6)	3
Headache	16 (30)	31	8 (15)	9
Nasopharyngitis	15 (28)	20	1 (2)	1
Fatigue	14 (26)	16	4 (7)	4
Alanine aminotransferase increased	11 (20)	12	9 (17)	10
Blood creatine kinase increased	8 (15)	10	4 (7)	6
Back pain	7 (13)	10	1 (2)	1
Influenza-like illness	7 (13)	11	7 (13)	8
Aspartate aminotransferase increased	7 (13)	8	5 (9)	6
Oropharyngeal pain	7 (13)	7	0	0
Pain in extremity	6 (11)	7	0	0
Diarrhea	6 (11)	6	2 (4)	2
Nausea	6 (11)	6	4 (7)	4
Cough	6 (11)	6	0	0

* *Medical Dictionary for Regulatory Activities* (version 24.1) terms are used to describe adverse events.
† The determination of whether an adverse event was related to treatment was made by the investigators.

markers were observed. All participants had an AAV5 humoral immune response within 6 weeks after treatment. Factor IX inhibitors did not develop in any participants.

DISCUSSION

In this study, etranacogene dezaparvovec was superior to routine factor IX prophylaxis with respect to the annualized bleeding rate (overall and factor IX–treated), factor IX activity, factor IX therapy consumption, factor IX infusion rate, and the annualized rates of spontaneous bleeding and joint bleeding. Increased factor IX activity was apparent from week 3 after treatment and was maintained over a period of 18 months, with no participant having elimination of factor IX transgene expression by hepatocyte-directed immunity.

In analyses of quality-of-life end points, no substantial differences were observed in the iPAQ total physical activity and EQ-5D-5L VAS scores between the lead-in period and month 12 after treatment. This is perhaps not surprising, in light of the demanding schedule in the first half of the year after gene therapy and the frequency and chronicity of musculoskeletal disease in these participants; more than 80% of the participants had a history of joint or bone disease, orthopedic surgery related to hemophilic arthropathy, or both. In addition, 10 of the 54 participants who received etranacogene dezaparvovec entered screening with a combined 22 active target joints. In exploratory analyses, apparent post-treatment improvements in the Hem-A-QoL score were observed. Reevaluation of health-related quality of life is planned for month 24 and at later time points.

In vitro and in vivo studies have shown the capacity of preexisting immunity to prevent successful AAV transduction.[22,34,35] Etranacogene dezaparvovec is unusual among AAV5-based gene therapies in that it sustains factor IX activity irrespective of preexisting neutralizing antibody status (for AAV5 neutralizing antibody titers ≤678). This observation supports the expectation that gene therapy with etranacogene dezaparvovec can be effective in most

patients irrespective of preexisting AAV5 neutralizing antibodies.[25,36]

In an early trial of liver-directed AAV gene therapy for hemophilia B, the efficiency of vector transduction of hepatocytes was concluded to be inversely related to the preexisting AAV neutralizing antibody titer.[23] Consequently, patients with preexisting AAV neutralizing antibodies have been excluded from most hemophilia gene-therapy trials.[12,14,16,17,19-24] The distribution of titers observed in our study is representative of the distribution in the general population, with AAV5 neutralizing antibody titers of 678 or higher present in approximately 6% of persons and a titer of 3212 or higher in approximately 4%.[25,36] In this study, preexisting AAV5 neutralizing antibody titers of 678 or lower did not predict treatment outcome with respect to the annualized bleeding rate, factor IX activity, elevations in aminotransferase levels, or other safety measures at 18 months after treatment. These observations suggest that AAV5 neutralizing antibodies at levels commonly seen in the general population do not affect the beneficial therapeutic outcomes of etranacogene dezaparvovec.

The AAV5 vector AMT-060 provides sustained wild-type factor IX expression and hemostatic protection for more than 5 years (9 of 10 participants discontinued routine factor IX prophylaxis; observation is ongoing), a result consistent with the 8 years of wild-type factor IX expression reported from the same expression cassette in a trial conducted by University College London and St. Jude Children's Research Hospital.[13,15,37] Because the same expression cassette was modified by only 2 nucleotides to direct expression of the factor IX Padua variant from etranacogene dezaparvovec, long-term expression is also expected with the use of the Padua variant. This expectation is consistent with the stable gene correction lasting for more than 2.5 years in a phase 2b study of etranacogene dezaparvovec involving 3 participants[29] and for 18 months in the current study.

At screening, participants had to be using continuous factor IX prophylaxis. At 18 months after treatment, 96% of the participants were free from continuous prophylaxis. The exceptions were one participant with a preexisting AAV5 neutralizing antibody titer of 3212 and one participant who received a partial etranacogene dezaparvovec dose (approximately 10% of the full dose). The near elimination of factor IX prophylaxis greatly reduces treatment burden.[9,10]

The incidence of serious adverse events was low in our study, and no serious adverse events were related to etranacogene dezaparvovec. In previous studies of other hemophilia A and B AAV vectors involving fewer participants, 20 to 82% received glucocorticoids to treat elevations in aminotransferase levels.[12,16,38-40] Although an increase in ALT levels was a notable common adverse event that was assessed as treatment-related in our study, only 17% of the participants received tapering courses of glucocorticoids for transient aminotransferase elevations. Factor IX activity in these participants remained in the mild hemophilia range; our study supports the dogma that vigilance for evidence of liver inflammation and timely implementation of glucocorticoid treatment may preserve *F9* gene expression.

Although the safety of the AAV5 vector etranacogene dezaparvovec dose used has been shown in this and earlier clinical studies,[18,29] it is understood that the use of this primarily nonintegrating viral vector, at the doses used in studies in humans, will result in genomic integration events. Many more years of planned follow-up are needed to fully understand any potential long-term risks of genotoxicity.[33]

In our study, etranacogene dezaparvovec had a favorable safety and efficacy profile in men with severe or moderately severe hemophilia B, including those with preexisting AAV5 neutralizing antibodies. Etranacogene dezaparvovec was superior to factor IX prophylaxis in terms of the annualized bleeding rate and was associated with sustained increased factor IX activity to the mild to near-normal range, with associated durable hemostatic improvement. Our findings suggest that gene therapy may reduce the burden of care and improve quality of life in patients with hemophilia B.

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APPENDIX

The authors' full names and academic degrees are as follows: Steven W. Pipe, M.D., Frank W.G. Leebeek, M.D., Ph.D., Michael Recht, M.D., Ph.D., Nigel S. Key, M.B., Ch.B., Giancarlo Castaman, M.D., Wolfgang Miesbach, M.D., Susan Lattimore, R.N., Kathelijne Peerlinck, M.D., Ph.D., Paul Van der Valk, M.D., Michiel Coppens, M.D., Ph.D., Peter Kampmann, M.D., Karina Meijer, M.D., Ph.D., Niamh O'Connell, M.B., B.Ch., B.A.O., Ph.D., K. John Pasi, M.B., Ch.B., Ph.D., Daniel P. Hart, M.B., Ch.B., Ph.D., Rashid Kazmi, M.B., B.S., Jan Astermark, M.D., Ph.D., Cedric R.J.R. Hermans, M.D., Ph.D., Robert Klamroth, M.D., Ph.D., Richard Lemons, M.D., Ph.D., Nathan Visweshwar, M.D., Annette von Drygalski, M.D., Pharm.D., Guy Young, M.D., Shelley E. Crary, M.D., Miguel Escobar, M.D., Esteban Gomez, M.D., Rebecca Kruse-Jarres, M.D., M.P.H., Doris V. Quon, M.D., Ph.D., Emily Symington, M.D., Michael Wang, M.D., Allison P. Wheeler, M.D., M.S.C.I., Robert Gut, M.D., Ph.D., Ying P. Liu, Ph.D., Ricardo E. Dolmetsch, Ph.D., David L. Cooper, M.D., Yanyan Li, Ph.D., Brahm Goldstein, M.D., M.C.R., and Paul E. Monahan, M.D.

The authors' affiliations are as follows: the Departments of Pediatrics and Pathology, University of Michigan, Ann Arbor (S.W.P.); the Department of Hematology, Erasmus University Medical Center, University Medical Center Rotterdam, Rotterdam (F.W.G.L.), Van Creveldkliniek, University Medical Center Utrecht, University Utrecht, Utrecht (P.V.V.), Vascular Medicine, Amsterdam University Medical Center, University of Amsterdam (M.C.), Amsterdam Cardiovascular Sciences, Pulmonary Hypertension and Thrombosis (M.C.), and uniQure Biopharma (Y.P.L.), Amsterdam, and University Medical Center Groningen, Groningen (K.M.) — all in the Netherlands; Yale University School of Medicine, New Haven, CT (M.R.); American Thrombosis and Hemostasis Network, Rochester, NY (M.R.); the Department of Medicine and UNC Blood Research Center, University of North Carolina at Chapel Hill, Chapel Hill (N.S.K.); the Center for Bleeding Disorders and Coagulation, Department of Oncology, Careggi University Hospital, Florence, Italy (G.C.); the Department of Hemostaseology and Hemophilia Center, Medical Clinic 2, Institute of Transfusion Medicine and Immunohematology, University Hospital Frankfurt, Frankfurt (W.M.), the Comprehensive Care Hemophilia Treatment Center, Vivantes Klinikum im Friedrichshain, Berlin, and the Institute of Experimental Hematology and Transfusion Medicine, University Hospital Bonn, Medical Faculty, University of Bonn, Bonn (R. Klamroth) — all in Germany; Oregon Health and Science University, Portland (S.L.); the Department of Vascular Medicine and Hemostasis, Hemophilia Center, University Hospitals Leuven, Leuven (K.P.), the Division of Hematology, Cliniques Universitaires Saint-Luc, Brussels (C.R.J.R.H.), and Université Catholique de Louvain, Louvain-la-Neuve (C.R.J.R.H.) — all in Belgium; the Department of Hematology, Rigshopitalet Copenhagen, Copenhagen (P.K.); National Coagulation Centre, St. James's Hospital, Dublin (N.O.); Barts and the London School of Medicine and Dentistry, Queen Mary University of London (K.J.P., D.P.H.), and the Royal London Hospital Haemophilia Centre, Barts Health NHS Trust (D.P.H.), London, University Hospital Southampton and National Institute for Health and Care Research Clinical Research Facility, Southampton (R. Kazmi), and Cambridge University NHS Foundation Trust, Addenbrooks Hospital, Cambridge (E.S.) — all in the United Kingdom; the Department of Translational Medicine, Lund University, and the Department of Hematology Oncology and Radiation Physics, Skåne University Hospital — both in Malmö, Sweden (J.A.); the Department of Pediatrics, University of Utah, and Primary Children's Hospital, Salt Lake City (R.L.); University of South Florida, Tampa (N.V.); the Department of Medicine, Hemophilia and Thrombosis Treatment Center, San Diego (A.D.), the Cancer and Blood Disorders Institute, Children's Hospital Los Angeles (G.Y.), the Orthopaedic Hemophilia Treatment Center, the Luskin Orthopaedic Institute for Children (D.V.Q.), and the University of Southern California Keck School of Medicine (G.Y.), Los Angeles, and the Center for Inherited Blood Disorders, Orange (E.G.) — all in California; Arkansas Children's Hospital, Pulaski, and University of Arkansas for Medical Sciences, Little Rock (S.E.C.); University of Texas Health Science Center, McGovern Medical School, and Gulf States Hemophilia and Thrombophilia Center — both in Houston (M.E.); Washington Center for Bleeding Disorders and University of Washington, Seattle (R.K.-J.); Hemophilia and Thrombosis Center, University of Colorado Anschutz Medical Campus, Aurora (M.W.); the Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville (A.P.W.); uniQure, Lexington, MA (R.G., R.E.D., D.L.C.); and CSL Behring, King of Prussia, PA (Y.L., B.G., P.E.M.).

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