ORIGINAL ARTICLE

Gene Therapy with Fidanacogene Elaparvovec in Adults with Hemophilia B

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ABSTRACT

BACKGROUND
Fidanacogene elaparvovec, an adeno-associated virus (AAV) gene-therapy vector for hemophilia B containing a high-activity human factor IX variant (FIX-R338L/FIX-Padua), was associated with sustained factor IX activity in a phase 1–2a study.

METHODS
We conducted a phase 3 open-label study of fidanacogene elaparvovec at a dose of 5×10^{11} vector genome copies per kilogram of body weight. Men 18 to 65 years of age with hemophilia B and a factor IX level of 2% or less were eligible for screening if they had received at least 6 months of therapy with prophylactic factor IX concentrate. The primary end point, tested for noninferiority, was the annualized bleeding rate (treated and untreated bleeding episodes) from week 12 to month 15 after treatment with fidanacogene elaparvovec as compared with the prophylaxis lead-in period. Superiority, additional efficacy end points, and safety were also assessed.

RESULTS
Of 316 men who underwent screening for the lead-in study, 204 (64.6%) were not eligible; 188 (59.5%) of those were ineligible owing to the presence of anti-AAV neutralizing antibodies. Of the 45 participants who received fidanacogene elaparvovec, 44 completed at least 15 months of follow-up. The annualized rate of bleeding for all bleeding episodes decreased by 71%, from 4.42 (95% confidence interval [CI], 1.80 to 7.05) at baseline to 1.28 (95% CI, 0.57 to 1.98) after gene therapy, a treatment difference of −3.15 episodes (95% CI, −5.46 to −0.83; P=0.008). This result shows the noninferiority and superiority of fidanacogene elaparvovec to prophylaxis. At 15 months, the mean factor IX activity was 26.9% (median, 22.9%; range, 1.9 to 119.0) by one-stage SynthASil assay. A total of 28 participants (62%) received glucocorticoids for increased aminotransferase levels or decreased factor IX levels (or both) starting between 11 and 123 days. No infusion-related serious adverse events, thrombotic events, development of factor IX inhibitors, or malignant conditions were observed.

CONCLUSIONS
Fidanacogene elaparvovec was superior to prophylaxis for the treatment of participants with hemophilia B, leading to reduced bleeding and stable factor IX expression. (Funded by Pfizer; BENEGENE-2 ClinicalTrials.gov number, NCT03861273.)

The current treatment for hemophilia B is the episodic intravenous administration of plasma-derived or recombinant factor IX replacement,[1] including products with extended half-lives,[2] to raise factor IX activity to prevent or treat bleeding. However, frequent intravenous injections impose a substantial burden on patients and their families.[3,4] Despite therapeutic advances in reducing the frequency of infusions, current prophylactic therapies are not curative and do not eliminate all symptoms, and joint damage still occurs.[5,6] Other agents that do not contain factor IX, such as small interfering RNA agents (e.g., fitusiran) that reduce antithrombin synthesis in hepatocytes[7] and monoclonal antibodies (e.g., concizumab[8] and marstacimab[9,10]) that bind and neutralize the inhibitory activity of tissue factor pathway inhibitor,[11] are in advanced development or have been approved. Once available, these agents may reduce the frequency of treatment and bleeding episodes, but they still require regular administration. Gene therapy could enable patients to live without the need for ongoing treatments and the burden of regular disease management.[12,13] The first gene-therapy product for hemophilia B has been approved in the United States and Europe.[14,15] The approved therapy is a single infusion (2×10^{13} genome copies per kilogram of body weight) of an adeno-associated virus (AAV) serotype 5 vector expressing a high-activity factor IX variant (FIX-R338L, also known as FIX-Padua).[16]

Fidanacogene elaparvovec is an AAV vector that is designed to deliver transgene production of FIX-R338L[17] for hemophilia B.[18] The transgene leverages the hepatic-control region of the gene encoding apolipoprotein E (*APOE*), a liver-specific human α_1-antitrypsin promoter, and a codon-optimized FIX-R388L minigene. In a phase 1–2a study and a longer-term follow-up study, treatment with fidanacogene elaparvovec resulted in sustained factor IX activity in the range of mild hemophilia to normal, with associated low occurrences of bleeding and a reduction in exogenous factor IX consumption.[19] These results were achieved with one of the lowest vector doses reported in hemophilia gene-therapy trials to date (5×10^{11} vector genome copies per kilogram).[16,20-22] Here, we present data from phase 3 of the clinical study of fidanacogene elaparvovec in persons with hemophilia B.

METHODS

STUDY DESIGN AND OVERSIGHT

BENEGENE-2 is a phase 3 study involving participants with moderately severe or severe hemophilia B conducted at 27 centers in 13 countries. The protocol (available with the full text of this article at NEJM.org) was approved by the relevant regulatory authorities and ethics committees responsible for each study site. The study was conducted in accordance with the Good Clinical Practice guidelines of the International Council for Harmonisation and the principles of the Declaration of Helsinki. The study began on July 29, 2019, and the primary completion date was November 16, 2022, with an updated data-cutoff date of August 30, 2023; safety and efficacy data collection will continue until each participant has had 6 years of follow-up. The study was designed and overseen by employees of the sponsor (Pfizer), who were responsible for site selection, site monitoring, data management, and data storage, in collaboration with a group of academic investigators (the scientific advisory committee) (Sections S1 and S2 in the Supplementary Appendix, available at NEJM.org).

Analyses were performed by the sponsor. The academic authors provided oversight for the accuracy and completeness of the data and could request additional analyses. The first draft of the manuscript was written by a medical writer contracted by Pfizer under the direction of the authors; all the authors critically reviewed the manuscript and provided substantive input during drafting. The authors vouch for the completeness and accuracy of the data and for the fidelity of the study to the protocol.

STUDY POPULATION

Full eligibility criteria are described in Section S3. Men 18 to 65 years of age with hemophilia B (factor IX level, ≤2%) who had received factor IX prophylaxis therapy for at least 6 months during the BENEGENE-1 lead-in study (ClinicalTrials.gov number, NCT03587116) and who agreed to suspend prophylaxis after fidanacogene elaparvovec infusion were eligible. Factor IX replacement therapy was permitted according to clinical need. Key exclusion criteria were detectable anti-AAV neutralizing antibodies; a history of or positive test for factor IX inhibitors; the presence of unstable or clinically significant disease other than hemophilia; a level of alanine aminotransferase, aspartate aminotransferase, or alkaline phosphatase that was more than twice the upper limit of the normal range; active hepatitis B or C status; and active human immunodeficiency virus (HIV) infection. All the participants provided written informed consent.

STUDY PROCEDURES

The study design is shown in Figure S1. On day 1, the participants received a single intravenous infusion of fidanacogene elaparvovec at a dose of 5×10^{11} vector genome copies per kilogram by means of an infusion pump. Additional study procedures are described in Section S4. Guidance that was supplied to investigators regarding the use of glucocorticoids, along with safety information, is shown in Section S5.

STUDY END POINTS

Details of the study end points, as well as the definition of target joints, are provided in Section S6. The primary end point was the annualized bleeding rate (treated and untreated bleeding episodes) from week 12 to month 15 after treatment with fidanacogene elaparvovec as compared with the prophylaxis lead-in period. Among the key secondary end points that were included in the statistical testing sequence were the annualized rate of bleeding for treated bleeding episodes, the annualized infusion rate of exogenous factor IX, and the activity level of factor IX (Section S7). Safety assessments included annual ultrasonography of the liver, measurement of vector shedding, and assessment of immune response directed at the AAV vector or the transgene product.

STATISTICAL ANALYSIS

All eligible participants were assigned to the study intervention. When 40 participants completed at least 15 months of follow-up, the protocol specified comparison of the annualized rate of bleeding for total bleeding episodes for noninferiority as compared with prophylaxis (margin, 3.0 episodes per year) and, if noninferiority was achieved, comparison for superiority. The primary and secondary efficacy end points were tested hierarchically to control for overall type I error. When 40 participants completed at least 15 months of follow-up, data for the annualized rate of bleeding for total bleeding episodes provided the study with 90% power, with a one-sided test at an alpha level of 0.025, to show noninferiority of fidanacogene elaparvovec with regard to the annualized rate of bleeding for total bleeding episodes, with the use of a repeated-measure negative binomial regression. A sensitivity analysis for factor IX activity was performed, with missing factor IX activity at month 15 imputed with the use of the average values from the preceding and following visits. Additional details of the statistical analyses are provided in Section S7 and the statistical analysis plan, available with the protocol. The primary analysis population for efficacy and safety analyses consisted of all the participants who received fidanacogene elaparvovec, regardless of the length of follow-up.

RESULTS

PARTICIPANTS

A total of 316 men with factor IX levels of 2% or less underwent screening for entry into the BENEGENE-1 lead-in study (Fig. 1); 204 (64.6%) of those screened were not eligible — 188 (59.5%) because they were positive for anti–AAV-neutralizing antibodies and 16 (5.1%) on the basis of other criteria (Table S1). Of the 102 men who were enrolled, 51 had completed the BENEGENE-1 study and underwent screening for the BENEGENE-2 study (Fig. 1), 51 did not undergo screening for the BENEGENE-2 study (40 were ongoing, 3 had withdrawn from the BENEGENE-1 study, and 8 had completed the BENEGENE-1 study but did not enroll in the BENEGENE-2 study). Of the 51 participants who underwent screening for the BENEGENE-2 study, 6 did not meet eligibility criteria (1 had anti–AAV-neutralizing antibodies detected at the BENEGENE-2 screening; the criteria for the other 5 who were deemed to be ineligible are listed in Fig. 1).

A total of 45 men were enrolled in BENEGENE-2 and received fidanacogene elaparvovec; the baseline demographic and clinical characteristics of the participants are shown in Table 1. The mean age was 33.2 years (range, 18 to 62), and 73% of the participants were White. At baseline, 2% of the participants had controlled HIV infection (determined by their medical history), 29% had positive serologic tests for previous hepatitis B infection, and 33% were positive for previous hepatitis C infection. Overall, 29% had target joints. The demographic characteristics of the participants in the BENEGENE-1 lead-in study were similar to those of the participants enrolled in BENEGENE-2 (Section S7).

FOLLOW-UP

Of the 45 participants enrolled in BENEGENE-2, 44 completed at least 15 months of follow-up, and the remaining participant had completed 12

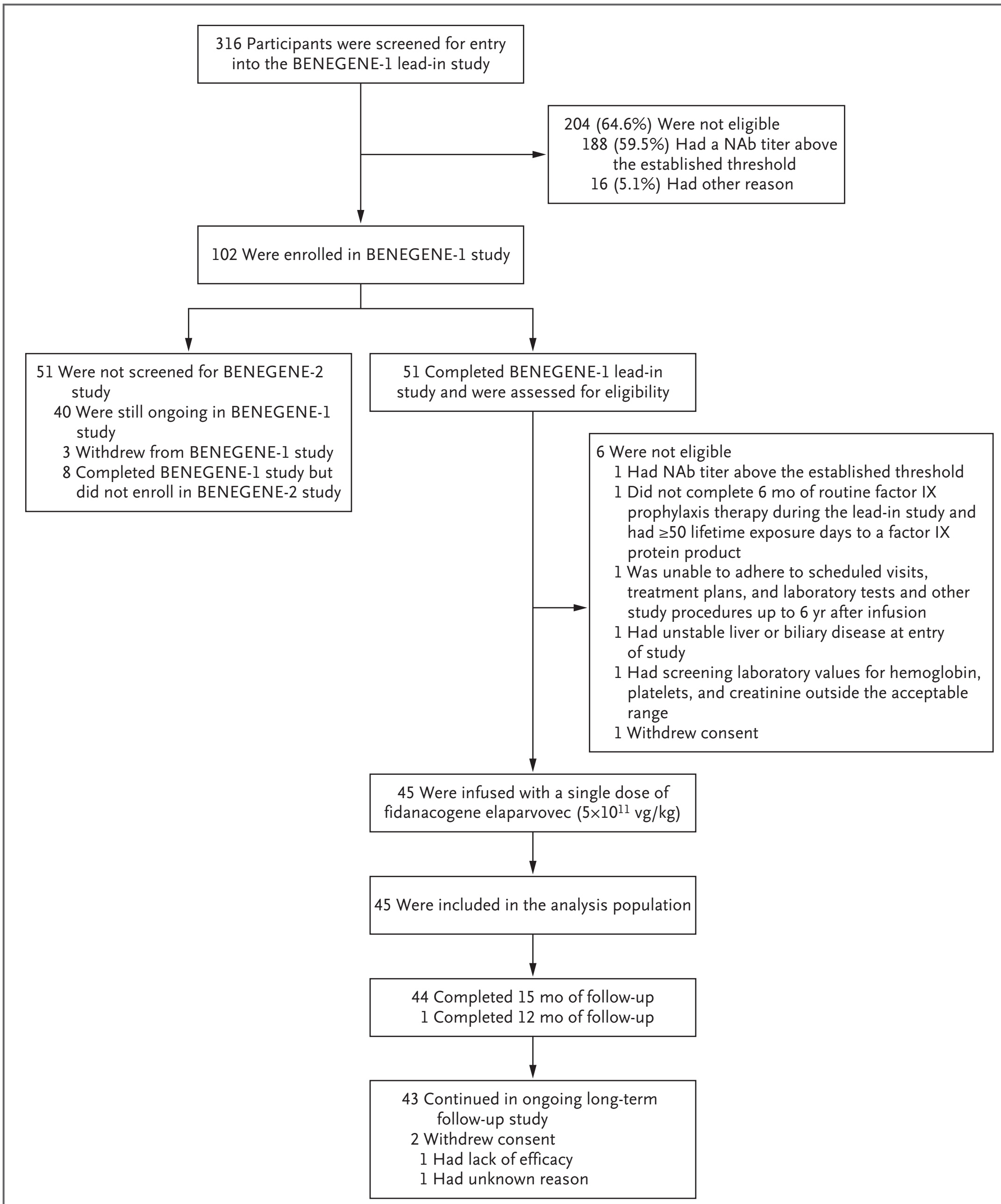


Figure 1. Screening in the BENEGENE-1 Study and Screening, Participation, and Follow-up in the BENEGENE-2 Study.

NAb denotes anti–adeno-associated virus neutralizing antibodies, and vg/kg vector genome copies per kilogram of body weight.

Table 1. Characteristics of the Participants at Baseline.*

Characteristic	Value (N=45)
Age — yr	
Mean	33.2±10.9
Median (range)	29 (18–62)
Male sex — no. (%)	45 (100)
Race — no. (%)†	
White	33 (73)
Asian	7 (16)
Black	1 (2)
Not reported	4 (9)
Geographic region — no. (%)	
Europe	13 (29)
North America	12 (27)
Middle East	9 (20)
Asia–Pacific	6 (13)
South America	3 (7)
Australia	2 (4)
Body-mass index‡	
Mean	27.9±5.5
Median (range)	28 (18–48)
Infection — no. (%)	
HIV infection§	1 (2)
Previous hepatitis B infection¶	13 (29)
Previous hepatitis C infection¶	15 (33)
Target joints — no. (%)	
Overall	13 (29)
One target joint	7 (16)
Two target joints	3 (7)
Three or more target joints	3 (7)
Category of factor IX therapy‖	
Extended half-life	29 (64)
Plasma-derived	2 (4)
Recombinant standard half-life	15 (33)

* Plus–minus values are means ±SD.
† Race was reported by the participant.
‡ Body-mass index is the weight in kilograms divided by the square of the height in meters.
§ Status with respect to human immunodeficiency virus (HIV) infection was determined from the medical history.
¶ Previous hepatitis B or C infection was determined from serologic results.
‖ Participants may have been prescribed more than one type of factor IX replacement therapy.

months of follow-up at the time of data cutoff. All the participants were included in the primary and secondary efficacy and safety analyses, including 2 who withdrew consent (Fig. 1). Detailed narratives regarding follow-up and safety end points are provided in Section S8.).

PRIMARY AND SECONDARY EFFICACY END POINTS

Table 2 summarizes key results. The primary end point of the annualized rate of bleeding for total bleeding episodes was 4.42 (95% confidence interval [CI], 1.80 to 7.05) in the prophylaxis period and 1.28 (95% CI, 0.57 to 1.98) from week 12 to month 15 after fidanacogene elaparvovec therapy, for a treatment difference estimate of −3.15 episodes (95% CI, −5.46 to −0.83; P=0.008), a finding that showed the noninferiority (primary end point) and superiority (secondary end point) of gene therapy as compared with factor IX prophylaxis (Fig. 2A). Fidanacogene elaparvovec therapy significantly reduced the mean annualized rate of bleeding for total bleeding episodes (by 71%) as compared with prophylaxis (P<0.001). The annualized rate of bleeding for treated bleeding episodes was 3.34 (95% CI, 1.70 to 4.98) in the prophylaxis period and 0.73 (95% CI, 0.23 to 1.23) after fidanacogene elaparvovec therapy, for an estimated treatment difference of −2.61 (95% CI, −4.27 to −0.96; P=0.002) (Fig. 2A). Fidanacogene elaparvovec therapy significantly reduced the mean annualized rate of bleeding for treated bleeding episodes (by 78%) as compared with prophylaxis (P<0.001), a change that was associated with a 92.3% reduction in the mean annualized infusion rate (Fig. 2B) and a 92.4% reduction in annualized total factor IX consumption.

The mean factor IX activity at month 15 was 26.9% (median, 22.9; range, 1.9 to 119.0) measured by the one-stage SynthASil assay (38 participants). Results of the factor IX activity sensitivity analyses (Fig. S4) were generally consistent with those shown in Figure 2C and Figure S2 (which along with Fig. S3 show factor IX activity during the 24 months after gene therapy). At 24 months after fidanacogene elaparvovec therapy, factor IX levels above 5% were maintained in 82% of the participants (Fig. 2D).

ADDITIONAL END POINTS

Other prespecified secondary end points are listed in Table 2. The percentage of participants

Table 2. Primary, Key Secondary, and Other Secondary End Points.*

End Point	Before Factor IX Gene Therapy, Prophylaxis Period (N=45)	After Factor IX Gene Therapy, Wk 12 to Mo 15 (N=45)	Treatment Difference	P Value	Percent Reduction
Primary end point of noninferiority: all bleeding episodes†					
Model-derived annualized bleeding rate (95% CI)‡§	4.42 (1.80 to 7.05)	1.28 (0.57 to 1.98)	−3.15 (−5.46 to −0.83)	0.008	71.12 (50.09 to 83.29)
Participants without any bleeding episodes — no. (%)	13 (29)	29 (64)	—		—
Key secondary superiority end points: treated bleeding episodes and annualized infusion rate¶					
Model-derived annualized bleeding rate (95% CI)‡§	3.34 (1.70 to 4.98)	0.73 (0.23 to 1.23)	−2.61 (−4.27 to −0.96)	0.002	78.15 (51.60 to 90.14)
Participants without any treated bleeding episodes — no. (%)	16 (36)	33 (73)	—		—
Mean annualized infusion rate‖	58.83±29.06	4.54±10.03	−54.29 (−63.58 to −45.01)	<0.001	92.3**
Other secondary end points					
Participants who resumed prophylaxis — no. (%)	NA	6 (13)	—		—
Mean annualized total factor IX consumption — IU/kg	3168.56±1635.55	239.39±539.62	−2929.17 (−3397.49 to −2460.85)	<0.001	92.4††

* CI denotes confidence interval, and NA not applicable.

† Both spontaneous and traumatic bleeding episodes (either treated or untreated) were counted, but procedural bleeding episodes were excluded. If the prophylaxis factor IX regimen was resumed for a participant, then the time period after the resumption of the prophylaxis regimen was excluded from the calculation of the end point of annualized bleeding rate.

‡ The treatment difference and P value were obtained from a repeated-measures generalized linear model with negative binomial distribution and identity-link function.

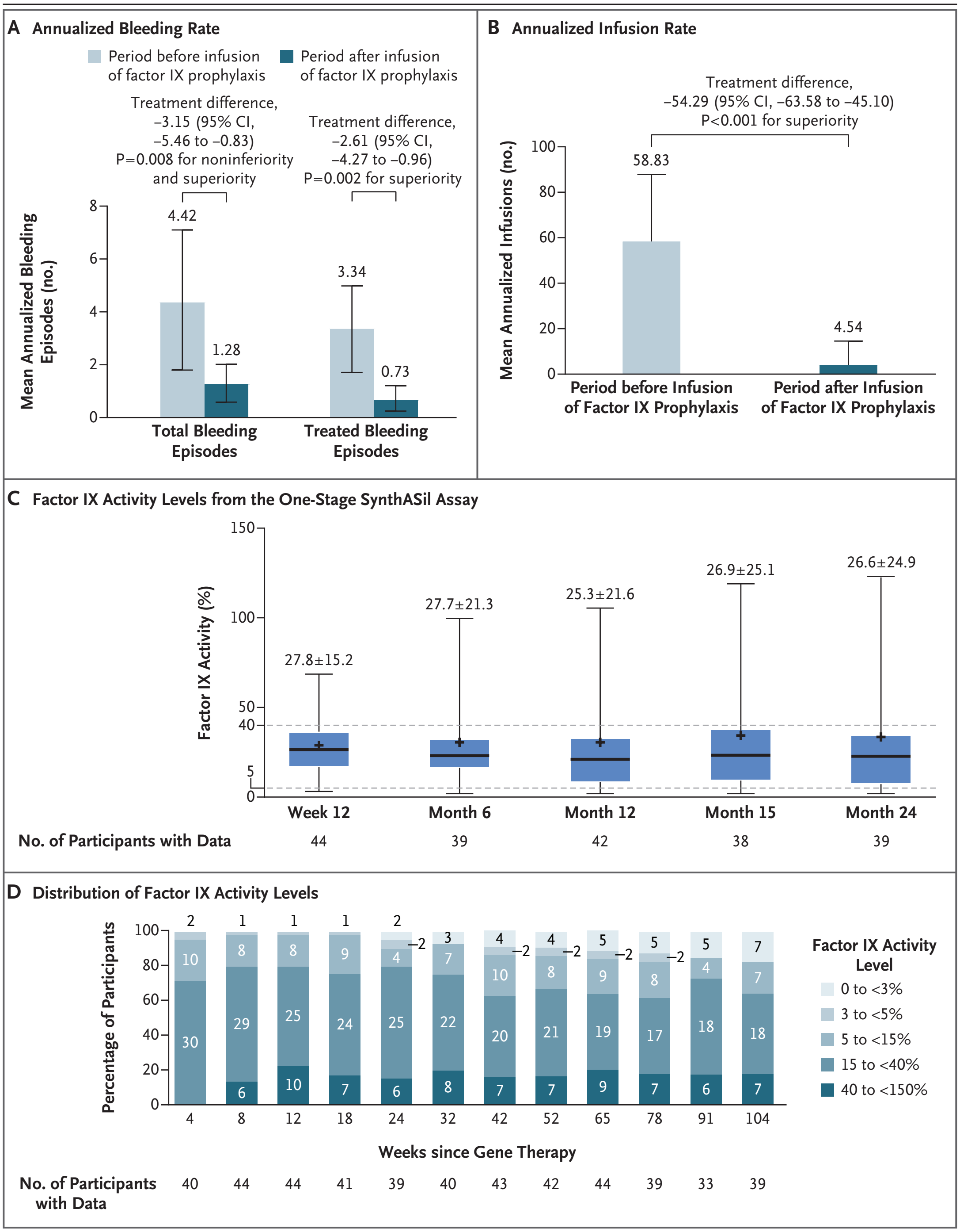
§ The percentage reduction was obtained from a repeated-measures generalized linear model with negative binomial distribution and log-link function.

¶ Both spontaneous and traumatic bleeding episodes that resulted in factor IX replacement treatment were counted, but procedural bleeding episodes were excluded. If the prophylaxis factor IX regimen was resumed for a participant, then the time period after the resumption of the prophylaxis regimen was excluded from the calculation of the end point of annualized bleeding rate. If the prophylaxis factor IX regimen was resumed for a participant, the time period after the resumption of the prophylaxis regimen was included in the calculation of the end point of annualized infusion rate.

‖ The treatment difference was calculated as the rate after fidanacogene elaparvovec therapy minus the rate before gene therapy. The estimated 95% confidence interval and P value were obtained from a paired t-test.

** The percent reduction was calculated as follows: 1 − (mean annualized infusion rate for factor IX from week 12 to month 15 after fidanacogene elaparvovec therapy / the mean annualized infusion rate during standard-care factor IX replacement therapy) × 100% (in which the annualized infusion rate = number of infusions [for any reason] during the given time period × 365.25 / [date of last day − date of first day + 1] in that time period).

†† The percent reduction was calculated as follows: 1 − (mean annualized factor IX consumption [in IU per kg] for factor IX from week 12 to month 15 after fidanacogene elaparvovec therapy / mean annualized factor IX consumption [in IU per kg] during standard-care factor IX replacement therapy) × 100%.



who had no bleeding episodes was 29% (13 participants) during the prophylaxis period before fidanacogene elaparvovec therapy and 64% (29 participants) from week 12 to month 15 after fidanacogene elaparvovec therapy. Similarly, the percentage of participants who had no treated bleeding episodes was 36% (16 participants) before fidanacogene elaparvovec therapy and 73% (33 participants) from week 12 to month 15 after fidanacogene elaparvovec therapy. After fidanacogene elaparvovec therapy, 13% of the participants (6 participants) resumed treatment with factor IX prophylaxis (5 owing to low factor IX activity and 1 owing to the frequency of bleeding

Figure 2 (facing page). Results of Primary and Key Secondary End-Point Analyses.
Panel A shows the annualized rate of bleeding for total bleeding episodes and treated bleeding episodes, and Panel B shows the annualized infusion rate (computed with the following formula: number of infusions [for any reason] during the given time period×365.25 / [date of last day−date of first day+1] in that time period). Panel C shows the factor IX activity levels from the one-stage SynthASil assay. For each period shown, the lower and upper edges of the blue box indicate the interquartile range, the heavy horizontal line at the middle of the box indicates the median, and the I bar indicates the minimum and maximum values, with the mean (±SD) factor IX activity value shown at the top of the I bar. The plus sign is the arithmetic mean, and the dashed horizontal lines at 5% and 40% indicate the range considered to be mild hemophilia; activity above 40% is considered to be normal. Panel D shows the percentage of participants according to their factor IX activity, with factor IX activity determined by the one-stage SynthASil assay. The analysis population for the primary end point and key secondary end points included all the participants who received fidanacogene elaparvovec.

episodes), with time to resumption ranging from 5.1 to 20.5 months. Details regarding the 6 participants who resumed factor IX prophylaxis are included in the Supplementary Appendix. In all six cases, the participants initially had a response to fidanacogene elaparvovec therapy, and all were treated with glucocorticoids. The mean annualized bleeding rate according to specific type is shown in Table S2. A total of 13 participants had target joints at baseline (7 participants had one target joint, 3 had two target joints, and 3 had at least three target joints). Within 15 months after fidanacogene elaparvovec therapy, at least one target joint had resolved (i.e., no bleeding episodes) in 12 of 13 participants (92%). A new target joint developed in 1 participant (2%) subsequent to fidanacogene elaparvovec therapy.

SAFETY END POINTS

A total of 38 participants (84%) reported 206 adverse events during the study; 7 (16%) reported serious adverse events (Table S3). Adverse events affecting at least 5% of the participants are shown in Table 3. The most common adverse event associated with fidanacogene elaparvovec therapy was an increased level of aminotransferase (in 24 participants [53%]), which was generally mild and asymptomatic. A total of 28 participants (62%) received glucocorticoids for increased aminotransferase levels or decreased factor IX levels (or both) (Table S4); 6 of the 28 participants (21%) resumed prophylaxis. No other immunosuppressive agents were used. The median time to glucocorticoid initiation was 37.5 days (range, 11 to 123), and the median duration of glucocorticoid treatment was 95.0 days (range, 41 to 276) (Table S5). Six participants (13%) received a second course of glucocorticoid therapy for a median duration of 62.0 days (range, 23 to 165). The results of liver-function tests are described in Section S9.

Table 3. Adverse Events.*

Event	Participants (N=45)
	no. (%)
Any adverse event	38 (84)
Serious adverse event	7 (16)
Adverse event leading to study discontinuation	0
Adverse event leading to death	0
Increased levels of aminotransferase†‡	24 (53)
Selected serious adverse event	
Infusion-related serious adverse event	0
Duodenal ulcer hemorrhage§	2 (4)
Anemia§	2 (4)

* Data shown are for all 45 participants who received fidanacogene elaparvovec.
† An increase in liver aminotransferase levels was considered to be an adverse event at the discretion of the investigator.
‡ An increase in the level of aminotransferases was the only adverse event that occurred in 5% or more of the participants. Investigators documented different preferred terms for adverse events relating to increased aminotransferase levels, and multiple overlapping events could be reported in a single participant. The following preferred terms were reported: increased alanine aminotransferase level (in 12 participants [27%]), abnormal hepatic function (in 5 [11%]), increased aspartate aminotransferase level (in 3 [7%]), increased hepatic-enzyme level (in 3 [7%]), increased aminotransferase level (in 3 [7%]), and abnormal liver-function test (in 1 [2%]).
§ Duodenal ulcer hemorrhage occurred in 2 participants. In 1 participant, two events from the same serious adverse event were assessed by the investigator as being related to treatment at the time of data cutoff and occurred within the first year after fidanacogene elaparvovec therapy: duodenal ulcer hemorrhage and associated anemia, which occurred in the context of glucocorticoid use without a concomitant gastric-acid–secretion inhibitor. After the data-cutoff date, the investigator changed the assessment of these events to adverse events unrelated to treatment. In the second participant, events involving anemia, duodenal ulcer, and upper gastrointestinal hemorrhage that occurred as a result of the same serious adverse event were assessed as unrelated to treatment at the time of data cutoff and occurred within the first year after fidanacogene elaparvovec therapy. Narratives regarding both participants are provided in Section S8 in the Supplementary Appendix.

Adverse events that were possibly related to glucocorticoid treatment are shown in Table S6; detailed narratives for serious adverse events that occurred during glucocorticoid therapy, including in 2 participants who had gastrointestinal hemorrhage in the absence of a gastric-acid inhibitor, are provided in the Supplementary Appendix. No thrombotic events or hepatic or other cancers related to fidanacogene elaparvovec were observed. No serious hypersensitivity events occurred. Full clearance of vector DNA (defined as the absence of shedding) was observed on average within 1 to 4 months after fidanacogene elaparvovec therapy except for peripheral blood mononuclear cells, in which case clearance took up to 6 months on average (Table S7). Anti–AAV-neutralizing antibodies developed in all the participants after fidanacogene elaparvovec therapy on the basis of an assay performed at week 52 and in all available samples beyond week 52 (up to year 3). Factor IX inhibitors developed in none of the participants.

DISCUSSION

After the phase 1–2a study of fidanacogene elaparvovec and a longer-term follow-up study involving 15 participants with hemophilia B[19] using one of the lowest reported doses of AAV-based gene therapy administered in hemophilia gene-therapy trials to date,[16,20-22] we conducted the BENEGENE-2 study to evaluate the efficacy and safety of fidanacogene elaparvovec in a larger patient cohort. All 45 participants received 5×10^{11} vector genome copies per kilogram, and all initially had a response to treatment, with post-therapy factor IX levels higher than baseline and similar to the levels observed in the phase 1–2a study.[19] These results show that fidanacogene elaparvovec therapy in appropriately selected participants leads to a sufficient increase in factor IX levels to discontinue prophylaxis. On the basis of these results, fidanacogene elaparvovec has been approved in Canada, the United States, and Europe for the treatment of adults with hemophilia B.[23-25]

In this study, factor IX levels after fidanacogene elaparvovec therapy, as compared with prophylaxis, were associated with an amelioration of the bleeding phenotype and a significant reduction in the annualized bleeding rate and the annualized total factor IX consumption. These findings offer additional evidence that transduction of the FIX-R338L variant can produce hemostatic competence at the reported factor IX activity level. The majority (>80%) of the participants had factor IX activity in the mild-hemophilia range for a sustained period of 15 to 24 months, a finding that shows durable efficacy similar to that observed in other trials of gene therapy for hemophilia B (but not hemophilia A).[20,26] Factor IX values measured with the use of different assays (one-stage vs. chromogenic) were consistent with findings in previous studies, with chromogenic and Actin-FSL assays showing similar but lower levels than the SynthASil assay.[27,28] Research into the potential mechanisms for the observed differences between assays is ongoing.[29,30] The participants who resumed prophylaxis had initially had a response to treatment, with factor IX activity levels subsequently decreasing. Predictors of this loss of response have not been identified. All the participants who resumed prophylaxis therapy were treated with at least one course of glucocorticoids, whereas 22 participants (79%) who were treated with glucocorticoids did not resume prophylaxis.

More than half the participants in this study were treated with glucocorticoids, predominantly for increased aminotransferase levels that were presumed to indicate cellular immune responses (although one participant started glucocorticoid therapy in response to a decrease in factor IX levels before the aminotransferase levels increased). Glucocorticoid use was higher in the phase 3 than in the fidanacogene elaparvovec phase 1–2a study[19] and higher than in another phase 3 gene-therapy trial for hemophilia B[16] but was lower than that observed in a phase 3 gene-therapy trial for hemophilia A.[20] Guidance on when to initiate glucocorticoids differs across studies, which makes comparisons challenging. Our phase 3 study had a relatively cautious approach and a low threshold for glucocorticoid initiation, an approach that was based on experience gained in the previous phase 1–2a study.[19] Increases in aminotransferase levels were generally mild, which suggests that any immune response was minor or treated promptly (or both).

Fidanacogene elaparvovec was generally safe. No safety signals emerged in this study, and there were no instances of inhibitor development, thrombosis, or cancer, although follow-up was limited. Vector DNA sequences were shed transiently in bodily fluids and cleared as previously observed.[19] Increased aminotransferase levels generally decreased over time during glucocorticoid treatment. Combined with the absence of clinically significant findings on routine liver ultrasonography, these findings suggest that liver health was maintained throughout follow-up, although longer follow-up will be needed to assess the effect on longer-term liver health. Two participants had gastrointestinal hemorrhage temporally associated with glucocorticoid therapy, a known side effect in the absence of acid blockers; no additional incidents of gastrointestinal hemorrhage were observed after a recommendation for proton-pump inhibitors was added to the protocol.

Our study evaluated a cohort of participants with hemophilia B receiving one of the lowest reported effective doses of an AAV gene-therapy vector for hemophilia B. Fidanacogene elaparvovec led to a sustained increase in factor IX levels and sustained phenotypic changes in nearly 90% of the participants. All the participants were negative for neutralizing antibodies and initially had a response to treatment. Given that another phase 3 gene-therapy trial for hemophilia B showed sustained factor IX activity with little correlation with preexisting levels of neutralizing antibodies,[16,31] a greater understanding of the association between preexisting neutralizing antibodies and outcomes is needed. Our study builds on results from previous studies and supports the widely held view that despite differences in serotype, promoters, or manufacturing protocols, or any combination of these, various gene therapies can result in similar efficacy and safety, albeit at different doses. Further work is needed to address the range of responses seen in our study and in most hemophilia gene-therapy trials and to examine why some participants resumed prophylaxis. The percentage of participants who resumed prophylaxis was higher in our study than in the previous phase 3 trial.[16] Because data regarding resumption of prophylaxis are relatively limited, further work is needed to explore whether this is a chance finding or whether it is associated with any product characteristics. Our study has several limitations, including the generalizability of the findings, limited follow-up, underrepresentation of Black patients, and the lack of a concurrent control group.

The findings of this phase 3 study showed that fidanacogene elaparvovec had a favorable benefit–risk profile providing efficacy at one of the lowest doses of AAV-based gene therapy studied for hemophilia B. An ongoing extended follow-up study to 15 years after gene therapy will provide further insights regarding the effects of fidanacogene elaparvovec.

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

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APPENDIX

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