ORIGINAL ARTICLE

Two-Year Outcomes of Valoctocogene Roxaparvovec Therapy for Hemophilia A

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ABSTRACT

BACKGROUND

Valoctocogene roxaparvovec delivers a B-domain–deleted factor VIII coding sequence with an adeno-associated virus vector to prevent bleeding in persons with severe hemophilia A. The findings of a phase 3 study of the efficacy and safety of valoctocogene roxaparvovec therapy evaluated after 52 weeks in men with severe hemophilia A have been published previously.

METHODS

We conducted an open-label, single-group, multicenter, phase 3 trial in which 134 men with severe hemophilia A who were receiving factor VIII prophylaxis received a single infusion of 6×10^{13} vector genomes of valoctocogene roxaparvovec per kilogram of body weight. The primary end point was the change from baseline in the annualized rate of treated bleeding events at week 104 after receipt of the infusion. The pharmacokinetics of valoctocogene roxaparvovec were modeled to estimate the bleeding risk relative to the activity of transgene-derived factor VIII.

RESULTS

At week 104, a total of 132 participants, including 112 with data that were prospectively collected at baseline, remained in the study. The mean annualized treated bleeding rate decreased by 84.5% from baseline ($P<0.001$) among the participants. From week 76 onward, the trajectory of the transgene-derived factor VIII activity showed first-order elimination kinetics; the model-estimated typical half-life of the transgene-derived factor VIII production system was 123 weeks (95% confidence interval, 84 to 232). The risk of joint bleeding was estimated among the trial participants; at a transgene-derived factor VIII level of 5 IU per deciliter measured with chromogenic assay, we expected that participants would have 1.0 episode of joint bleeding per year. At 2 years postinfusion, no new safety signals had emerged and no new serious adverse events related to treatment had occurred.

CONCLUSIONS

The study data show the durability of factor VIII activity and bleeding reduction and the safety profile of valoctocogene roxaparvovec at least 2 years after the gene transfer. Models of the risk of joint bleeding suggest that the relationship between transgene-derived factor VIII activity and bleeding episodes is similar to that reported with the use of epidemiologic data for persons with mild-to-moderate hemophilia A. (Funded by BioMarin Pharmaceutical; GENEr8-1 ClinicalTrials.gov number, NCT03370913.)

The X-linked bleeding disorder hemophilia A is caused by a deficiency in functional clotting protein factor VIII. Severe hemophilia A (factor VIII activity level, <1 IU per deciliter) is associated with spontaneous bleeding[1] and painful, disabling arthropathy.[1-3] Standard care for patients with severe hemophilia A is prophylaxis with factor VIII or emicizumab[1]; however, breakthrough bleeding still occurs with both therapies,[4-6] and frequent infusions are associated with a high treatment burden.[1,7-9] Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) uses an adeno-associated virus (AAV) vector to transfer a B-domain–deleted human factor VIII coding sequence controlled by a liver-selective promoter, providing endogenous factor VIII protein production in hepatocytes of persons with hemophilia A.[10-15]

In the open-label, single-group, multicenter, phase 3 GENEr8-1 study, 134 men who were receiving regular prophylaxis with exogenous factor VIII for at least 6 months received valoctocogene roxaparvovec in one dose of 6×10^{13} vector genomes (vg) per kilogram of body weight administered as a peripheral vein infusion.[15] At 49 to 52 weeks after the infusion, participants had significantly higher factor VIII activity than the imputed baseline value of 1 IU per deciliter and significantly reduced rates of treated bleeding episodes and factor VIII use.[15] The most common adverse events were transient elevations in alanine aminotransferase levels.[15]

Here, we present the results of the primary analysis of the change in the annualized treated bleeding rate at 2 or more years after gene transfer. We also performed pharmacokinetic modeling of factor VIII activity over a 5-year period and analyzed the relationship between endogenous, transgene-derived factor VIII activity and bleeding episodes.

METHODS

STUDY DESIGN

Details of the single-group, open-label, phase 3 GENEr8-1 trial have been published previously.[15] Eligible participants were men who were at least 18 years of age, had severe hemophilia A (factor VIII activity level, <1 IU per deciliter), were receiving regular prophylaxis with exogenous factor VIII, and had no history of factor VIII inhibitors or anti-AAV5 antibodies. Participants either enrolled directly or were enrolled from the prospective, noninterventional, observational 270-902 study.[6] Analysis populations included intention-to-treat (all the participants who received valoctocogene roxaparvovec), modified intention-to-treat (participants in the intention-to-treat population who were negative for human immunodeficiency virus [HIV] infection), and rollover population (participants in the modified intention-to-treat population who had been enrolled in the 270-902 study).[15] Participants received valoctocogene roxaparvovec in one dose of 6×10^{13} vg per kilogram. Glucocorticoids or other immunosuppressants were used in response to elevations in alanine aminotransferase level, as previously described.[15]

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The sponsor, BioMarin Pharmaceutical, designed the study with input from the authors. The study was performed in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines; all the participants provided written informed consent. Data analyses were performed by authors who are employees of the sponsor, and all the authors contributed critical interpretation of the results. All the authors vouch for the accuracy and completeness of the data and for the fidelity of the study to the protocol (available with the full text of this article at NEJM.org). The first draft of the manuscript was written by a medical writer, contracted by the sponsor, under the direction of the authors; the authors critically reviewed the manuscript and provided substantive input during drafting.

END POINTS

Bleeding episodes and treatments with factor VIII were reported by participants without a requirement for adjudication or imaging. Traumatic bleeding events included any bleeding episode with an identifiable cause; all others were considered to be spontaneous bleeding events. The category of "all bleeds" included every bleeding episode recorded; if standard half-life, extended half-life, or plasma-derived factor VIII products were used up to 72 hours after a bleeding episode, the episode was considered to be treated. Bleeding episodes that were associated with surgery or other medical procedures were excluded from analyses; all factor VIII use, including use related to surgery, was included in the analysis.

Bleeding and factor VIII use were assessed in the postprophylaxis period, which, as specified

in the protocol, could begin at the start of week 5 after the transgene infusion (when standard factor VIII prophylaxis was scheduled to end) or 3 days after standard factor VIII prophylaxis ended, whichever was later, and lasted until the data-cutoff date or participant withdrawal, whichever was earlier. Annualized rates of bleeding episodes and factor VIII use are reported for the rollover population.

Factor VIII activity was assessed with the use of a chromogenic substrate assay and one-stage assay.[15] Values below the lower limit of quantitation (3.0 IU per deciliter for the chromogenic substrate assay or 1.0 IU per deciliter for the one-stage assay) were imputed as 0 IU per deciliter. The median factor VIII activity was calculated for 4-week or 6-week windows; measurements that were obtained within 72 hours after receipt of exogenous factor VIII were excluded. Factor VIII activity was assessed beginning at week 5 after transgene infusion and up to the data-cutoff date, withdrawal from the study, or resumption of standard prophylaxis with factor VIII or emicizumab, whichever occurred first. Factor VIII activity was assessed in the modified intention-to-treat population, including in a subgroup of participants who received an infusion of valoctocogene roxaparvovec at least 3 years before the data-cutoff date. Safety was assessed with the use of recorded adverse events and laboratory testing in the intention-to-treat population.

MODELING ANALYSES

To estimate the half-life of transgene-derived factor VIII production beyond 2 years after gene transfer, we developed a quantitative pharmacokinetic model and used a linear mixed-effects approach to extrapolate mean and median endogenous factor VIII activity. Details are provided in the Supplementary Appendix, available at NEJM.org.

To estimate the risk of treated joint bleeding events according to factor VIII activity, we paired the number of observed treated joint bleeding events with median factor VIII activity in 4-week or 6-week intervals. A binomial regression model was used to predict the annualized joint bleeding rate and 95% confidence interval for any given factor VIII activity; results were compared with published epidemiologic data from persons with hemophilia A.

STATISTICAL ANALYSIS

The sample-size and power estimates have been described previously.[15] A prespecified statistical analysis plan was established for week 104. End points were tested hierarchically to control for the overall type I error (Table S1 in the Supplementary Appendix). The primary efficacy end point was previously the change from baseline in factor VIII activity. The primary efficacy end point was amended (in response to Food and Drug Administration [FDA] feedback) to the change from baseline in the annualized rate of treated bleeding events during the postprophylaxis period in the rollover population and was first tested for noninferiority (margin, 3.5 episodes per year; estimated from pivotal studies of factor VIII replacement products) as compared with baseline.

A 95% confidence interval for the mean change from baseline in the rate of treated bleeding events was constructed with a two-sided significance level of 0.05. Superiority was subsequently assessed with the use of the confidence interval in a one-sample t-test against the null hypothesis that the change from baseline was greater than or equal to zero. Any missing data with regard to a change from baseline were imputed as the median value of all the participants' observed changes from baseline. Mean and median annualized rates of bleeding episodes were reported, since this end point was assumed to follow a negative binomial distribution and the sample mean was approximately normally distributed.

The first secondary efficacy end point was the change from baseline to week 104 in factor VIII activity (as measured with a chromogenic assay) in the modified intention-to-treat population with the use of a one-sample t-test against the null hypothesis that the change was less than or equal to zero. Median and mean factor VIII activity were reported; although the end point was not normally distributed, the sample mean (the basis of the hypothesis test) was approximately normally distributed given the large sample size. Distribution summaries for subgroups with small sample sizes are provided for descriptive purposes only. Full details of the secondary efficacy end points are provided in the Supplementary Appendix.

RESULTS

PARTICIPANTS

At 104 weeks after the gene transfer, 132 of 134 participants who received valoctocogene roxaparvovec remained in the study; 1 participant was lost to follow-up at week 66, and 1 participant died at week 96 by suicide that was considered by the investigators to be unrelated to treatment (Fig. S1). The participants' characteristics at baseline have been described previously.[15] All the participants were included in the intention-to-treat population, 132 HIV-negative participants were included in the modified intention-to-treat population, and 112 participants who were previously enrolled in a noninterventional study were included in the rollover population.[15] As of the data-cutoff date, the median follow-up was 110.9 weeks (range, 66.1 to 197.4) among all the participants, including 17 HIV-negative participants who received an infusion of valoctocogene roxaparvovec at least 3 years before the data-cutoff date.

ANNUALIZED RATE OF TREATED BLEEDING EPISODES

Among the rollover participants, the mean change in the annualized treated bleeding rate in the postprophylaxis period as compared with baseline, when the participants were receiving factor VIII prophylaxis, was −4.1 bleeding events (95% confidence interval [CI], −5.3 to −2.9) per year (−84.5%, P<0.001). This change exceeded the noninferiority margin of 3.5 and showed the superiority of valoctocogene roxaparvovec over factor VIII prophylaxis (Fig. 1A).

ANNUALIZED RATES OF FACTOR VIII USE AND ALL EPISODES OF BLEEDING

The annualized rate of factor VIII use changed −98.2% from baseline to postprophylaxis in the rollover population, a mean of −3891.3 IU per kilogram per year (95% CI, −4221.0 to −3561.5; P<0.001) (Fig. 1B). One-time prophylaxis use was limited (21 participants) and clinically appropriate according to individual risk. The mean (±SD) and median annualized rates of one-time use of factor VIII prophylaxis in the intention-to-treat population were 15.5±72.1 IU per kilogram per year and 0.0 IU per kilogram per year (range, 0.0 to 639.2), respectively. The annualized occurrence of all bleeding events during the postprophylaxis period changed from baseline in the rollover population by a mean of −4.1 (95% CI, −5.4 to −2.8) bleeding events per year (−77.0%, P<0.001), which showed superiority over prophylaxis (Fig. 1C). Year 3 rates of bleeding and factor VIII use remained consistent with those observed in years 1 and 2 (Table S2) among the 17 HIV-negative participants who had received an infusion at least 3 years before the data-cutoff date.

FACTOR VIII ACTIVITY

Factor VIII activity increased from baseline (imputed at 1 IU per deciliter) to week 104 by a mean of 22.0 IU per deciliter (95% CI, 16.4 to 27.7; P<0.001) as measured with a chromogenic assay in the modified intention-to-treat population and by 35.1 IU per deciliter (95% CI, 26.9 to 43.2) as measured with a one-stage assay (Fig. S2). Data for two participants who did not complete week 104 were imputed as 0 IU per deciliter. Additional details regarding factor VIII activity are provided in Table S3.

Modeling was used to estimate future factor VIII activity. The trajectory of factor VIII activity was consistent with first-order elimination kinetics starting at week 76, which facilitated the development of a linear mixed-effects model. Model parameter estimates are shown in Table S4; diagnostic plots showed no major deficiencies (Figs. S3 and S4). The estimated typical half-life of the transgene-derived factor VIII production system as calculated from the slope was 123 weeks (95% CI, 84 to 232). Factor VIII activity was extrapolated to 5 years after the gene transfer (Table 1). At week 260, the estimated mean and median factor VIII levels (11.8 IU per deciliter and 5.7 IU per deciliter, respectively) as measured with a chromogenic assay were consistent with a mild hemophilia A phenotype.

CHARACTERIZATION OF BLEEDING EVENTS

After the postprophylaxis bleeding rate was determined to be significantly reduced from baseline, bleeding events were further characterized. In the rollover population, 42.0% of the participants reported no bleeding events during the postprophylaxis period (as compared with 30.4% during the 6-month baseline data-collection period), and 74.1% reported no treated bleeding events (as compared with 32.1% during the base-

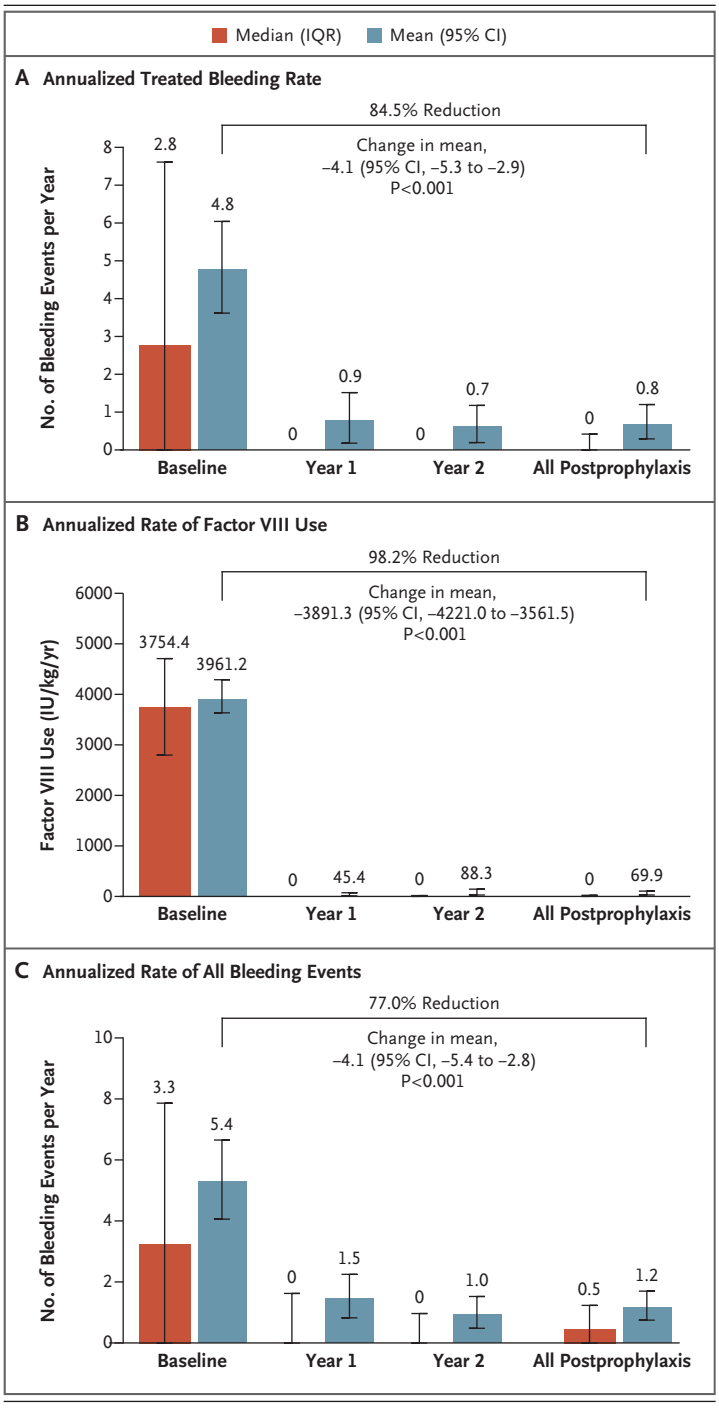

Figure 1. Changes from Baseline in Annualized Bleeding Rate and Factor VIII Use after Infusion and at Years 1 and 2 of Follow-Up (Rollover Population).

The rollover population included 112 participants from the modified intention-to-treat population who had at least 6 months of data from their participation in the noninterventional 270-902 study. Baseline values were obtained from 6 months of prospective data collected in the 270-902 study when participants were receiving regular prophylaxis with exogenous factor VIII. Year 1 values included the period beginning either at the start of week 5 or 3 days after the end of factor VIII prophylaxis (whichever was later) and ending at week 52. Year 2 values included week 53 to week 104. All postprophylaxis values included the period that began either at the start of week 5 or 3 days after the end of factor VIII prophylaxis (whichever was later) and ended at the data-cutoff date or when a participant withdrew from the study, whichever was earlier. Treated bleeding events were defined as bleeding events followed by the use of standard half-life, extended half-life, or plasma-derived factor VIII products within 72 hours after the event. Bleeding events that were associated with surgery or medical procedures were excluded from the analysis. The annualized bleeding rate was defined as follows: (number of bleeding episodes ÷ total number of days) × 365.25. The annualized rate of factor VIII use was defined as follows: (IU per kilogram of factor VIII used ÷ total number of days) × 365.25. P values are from two-sided one-sample t-tests against the null hypothesis that the change from baseline would be greater than or equal to zero. I bars indicate 95% confidence intervals for means and the interquartile range (IQR) for medians.

line data-collection period). The percentages of participants in the rollover population with no bleeding events at all and no treated bleeding events were 58.0% and 82.1%, respectively, in year 1 and 67.0% and 83.9%, respectively, in year 2 (Fig. 2A). The percentage of participants in the intention-to-treat population who reported bleeding events decreased from years 1 to 2 (Fig. 2B and 2C). Lower mean factor VIII activity was associated with more treated spontaneous bleeding events (3.6 IU per deciliter on chromogenic assay and 5.6 IU per deciliter on one-stage assay) and traumatic bleeding events (6.2 IU per deciliter on chromogenic assay and 10.1 IU per deciliter on one-stage assay) than untreated spontaneous (14.8 IU per deciliter on chromogenic assay and 23.5 IU per deciliter on one-stage assay) and traumatic bleeding events (28.6 IU per deciliter on chromogenic assay and 43.4 IU per deciliter on one-stage assay) (Fig. S5). Details of joint bleeding events are shown in Figure S6.

A negative binomial regression model evaluated the association between postprophylaxis treated joint bleeding and transgene-derived factor VIII activity. Using data from all the participants, the model predicted 1.0 treated joint bleeding event (95% CI, 0.7 to 1.5) per year with factor VIII activity of 5 IU per deciliter as measured with a chromogenic assay. At 5 IU per

Table 1. Extrapolated Factor VIII Activity.*

Time after Infusion	Factor VIII Level	
	Mean	Median (Range)
	IU per deciliter	
Wk 104	22.3±29.7	11.1 (BLQ–171)
Wk 156	16.9±25.0	8.9 (BLQ–156)
Wk 208	13.6±22.4	7.2 (BLQ–143)
Wk 260	11.8±21.0	5.7 (BLQ–131)

* Plus–minus values are means ±SD. A linear mixed-effects approach was used to obtain estimates of factor VIII activity half-life in order to extrapolate mean and median factor VIII activity levels beyond the 2-year period. Measurements were obtained with the use of a chromogenic substrate assay. BLQ denotes below the limit of quantitation.

deciliter as measured with a one-stage assay (a more sensitive assay), the model predicted 1.4 treated joint bleeding events (95% CI, 0.9 to 2.2) per year. The model-predicted relationship between factor VIII activity and bleeding events is consistent with the relationship shown in published data regarding persons with mild or moderate hemophilia A (Fig. 3).[16]

At week 104, a total of 31 of the participants remaining in the study had factor VIII activity of less than 5 IU per deciliter as measured with a chromogenic assay (Table S5). For most of these 31 participants, bleeding rates were low and did not tend to increase over time (Fig. S7). As in the results from the negative binomial regression analysis, factor VIII activity of less than 5 IU per deciliter as measured with a one-stage assay in these participants aligned better with the phenotypic expectation of bleeding risk than factor VIII activity of less than 5 IU per deciliter as measured with a chromogenic assay (Fig. S8); specifically, factor VIII activity of 3 to 5 IU per deciliter measured with chromogenic assay was more consistent with a mild than a moderate phenotype, despite being less than 5 IU per deciliter. At year 2, a one-stage assay showed that 4.5%, 9.1%, and 59.8% of participants had factor VIII activity consistent with severe, moderate, and mild hemophilia, respectively, and 26.5% had factor VIII activity greater than 40 IU per deciliter. Of 6 participants who reinitiated prophylaxis (5 with factor VIII prophylaxis and 1 with emicizumab), 5 were in the group of 31 who had factor VIII activity of less than 5 IU per deciliter as measured with a chromogenic assay (Table S6). Additional details are provided in the Supplementary Appendix.

SAFETY

No new safety signals were observed at 2 years after the infusion; safety results up to 52 weeks after the infusion were published previously.[15] The most common adverse event at 2 years remained elevated alanine aminotransferase levels (in 88.8% of the participants), which were treated with immunosuppressants; the median duration of elevation in alanine aminotransferase levels was 21.0 days (range, 1 to 498) (Table 2 and Table S7). By week 104, 99% of the participants who had received immunosuppressants at any time for elevation in alanine aminotransferase levels were no longer receiving those agents. The mean total duration of glucocorticoid use per participant was 34.7 weeks and the median duration was 32.9 weeks (Table S8). A total of 81 participants (60.4%) had adverse events related to glucocorticoid therapy, and 15 (11.2%) had adverse events related to receipt of other immunosuppressants; no serious adverse events related to immunosuppression occurred in year 2.

A total of 123 participants (91.8%) had an adverse event related to valoctocogene roxaparvovec. As of the latest data-cutoff date, 24 participants (17.9%) had a serious adverse event and 5 (3.7%) had a serious adverse event that was related to valoctocogene roxaparvovec. All serious adverse events that were related to valoctocogene roxaparvovec occurred within the first 52 weeks after the infusion.[15] Four new serious adverse events that were considered by the investigator to be unrelated to treatment occurred after the previous data-cutoff date: apnea, anaphylactic reaction, coronary artery disease, and death by suicide. Additional details on serious adverse events, elevations in alanine aminotransferase levels, and immunosuppressant use are provided in the Supplementary Appendix. Antibodies to the AAV vector developed in all the participants.

DISCUSSION

Among adult men with severe hemophilia A, a significant reduction in the annualized bleeding rates as compared with those reported with previous prophylaxis was observed for at least 2 years after AAV-mediated gene transfer with valoctocogene roxaparvovec. Clinical efficacy remained

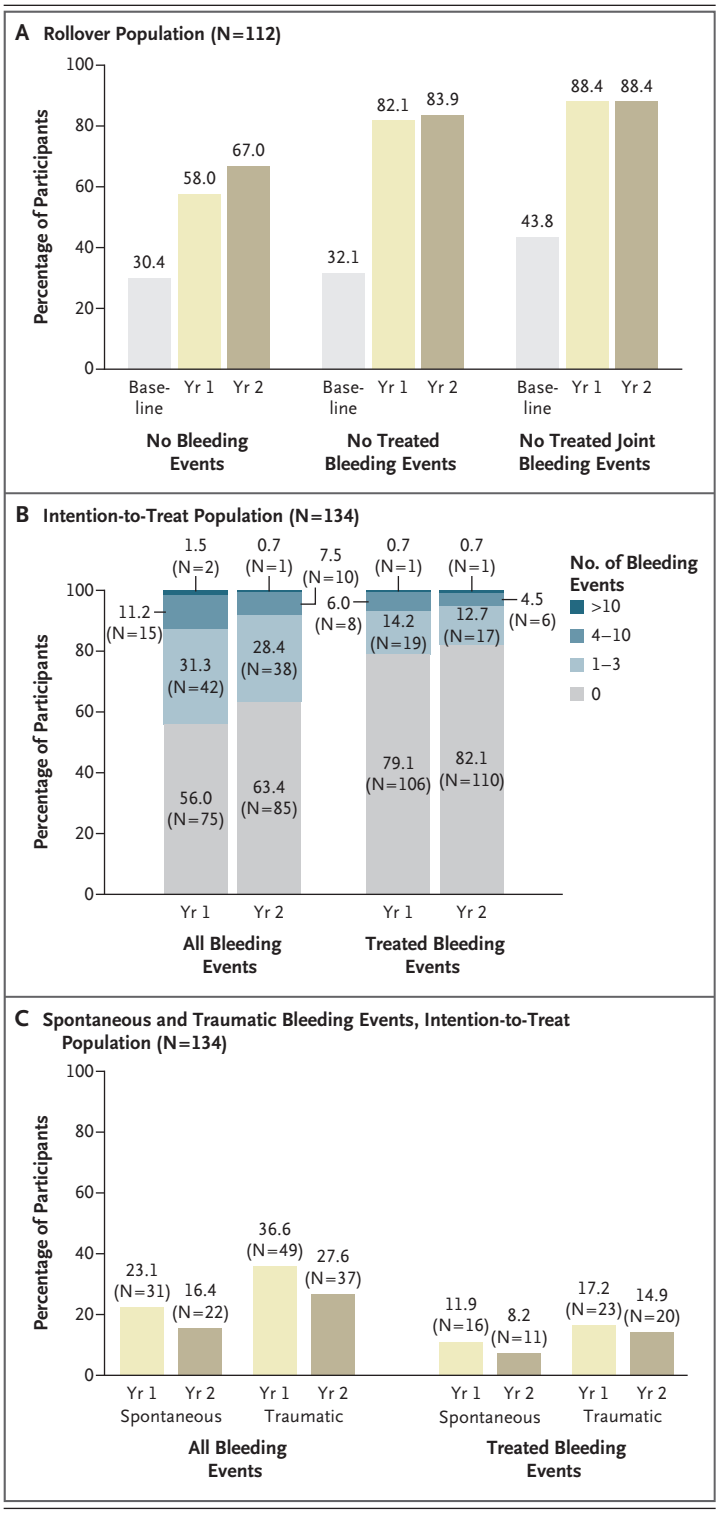

Figure 2. Participant-Reported Bleeding Episodes.

The percentages shown for bleeding events at baseline represent participants in the noninterventional 270-902 study who had bleeding events during the 6-month period of prospective data collection while they were receiving regular prophylaxis with exogenous factor VIII. Year 1 values included the period beginning either at the start of week 5 or 3 days after the end of factor VIII prophylaxis (whichever was later) and ending at week 52. Year 2 values were from week 53 to week 104. The type of bleeding event (spontaneous or traumatic) was classified on the basis of participant reporting without additional adjudication. Bleeding events that were associated with surgeries or medical procedures were excluded from analyses. All bleeding included every bleeding episode reported by participants, regardless of whether treatment with factor VIII products was used within 72 hours. A treated bleeding event was defined as a bleeding event followed by use of standard half-life, extended half-life, or plasma-derived factor VIII products within 72 hours after the event.

consistent during year 3 among 17 HIV-negative participants who had received gene therapy at least 3 years before the data-cutoff date. This finding of sustained bleeding control is in line with results from the phase 1–2 trial, in which reduced rates of treated bleeding were maintained beyond 5 years.[12-14] In 7 participants in the phase 1–2 trial who received a dose of 6×10^{13} vg per kilogram, the relative rate of decrease in factor VIII activity was similar to that seen in this phase 3 study[14]; the median factor VIII levels in that phase 1–2 study, measured with a chromogenic assay, were 60.3, 26.2, 19.9, 14.5, and 8.2 IU per deciliter at weeks 52, 104, 156, 208, and 260, respectively. In the current study, the mean and median extrapolated factor VIII activity levels measured with a chromogenic assay at week 260 were estimated to be 11.8 and 5.7 IU per deciliter, respectively. The pharmacokinetics of factor VIII activity presented here cannot be compared with data from other studies[17] owing to differences in study designs, populations, methods of calculation (logarithmic vs. linear), and products used.

The number of all bleeding events and bleeding events that were treated with exogenous factor VIII differed over 2 years of follow-up. Since bleeding events in this study were reported by the participants, data on the severity and circumstances around reported bleeding events and the rationale for nontreatment were not captured. However, untreated bleeding events tended to occur at a considerably higher level of transgene-derived factor VIII activity than treated bleeding events; this could indicate that participants considered that the nature of the bleeding (e.g., small cut or scrape) did not warrant

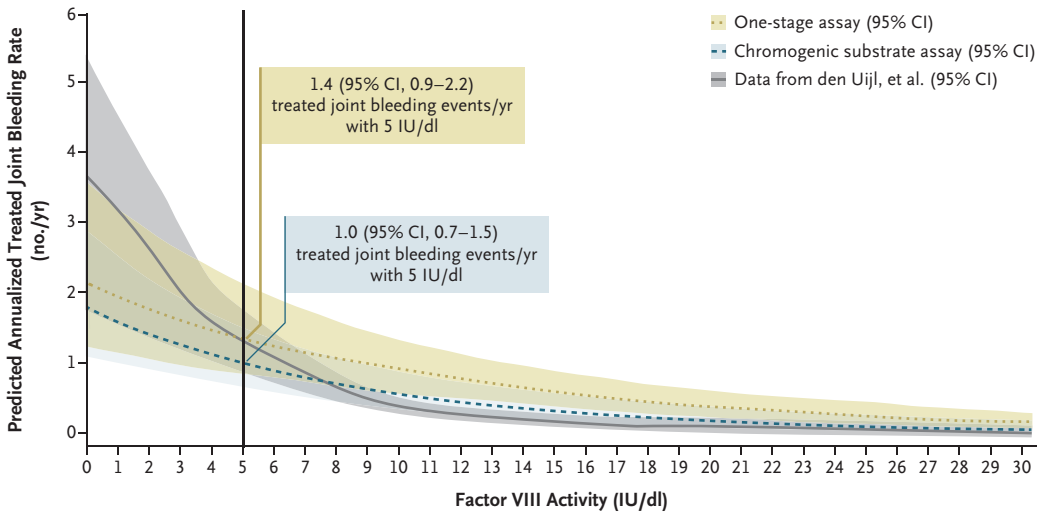

Figure 3. Comparison of Epidemiologic Estimates of Annualized Treated Joint Bleeding Rates.

Estimates that were obtained from a negative binomial model by den Uijl et al.[16] associating joint bleeding rates with factor VIII activity in 433 participants with mild and moderate hemophilia A are compared with annualized treated joint bleeding rates for transgene-derived factor VIII activity measured with a chromogenic substrate assay and a one-stage assay as estimated with the use of a negative binomial regression model with data from 134 participants in this trial.[16] The vertical line indicates where the curves intersect factor VIII activity of 5 IU per deciliter. The shaded areas around the curves represent the 95% confidence interval.

treatment. For existing problem joints, the decision to report a bleeding event and whether to administer treatment may have been complicated by difficulty in distinguishing between residual pain and bleeding. Similar differences between rates of participant-reported all bleeding events and treated bleeding events were observed in a noninterventional study involving persons with hemophilia A who were receiving standard factor VIII prophylaxis[18] and persons with hemophilia A who had received emicizumab.[4,5,19-21] Overall, these results suggest that some bleeding episodes did not warrant the administration of exogenous factor VIII. Treated bleeding episodes that were reported by the participants may provide a more meaningful and reliable outcome measure for the evaluation of new therapies than all bleeding episodes.[18,19]

Although nearly two thirds of the participants in our study who were evaluated at week 104 had factor VIII activity in the range traditionally associated with mild hemophilia A (5 to 40 IU per deciliter) and typically had the expected control of bleeding, hemostasis was also largely maintained in the minority of participants with factor VIII activity of less than 5 IU per deciliter. As compared with previously published epidemiologic data analyzed with the use of binomial regression,[16] factor VIII activity of 3 to 5 IU per deciliter (measured with a chromogenic assay) was more consistent with a mild phenotype than a moderate one, despite the levels being less than 5 IU per deciliter. Among 18 participants who remained in the study with factor VIII activity of less than 3 IU per deciliter (measured with a chromogenic assay) at week 104, a total of 83% had postprophylaxis annualized rates of treated bleeding that were the same as or lower than the rates at baseline, including 22% without treated bleeding events who did not resume prophylaxis therapy. As compared with trough fluctuations that are associated with exogenous factor VIII administration, constitutively expressed endogenous factor VIII at consistent levels may provide better protection from bleeding, as shown by the relatively higher annualized bleeding rates seen in real-world studies of bleeding and factor VIII prophylaxis.[6,18,22,23] Rates of joint bleeding that are estimated from epidemiologic studies of persons with hemophilia A vary[24] because individual bleeding risk is influenced by factor VIII levels

Table 2. Adverse Events in 134 Participants (Intention-to-Treat Population).*

Event	Year 1	Year 2	Total
		number (percent)	
Any adverse event	134 (100)	112 (83.6)	134 (100)
Adverse event occurring in ≥30% of participants†			
Alanine aminotransferase increase	—	—	119 (88.8)
Headache	—	—	55 (41.0)
Arthralgia	—	—	53 (39.6)
Nausea	—	—	51 (38.1)
Aspartate aminotransferase increase	—	—	47 (35.1)
Serious adverse event	21 (15.7)	5 (3.7)‡	24 (17.9)
Adverse event grade ≥3	31 (23.1)	13 (9.7)	42 (31.3)
Fatal adverse event	0	1 (0.7)	1 (0.7)
Treatment-related adverse event			
Any adverse event	123 (91.8)	28 (20.9)	123 (91.8)
Serious adverse event	5 (3.7)	0	5 (3.7)
Adverse event related to glucocorticoids			
Any adverse event	80 (59.7)	9 (6.7)	81 (60.4)
Serious adverse event	3 (2.7)	0	3 (2.2)
Adverse event related to nonsteroidal immunosuppressants			
Any adverse event	12 (9.0)	2 (1.5)	15 (11.2)
Serious adverse event	1 (3.0)	0	1 (0.7)
Adverse event of special interest			
Alanine aminotransferase increase§	114 (85.1)	39 (29.1)	119 (88.8)
Alanine aminotransferase increase of grade ≥3	11 (8.2)	1 (0.7)	11 (8.2)
Adverse event related to liver function	116 (86.6)	39 (29.1)	119 (88.8)
Potential Hy's law case¶	0	0	0
Infusion-related reaction‖	12 (9.0)	0	12 (9.0)
Infusion-associated reaction**	50 (37.3)	0	50 (37.3)
Systemic hypersensitivity	7 (5.2)	0	7 (5.2)
Anaphylactic or anaphylactoid reaction	3 (2.2)	0	3 (2.2)
Thromboembolic event	0	0	0
Anti–factor VIII neutralizing antibodies	0	0	0
Cancer, except nonmelanoma skin cancer	0	0	0

* Year 1 data are adverse events that occurred from infusion through week 52. Year 2 data are adverse events that occurred from week 53 through week 730. Total data are adverse events that occurred between infusion and the most recent data-cutoff date. Adverse events were coded according to the *Medical Dictionary for Regulatory Activities*, version 24.0, and were graded for severity with the use of Common Terminology Criteria for Adverse Events, version 4.03. The determination of whether an event was related to the study drug was made by the investigator.

† These events were assessed only for the total follow-up period.

‡ Some serious adverse events that were assigned to year 2 were captured in the previous data-cutoff period.[15]

§ The threshold for alanine aminotransferase elevation as an adverse event of special interest evolved during the trial. First, the threshold was defined as an alanine aminotransferase level that was greater than or equal to 1.5 times the upper limit of the normal range (ULN; 43 U per liter). The threshold was amended to include elevations to greater than the ULN when the alanine aminotransferase level was also greater than 2 times the baseline level. Finally, the threshold was amended again to include an elevation greater than the ULN or greater than or equal to 1.5 times the baseline level.

¶ Hy's law cases have three components: elevations in alanine aminotransferase or aspartate aminotransferase level to greater than 3 times the ULN, often much greater (to >5 times or >10 times the ULN); total bilirubin elevations to greater than 2 times the ULN without findings of obstruction (e.g., an elevated alkaline phosphatase level), cancer, or impaired glucuronidation capacity; and absence of another explanation for the combination of increased alanine aminotransferase or aspartate aminotransferase and total bilirubin levels (e.g., viral hepatitis or preexisting liver disease).

‖ Infusion-related reactions were defined as adverse events that occurred during infusion or within 6 hours after infusion, irrespective of a causal association with valoctocogene roxaparvovec.

** Infusion-associated reactions were defined as adverse events occurring within 48 hours after infusion, irrespective of causal association with valoctocogene roxaparvovec.

as well as factor VIII protein quality and lifestyle characteristics.[25-27]

Research to further characterize factors that affect expression of the transgene from transduced hepatocytes is ongoing. Analyses to assess the effect of fewer treated bleeding episodes on quality of life in participants who receive treatment with valoctocogene roxaparvovec are under consideration. Among the six participants in our study who reinitiated prophylaxis, most continued to have treatment benefit from valoctocogene roxaparvovec, with fewer bleeding events reported after resumption of prophylaxis than while they were receiving prophylaxis before the gene transfer. Additional investigation and follow-up may provide further insights into clinical benefits provided by low levels of transgene-derived factor VIII, including more detailed data on joint health.

At 2 years after the infusion, no new safety signals were identified and no delayed serious adverse events were attributed to valoctocogene roxaparvovec or immunosuppressant use. The most common treatment-associated adverse events, including infusion-related reactions and elevation in alanine aminotransferase levels, occurred soon after the infusion, as described previously.[15] Most elevations in alanine aminotransferase levels occurred within 26 weeks after infusion; all but one participant completed immunosuppressant use by week 104. Immunosuppressant-related adverse events were infrequent in year 2, although more than 60% of the participants had adverse events related to immunosuppressant therapy in year 1.[15] Through 5 years of follow-up in the phase 1–2 trial, treatment-related adverse events were also uncommon after year 1.[12-14] Additional research involving the interrelationship of elevations in alanine aminotransferase level, factor VIII activity, and immune suppression is under way.

Anti-AAV5 antibodies developed in all the participants after receipt of treatment with valoctocogene roxaparvovec, precluding retreatment; there was no apparent relationship between the development of anti-AAV5 antibodies and factor VIII activity.[28] Research on the effect of preexisting low concentrations of anti-AAV5 antibodies on vector transduction and factor VIII expression is ongoing in a phase 1–2 valoctocogene roxaparvovec titer-escalation study (ClinicalTrials.gov number, NCT03520712).

Limitations to this study include the reporting of bleeding events and subsequent treatment by participants with no additional adjudication or standardized imaging; the classification of spontaneous bleeding as compared with traumatic bleeding on the basis of participant information alone; the lack of assessment of physical activity or risk-taking in the context of bleeding episodes; the lack of formal joint-health scoring after baseline, precluding assessment of changes in joint status; uncertainty regarding the significance of the discrepancy between chromogenic and one-stage assay results; and the counting of only exogenous factor VIII products as treatment for bleeding events. Enrollment may also have been biased toward young participants, since persons with HIV infection or chronic hepatitis B or C and those with factor VIII inhibitors were excluded; the generalizability of these results has been discussed previously.[15]

Although further follow-up will be useful to show the long-term safety and efficacy of gene therapy in severe hemophilia A, the data from our study advance our understanding of transgene-derived factor VIII expression dynamics and the persistence of hemostatic efficacy despite transient elevations in alanine aminotransferase levels. Over a 2-year period, valoctocogene roxaparvovec infused as a single dose of 6×10^{13} vg per kilogram provided control of bleeding that was superior to factor VIII prophylaxis in adult men with severe hemophilia A; more than 80% of the participants in the rollover population had no treated bleeding events each year after infusion without routine prophylaxis, and a 98% reduction from baseline in mean use of exogenous factor VIII was observed. Results of pharmacokinetic modeling indicated that factor VIII activity levels in study participants will remain in the mild hemophilia range for at least 5 years after gene transfer. Although valoctocogene roxaparvovec may not eliminate bleeding, it potentially provides more consistent protection than factor VIII prophylaxis with less treatment burden.

Recently, valoctocogene roxaparvovec was granted conditional marketing authorization by the European Medicines Agency and is under consideration by the FDA for approval in the United States. Ongoing follow-up of the participants in this study and others may provide further insights regarding the effects of valoctocogene roxaparvovec use in participants up to 15 years after infusion.

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APPENDIX

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