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ORIGINAL ARTICLE

Three-year outcomes of valoctocogene roxaparvovec gene therapy for hemophilia A

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Abstract

Background: Valoctocogene roxaparvovec transfers a human factor (F)VIII coding sequence into hepatocytes of people with severe hemophilia A to provide bleeding protection.

Objectives: To present 3-year efficacy and safety in the multicenter, open-label, single-arm, phase 3 GENEr8-1 trial.

Methods: GENEr8-1 enrolled 134 adult males with severe hemophilia A who were receiving FVIII prophylaxis. Efficacy endpoints included annualized bleeding rate, annualized FVIII utilization, FVIII activity (chromogenic substrate assay; imputed as 1 IU/dL at baseline and 0 IU/dL after discontinuation), and the Haemophilia-Specific Quality of Life Questionnaire for Adults. Safety was assessed by adverse events (AEs).

Results: At week 156, 131 of 134 participants remained in the study; overall, 17 of 134 resumed prophylaxis. Mean annualized bleeding rate for treated bleeds decreased from 4.8 (SD, 6.5) bleeds/y at baseline to 0.8 (SD, 2.3; $P < .0001$) bleeds/y after prophylaxis (prophylaxis cessation to last follow-up) and 0.97 (SD, 3.48) bleeds/y during year 3. Annualized FVIII utilization decreased 96.8% from baseline after prophylaxis and 94.2% during year 3. At week 156, mean and median FVIII activity were 18.4 (SD, 30.8) and 8.3 IU/dL, respectively. FVIII activity decrease was lower between years 2 and 3 than between years 1 and 2. At the end of year 3, clinically meaningful improvements in the Haemophilia-Specific Quality of Life Questionnaire for Adults Total Score were observed (mean change from baseline, 6.6; 95% CI, 4.24-8.87; $P < .0001$). Mild alanine aminotransferase elevations remained the most common AE during year 3 (23.7% of participants). A serious AE of B-cell acute lymphoblastic leukemia was considered unrelated to treatment.

Conclusion: Hemostatic efficacy was maintained, and safety remained unchanged from previous years.

KEYWORDS
adeno-associated virus, clinical trial, gene therapy, health-related quality of life, hemophilia A, phase 3

1 | INTRODUCTION

Hemophilia A (HA) is an X-linked bleeding disorder caused by deficiency in the clotting protein factor (F)VIII [1]. Severe HA (FVIII activity, <1 IU/dL) is associated with spontaneous bleeding that causes pain, joint stiffness, and arthropathies, with deleterious impacts on daily life [1–7]. Current standard of care for HA is exogenous FVIII prophylaxis or nonfactor replacement therapies, such as emicizumab

[1]. These therapies require lifelong administration regimens, creating treatment burdens [1,8–13]. Breakthrough bleeds can still occur despite good treatment compliance [1,8,10,11].

Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) uses an adeno-associated virus (AAV) vector to transfer a B-domain–deleted human FVIII coding sequence controlled by a hepatocyte-selective promoter, resulting in endogenous FVIII production and bleeding protection [14–19]. In the multicenter, open-label, single-arm, phase 3 GENEr8-1 trial (NCT03370913), 134 adult males with severe HA who had previously been receiving regular exogenous FVIII prophylaxis for at least 12 months received a single 6×10^{13} vg/kg dose of valoctocogene roxaparvovec [19,20]. As previously reported, participants had significantly higher FVIII activity compared with the baseline imputed value of 1 IU/dL, which provided significantly reduced rates of treated bleeding episodes compared with baseline over 2 years [19,20]. The most common adverse events (AEs) were transient alanine aminotransferase (ALT) elevations and AEs related to corticosteroids used to control them [19,20].

Valoctocogene roxaparvovec has been approved by the US Food and Drug Administration and conditionally approved by the European Medicines Agency [21,22]. Bleeding risk is multifactorial, and the minimum FVIII activity necessary for hemostatic control varies between individuals with HA [23–25]. Understanding the level of FVIII activity required to provide bleeding protection in the context of gene therapy is paramount. Spontaneous bleeds are uncommon when FVIII activity levels are >15 IU/dL [1]; however, bleeding and FVIII activity analyses in participants who received valoctocogene roxaparvovec in phase 1/2 and phase 3 trials suggest that endogenously produced FVIII may provide effective hemostatic control at lower levels than this [20]. As GENEr8-1 follow-up increases, more data become available to evaluate the association between transgene-derived FVIII activity and bleeding rates in participants.

Here, we present 3-year follow-up data from GENEr8-1 evaluating the efficacy and safety of valoctocogene roxaparvovec for severe HA. We demonstrate continued hemostatic efficacy, explore the relationship between FVIII activity and bleeding protection, examine factors underlying return to regular prophylaxis, and evaluate self-reported health-related quality of life (HRQOL). We also assess safety over 3 years.

2 | METHODS

2.1 | Study design

The detailed protocol for the multicenter, single-arm, open-label, phase 3 GENEr8-1 trial (NCT03370913) was published previously [19,20]. Briefly, adult (>18 years) men with severe HA (FVIII activity, ≤1 IU/dL) who were receiving long-term (≥12 months) prophylactic exogenous FVIII and had no history of FVIII inhibitors or anti-AAV5 antibodies were eligible to either enroll directly or roll over from a prospective, noninterventional, observational study (270-902) [10]. Of the 134 participants who received a single dose of 6×10^{13} vg/kg valoctocogene roxaparvovec (intent-to-treat [ITT] population), 112 enrolled from the 270-902 trial (rollover population) and 22 enrolled directly in GENEr8-1 (Supplementary Figure S1). Of the 22 participants who enrolled directly, 2 were HIV-positive. In GENEr8-2 (NCT03392974), an HIV-positive participant who was receiving highly active antiretroviral therapy developed markedly elevated liver enzyme levels after receiving 4×10^{13} vg/kg valoctocogene roxaparvovec; therefore, out of an abundance of caution for long-term liver health, further enrollment of HIV-positive participants was suspended in GENEr8-1, and these 2 participants were excluded from the modified ITT (mITT) population. A case study of the 2 HIV-positive participants has been published previously [26]. For FVIII activity and HRQOL, the primary analysis population was the mITT population, which included 20 directly enrolled HIV-negative participants and 112 rollover participants. For annualized bleeding rate (ABR) and FVIII infusion endpoints, the 112 rollover participants were the primary analysis population. Regular FVIII prophylaxis was scheduled to end 4 weeks after valoctocogene roxaparvovec infusion. Efficacy evaluation periods presented here include the entire postprophylaxis period (cessation of prophylaxis to last follow-up) and year-specific periods (cessation of prophylaxis to week 52, weeks 53-104, and weeks 105-156). Data on FVIII activity and return to prophylaxis (RTP) after week 156 are provided for participants with sufficient follow-up.

2.2 | Endpoints

2.2.1 | Predefined efficacy endpoints

ABR of treated and all bleeds were assessed in the rollover population and separately for participants who resumed prophylaxis. Bleeding episodes were reported by participants without adjudication and categorized as traumatic (identifiable cause) or spontaneous; both categories were included in ABR calculations. "All bleeds" included all bleeds except those related to surgery; if exogenous FVIII was used within 72 hours to treat a bleed, the bleeding episode was considered treated. Bleeds related to invasive procedures have been reported separately [27]. Reported ABRs include data after participants resumed prophylaxis, and the change from baseline in ABRs was tested with a pairwise *t*-test.

FVIII activity was assessed with a chromogenic substrate assay (CSA; lower limit of quantitation [LLOQ], 1.5 IU/dL) and one-stage assay (OSA; LLOQ, 1 IU/dL). The CSA LLOQ changed from 3.0 to 1.5 IU/dL between years 1 and 2. Median FVIII activity was calculated for 4- or 6-week windows. FVIII activity was imputed as 1 IU/dL at baseline and 0 IU/dL if a participant discontinued the study. FVIII activity was excluded after a participant resumed prophylaxis, and missing values were imputed as the smaller of the median values of the previous or next 4- or 6-week window (or via linear extrapolation if the next window was missing). Measurements within 72 hours of exogenous FVIII treatment were excluded; values below the LLOQ were imputed as 0 IU/dL. Median FVIII activity is reported for the mITT population beginning 5 weeks after infusion up to the cutoff, withdrawal, or RTP; it is also reported separately for participants who resumed prophylaxis. Annualized FVIII utilization (AFU) and

annualized FVIII infusion rates were assessed in the rollover population and separately for rollover participants who resumed prophylaxis.

Participants completed HRQOL instruments at baseline and at weeks 4, 12, 26, 52, 76, 104, 128, and 156; change from baseline to years 1, 2, and 3 in mean scores was assessed for the mITT population. Missing data were not imputed. The Haemophilia-Specific Quality of Life Questionnaire for Adults (Haemo-QOL-A) is a patient-reported outcome measure suitable for use in people who have received gene therapy [28]. It comprises 6 domains and a Total Score and yields values ranging from 0 to 100 (higher values indicate better HRQOL) [28–30]. Haemo-QOL-A scores are reported for the mITT population, for the mITT population with data excluded after a participant resumed prophylaxis, and separately for mITT participants who resumed prophylaxis. The EuroQol 5 Dimension 5 Level (EQ-5D-5L) Utility Index Score measures general health status from 0 to 1, with higher scores indicating better HRQOL [31,32]. The Haemophilia Activities List (HAL) measures self-perceived function related to everyday activities (range, 0-100); higher scores indicate better HRQOL [33]. The Work Productivity and Impairment plus Classroom Impairment Questions: Hemophilia Specific (WPAI + CIQ:HS) measures hemophilia-related impairments in work or classroom productivity (participants completed the relevant element) and activity (completed by all participants), with scores provided as percent impairment [34,35].

2.2.2 | Post hoc efficacy analyses

Subgroup analyses comparing ABR for treated bleeds and AFU between participants stratified by week 156 FVIII activity were performed in the rollover population. Subgroups examined were participants with week 156 FVIII activity ≥40 IU/dL, >15 to <40 IU/dL, >5 to ≤15 IU/dL, ≥3 to ≤5 IU/dL, and <3 IU/dL; the lower cutoff of <3 IU/dL was chosen to match the previous LLOQ of the CSA. ABR data were up to a participant's RTP. Analyses of RTP were performed for the ITT population for descriptive purposes.

2.2.3 | Safety

Safety was reported for the ITT population, including AEs, serious AEs (SAEs), and concomitant medications such as immunosuppressants. One case of B-cell acute lymphoblastic leukemia (B-ALL) was diagnosed by bone marrow biopsy, and standard-of-care genetic testing was performed on, and compared between, enriched populations of leukemic and nonleukemic blood cells to determine vector-host integration sites and contribution of valoctocogene roxaparvovec treatment to the malignancy.

2.3 | Statistics

Formal hypothesis testing was completed during the year 1 and 2 analyses [19,20]; similar hypothesis tests were performed at a nominal significance level of .05 for descriptive purposes. The primary efficacy endpoint was change from baseline in ABR for all bleeds during the postprophylaxis period (superiority null hypothesis, change ≥0). Secondary efficacy endpoints were change from baseline in ABR for treated bleeds after prophylaxis (first against noninferiority using CIs [margin, 3.5], then for superiority [null hypothesis, change ≥0]); change from baseline (1 IU/dL) in FVIII activity (CSA) at week 156 (null hypothesis, change ≤0); change from baseline in exogenous FVIII utilization after prophylaxis (null hypothesis, change ≥0); and change from baseline in Haemo-QOL-A Total Score and domain scores for Physical Functioning, Consequences of Bleeding, and Role Functioning at week 156 (null hypothesis, change ≤0). Tertiary efficacy endpoints were change from baseline to week 156 on additional HRQOL measures. Descriptive statistics are reported.

3 | RESULTS

3.1 | Participants

At 156 weeks, 131 of 134 participants who received valoctocogene roxaparvovec remained in the study (Supplementary Figure S1). Two participants were lost to follow-up (1 each at weeks 66 and 104), and 1 participant died at week 96 in an event considered unrelated to treatment. As of the cutoff date, median follow-up was 162.4 (range, 66.1-255.0) weeks among all participants, including mITT participants who received valoctocogene roxaparvovec at least 4 years before the cutoff date. Baseline participant characteristics were published previously [19]. During the entire postprophylaxis period, 17 of 134 participants resumed regular prophylaxis.

3.2 | ABRs

In the rollover population (N = 112), mean ABR for treated bleeds was 0.8 (SD, 2.3) bleeds/y and mean change from baseline in ABR for treated bleeds was −4.0 (95% CI, −5.2 to −2.8; $P < .0001$) bleeds/y during the entire postprophylaxis period, an 82.9% reduction from baseline (Figure 1A). Mean ABR for treated bleeds was 1.0 (SD, 3.5) bleeds/y during year 3, representing a mean change of −3.8 (SD, 7.4) bleeds/y and an 80% decrease from baseline ($P < .0001$). During year 3, 74.5% of rollover participants had 0 treated bleeds compared with 32.1% during baseline on FVIII prophylaxis, 82.1% during year 1, and 83.9% during year 2. For the subset of 17 participants from the mITT population who had been dosed ≥4 years prior to the data cut, mean treated ABR was 0.8 (SD, 1.4) bleeds/y during year 4 (Supplementary Table S1).

The mean change from baseline in ABR for all bleeds during year 3 was −3.9 (SD, 7.8; $P < .0001$) bleeds/y and −4.1 (SD, 7.0; $P < .0001$) bleeds/y during the entire postprophylaxis period (Figure 1B). No bleeds were reported by 30.4% of participants in the rollover population during baseline, 58.0% during year 1, 66.1% during year 2, and 62.7% during year 3. For the subset of mITT participants dosed at least 4 years prior to the data cut, mean (95% CI) change from

baseline in ABR for all bleeds from the end of prophylaxis to week 208 was −8.7 (−21.9 to 4.5) per year (−84.5% from baseline; P = .1816).

3.3 | FVIII activity

Mean week 156 FVIII activity was 18.4 (SD, 30.8) IU/dL per CSA (median, 8.3 IU/dL; IQR, 3.0-17.2 IU/dL) for the mITT population, in line with the predicted mean week 156 FVIII activity of 16.9 (SD, 25.0; median, 8.9) IU/dL previously extrapolated with a linear mixed-effects model [20]. Mean week 156 FVIII activity per OSA was 29.7 (SD, 43.5) IU/dL (median, 16.2 IU/dL; IQR, 5.5-31.7 IU/dL; Figure 2 and Supplementary Table S2) for the mITT population. At week 156, 2 of 132 mITT participants had FVIII activity above the normal range (150 IU/dL) per CSA. As of the data cutoff date, FVIII activity at week 208 for 17 participants dosed at least 4 years before the data cut was 15.2 (SD, 21.5) IU/dL by CSA (median, 7.4 IU/dL; IQR, 4.7-11.9 IU/dL) and 22.1 (SD, 26.3) IU/dL by OSA (median, 13.2 IU/dL; IQR, 8.6-20.5 IU/dL; Supplementary Table S1).

FVIII activity levels above the upper limit of normal (ULN; 170 IU/dL for CSA; 150 IU/dL for OSA) were observed in 38 of 134 (28.4%) participants; 6 of these participants had FVIII activity assessments >ULN per CSA (range of >ULN duration, 0.7-62.9 weeks). No thrombotic events were reported, and no participants used intermittent anticoagulation for thromboembolic event prevention.

3.4 | Exogenous FVIII use

During year 3, mean AFU rates declined 94.2% to 228.1 (SD, 589.3) IU/kg/y compared with baseline; during the entire postprophylaxis period, the mean AFU declined 96.8% to 124.9 (SD, 316.4) IU/kg/y (Figure 1C). Mean change from baseline in AFU was −3753.5 (SD, 1865.9; P < .0001) IU/kg/y during year 3 and −3836.3 (SD, 1776.8; P < .0001) IU/kg/y across the entire postprophylaxis period. For the subset of participants from the mITT population who had been dosed at least 4 years prior to the data cut, mean (95% CI) change from baseline in AFU from the end of prophylaxis to year 4 was −4703.2 (−5463.3 to −3943.1) IU/kg/y (−97.3% from baseline; P < .0001).

One-time FVIII prophylaxis not due to invasive procedures (eg, in preparation for intense physical activity) was used by 13 of 134 ITT participants in year 3 (Supplementary Table S3). In the rollover population, median AFU for 1-time prophylaxis was low during year 3 and the entire postprophylaxis period (data not shown). FVIII prophylaxis was used for an invasive procedure by 20 and 34 rollover participants during year 3 and during the entire postprophylaxis period, respectively.

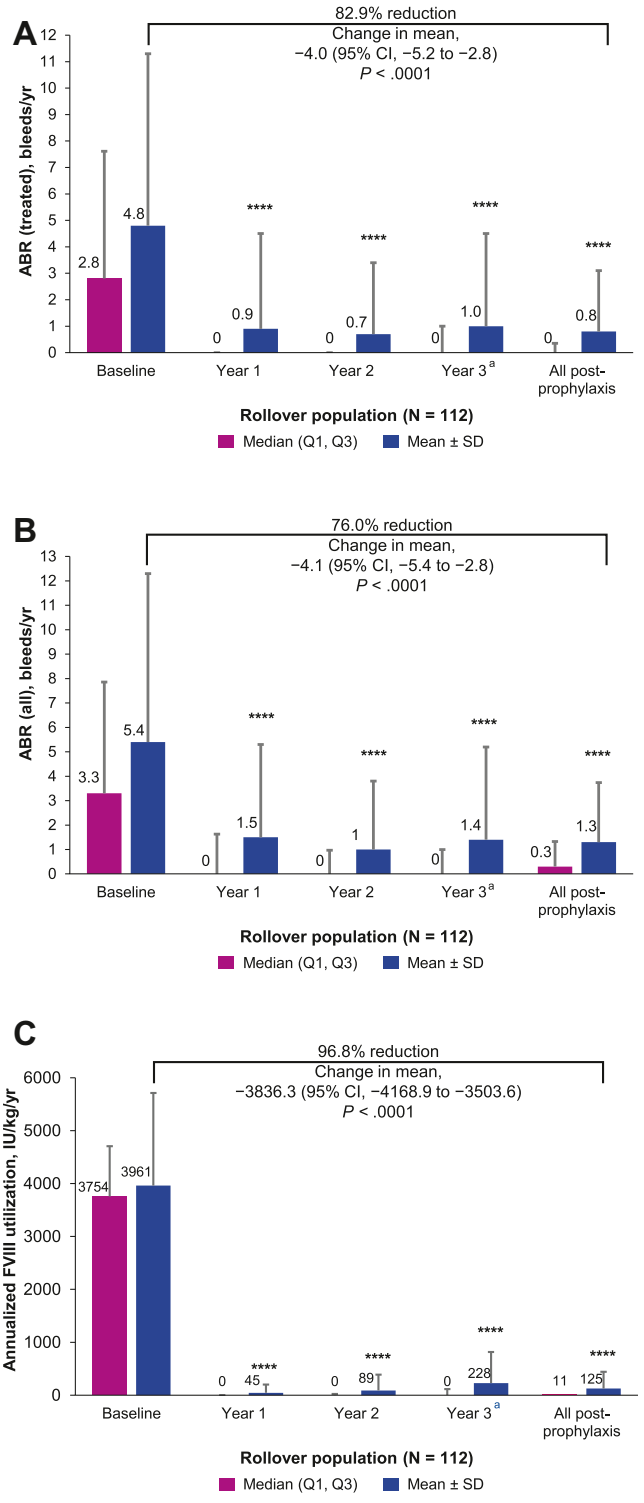


FIGURE 1 Changes from baseline after prophylaxis in the rollover population (N = 112) in (A) annualized bleeding rate (ABR) for treated bleeds, (B) annualized rate of all bleeding events, and (C) annualized rate of factor (F)VIII use. [a]Year 3 data were based on N = 110 due to participants who discontinued the study. ****P < .0001 compared with baseline. Q, quartile.

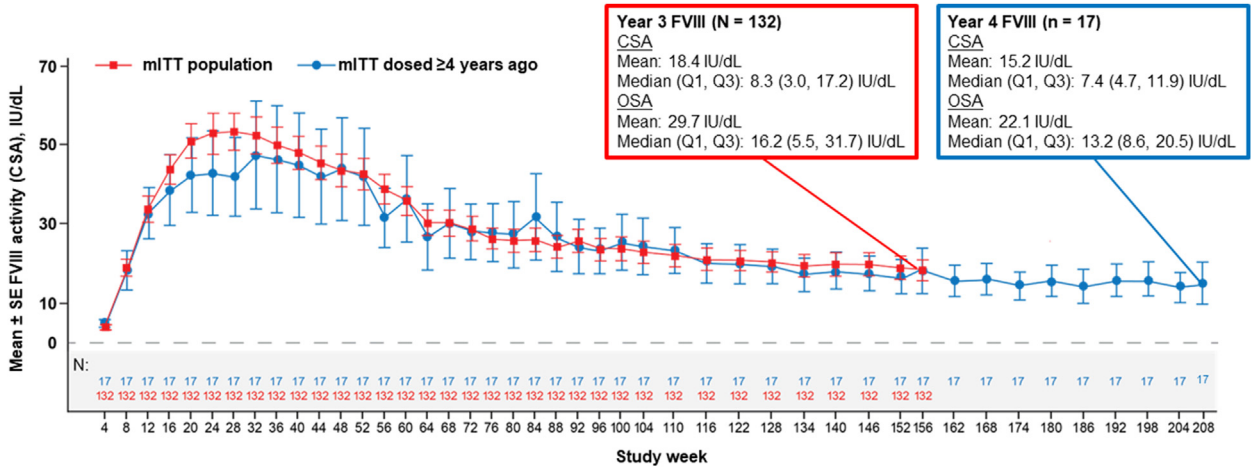


FIGURE 2 Factor (F)VIII activity in the modified intent-to-treat (mITT) population. Median FVIII activity was calculated for 4- or 6-week windows. FVIII activity was imputed as 1 IU/dL at baseline, 0 IU/dL if the participant discontinued the study, and the smaller of the median values of the previous or next 4- or 6-week window for other missing values (or via linear extrapolation using the values of the previous 2 windows if the next window was missing and capped at the value of the previous window). CSA, chromogenic substrate assay; OSA, one-stage assay; Q, quartile.

3.5 | Outcomes by week 156 FVIII activity

At the end of year 3, 14 (10.6%) mITT participants had FVIII activity considered normal or above normal levels (≥40 IU/dL; 2/14 had FVIII activity > 150 IU/dL), 74 (56.1%) had FVIII activity in the mild hemophilia range (>5 to <40 IU/dL), 12 (9.1%) had FVIII activity in the moderate hemophilia range (≥3 to ≤5 IU/dL), and 32 (24.2%) had FVIII activity below the CSA LLOQ (<3 IU/dL, moderate-to-severe hemophilia). In the 17 mITT participants who were dosed ≥4 years before the data cut, 11.8% had FVIII activity at normal levels, 58.8% had activity in the mild hemophilia range, 11.8% had activity in the moderate hemophilia range, and 17.6% had activity in the moderate-to-severe range (<3.0 IU/dL). Mean FVIII activity trajectories throughout the study period were similar when stratified by week 156 FVIII activity levels (Supplementary Figure S2).

Treated ABR was generally similar at baseline for all week 156 FVIII activity ranges and had notably decreased after gene transfer for each year 3 FVIII category, including those with week 156 FVIII <3 IU/dL (Supplementary Figure S3A). The proportion of participants with no treated bleeds was lowest for the subgroup with week 156 FVIII activity <3 IU/dL (Supplementary Figure S3B); similarly, the <3 IU/dL subgroup had the highest median AFU during year 3, though this value was still substantially decreased from baseline (Supplementary Figure S3C).

The 34 participants with median week 156 FVIII activity <5 IU/dL (range, <1.5 to 4.9 IU/dL) who did not RTP had ABR for treated bleeds between 0 and 12.0 bleeds/y during the entire postprophylaxis period (Supplementary Figure S4). Of these 34 participants, 4 had higher ABR for treated bleeds during the postprophylaxis period than during baseline; most had lower ABR for treated bleeds compared with baseline, low postprophylaxis ABRs for treated bleeds, or no substantial treated spontaneous bleeds.

3.6 | RTP

By the end of year 3, 122 of 132 (92%) mITT participants remained off regular prophylaxis (which was defined in the protocol as usual FVIII prophylaxis administered once per week for ≥4 consecutive weeks or ≥2 doses of emicizumab injections in 31 days) and 10 resumed prophylaxis (Figure 3A). Another 7 participants resumed prophylaxis after week 156, 1 of whom resumed prophylaxis temporarily. ABR and AFU for these participants are provided in Supplementary Table S4. Participants who resumed prophylaxis by the cutoff date tended to have lower peak FVIII activity within the first 52 weeks after infusion (Supplementary Figure S5).

Of the 17 participants who resumed prophylaxis, 12 had FVIII activity at or below the recommended threshold at which hemostatic agents should be considered (≤5 IU/dL) at the time point closest to RTP (Figure 3B) [1]. ABR for treated bleeds for these 12 participants ranged from 0 to 13.4 bleeds/y during the postprophylaxis period up to RTP. Furthermore, ABR for treated bleeds was higher during the postprophylaxis period up to RTP than at baseline for 3 of 12 of these participants.

For the 5 participants with FVIII activity >5 IU/dL proximal to RTP (range, 5.5-12.4 IU/dL), ABR in the postprophylaxis period up to RTP ranged from 0 to 15.6 bleeds/y and was higher than at baseline for 2 of 5 participants. Traumatic treated bleeds due to exercise occurred in 2 of these 5 participants; 1 participant had multiple treated spontaneous ankle bleeds and 1 untreated spontaneous ankle bleed, and 1 participant transiently reinitiated FVIII prophylaxis for 6 weeks due to ankle pain. Overall, the decisions to resume prophylaxis were individual, based on participant-investigator shared decision making, and depended on factors such as bleeds, FVIII activity, and lifestyle choices (eg, physical activity).

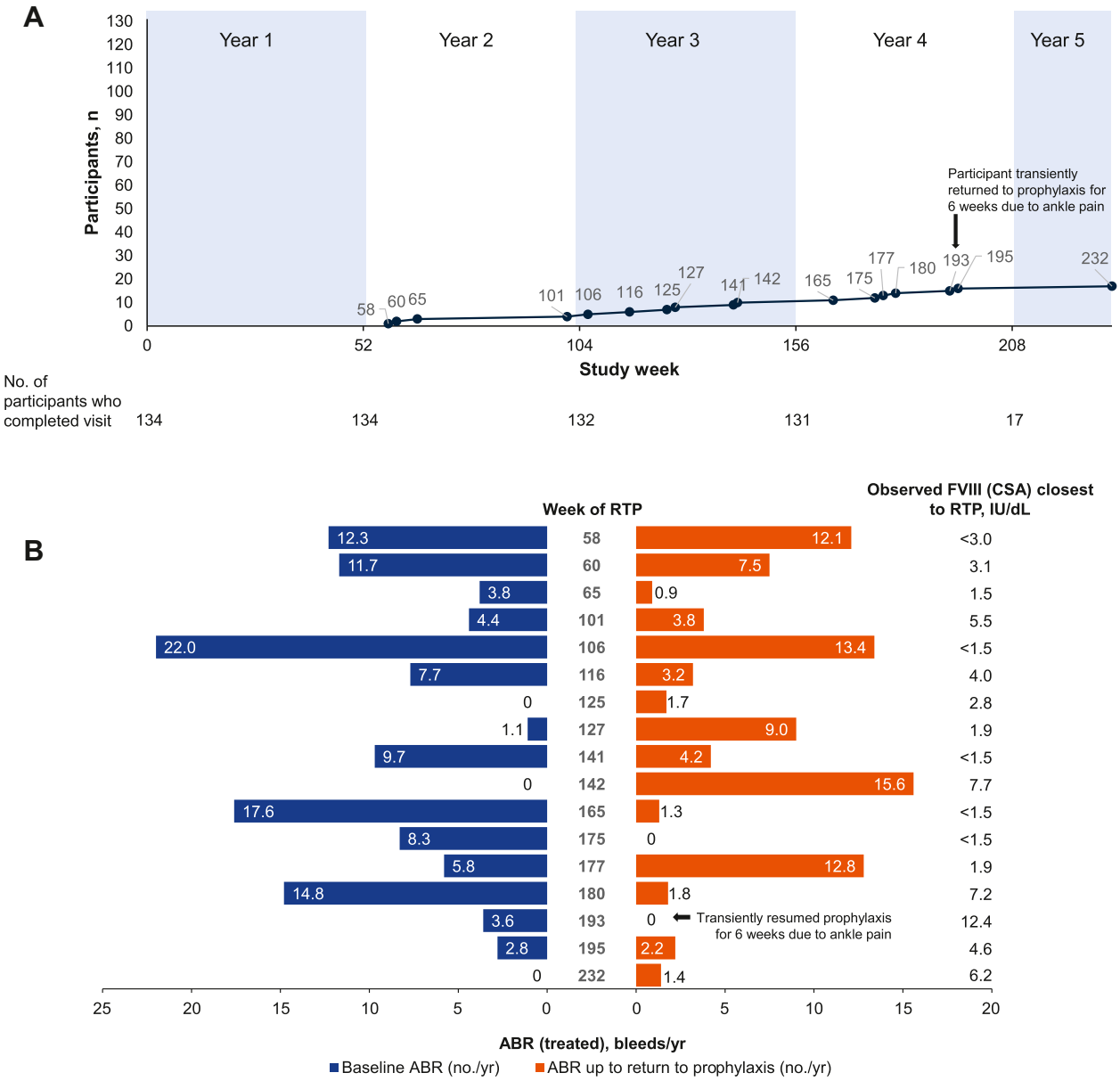


FIGURE 3 Participants who returned to prophylaxis after receiving gene therapy. (A) Timing of participants' return to prophylaxis (RTP). (B) Annualized bleeding rate (ABR, for treated bleeds) at baseline and up to RTP and factor (F)VIII activity. Prophylaxis was defined as a FVIII infusion categorized as "usual FVIII prophylaxis" administered at least once a week for ≥4 consecutive weeks or ≥2 emicizumab injections in 1 month. The valid FVIII measurement closest to RTP, but not after, was plotted. The lower limit of quantitation for chromogenic substrate assay (CSA) was 1.5 IU/dL (previously 3 IU/dL). For median FVIII at week 156, values below the lower limit of quantitation were imputed as 0 IU/dL.

3.7 | HRQOL

For participants in the mITT population with available measurements at both time points, mean increase from baseline to week 156 in Haemo-QOL-A Total Score (which measures HRQOL in adults with hemophilia) was 6.6 (95% CI, 4.2-8.9; n = 122; $P < .0001$), greater than the anchor-based clinically important difference (CID) estimate of 5.5 [28]. Values exceeding the CID estimate were first observed at week 12 (n = 128; mean, 5.5; 95% CI, 4.0-7.0; $P < .0001$). Change from baseline to week 156 in Haemo-QOL-A Role Functioning (n = 122; mean, 7.5; 95% CI, 4.6-10.4; $P < .0001$) and Consequences of Bleeding (n = 123; mean, 10.0; 95% CI, 6.8-13.1; $P < .0001$) domains also exceeded the CID estimate of 6.0 for each domain [28]. Mean change from baseline in the Physical Functioning domain score was 4.7 (95% CI, 2.1-7.4; n = 123; $P = .0005$) but did not reach the domain score CID threshold of 6.0. Haemo-QOL-A results were similar when excluding participant data after resumption of prophylaxis (Figure 4A). Haemo-QOL-A data for participants who resumed prophylaxis are provided in Supplementary Table S5.

Results for the EQ-5D-5L Utility Index Score in the mITT population, designed to assess general health status in adults and adolescents, demonstrated a mean increase of 0.02 (95% CI, −0.01 to 0.05;

n = 121; P = .1108) from baseline to year 3, less than the CID of 0.03 (Figure 4B). Mean HAL summary scores (assessing self-perceived functioning related to movement and everyday activities) also increased from baseline to year 3 in the mITT population (2.2; 95% CI, −0.3 to 4.7; n = 123; P = .0871; Figure 4C). Furthermore, mean percent impairment measured by WPAI + CIQ:HS decreased 4.7% (95% CI, −9.3% to −0.1%; n = 106; P = .0444) in the mITT population, suggesting lower disease impact on work and classroom productivity (Figure 4D). Overall, at year 3, the magnitude of improvement in the EQ-5D-5L Utility Index Score, HAL summary score, and WPAI + CIQ:HS decreased slightly from years 1 and 2.

3.8 | Safety

After 3 years, the safety profile of valoctocogene roxaparvovec remains unchanged from previous reports (Table 1). All 134 ITT participants have experienced at least 1 AE in the GENEr8-1 trial. No treatment-related SAEs occurred after year 2. Safety results up to 104 weeks after infusion, including cases of systemic hypersensitivity and infusion-related reactions, have previously been published [19,20].

3.8.1 | B-ALL

At week 154, 1 participant was diagnosed with B-ALL by bone marrow biopsy almost 3 years after infusion of 6×10^{13} vg/kg valoctocogene roxaparvovec. Genetic testing and whole-genome sequencing were performed on enriched populations of leukemic and healthy blood cells. A known mutational driver of acute lymphoblastic leukemia (cytokine receptor-like factor 2 rearrangement, a Philadelphia-like chromosomal translocation) was detected in 85% of bone marrow cells by standard-of-care genetic workup and subsequent tumor-focused gene panel at the clinical site. Extremely low levels of valoctocogene roxaparvovec vector DNA were detected (<1 copy/100 cells) in 5 cell populations (2 leukemic cell-enriched and 3 healthy) that underwent genomic analysis by multisite droplet digital polymerase chain reaction, with leukemic cells containing the lowest levels. No vector-host integration sites were identified by whole-genome sequencing in any samples. Based on these analyses, this instance of B-ALL was very likely not related to valoctocogene roxaparvovec. Moreover, a review of the available information conducted by an independent Data Monitoring Committee did not result in any change in the conduct of ongoing valoctocogene roxaparvovec trials.

3.8.2 | Transaminitis and immunosuppressant use

ALT elevation remained the most common AE, having occurred in 31 of 131 (23.7%) ITT participants during year 3. Most (90.3%) year 3 ALT elevations were grade 1, and 64.5% of year 3 ALT elevations were determined to not be related to the study drug (all ALT elevations related to the study drug were grade 1). Of the ALT elevations determined to be related to valoctocogene roxaparvovec, the duration of the elevations ranged from 8 to 138 days (1 ALT elevation was ongoing as of the cutoff date). During all follow-ups, median duration of ALT elevation above the ULN was 10.0 (range, 0.6-144.6) weeks. No participant initiated immunosuppressant use to manage ALT elevations during year 3, and no participants who continued corticosteroid use from prior to year 3 experienced corticosteroid-related AEs; 3 participants used corticosteroids for purposes other than ALT elevation, and 1 participant continued corticosteroid use initiated to manage ALT elevation near the start of year 2, despite normalization of ALT levels.

Over all follow-ups, 79% of participants used corticosteroids for ALT elevations for a median total duration of 33 (range, 3-120) weeks and a median total dose of 6420 (range, 960-31 760) mg (Table 2). In total, 83 of 134 ITT participants experienced an AE related to immunosuppressant use; 81 participants experienced an AE related to steroidal immunosuppressant use, and 16 experienced an AE related to nonsteroidal immunosuppressant use. The alternative immunosuppressants used in year 3 were mycophenolate mofetil, budesonide, and tacrolimus. AEs and SAEs related to corticosteroid use that occurred in years 1 and 2 have previously been described [19,20].

4 | DISCUSSION

Valoctocogene roxaparvovec, which aims to reduce or eliminate spontaneous bleeding and the need for routine prophylaxis, is the first approved gene therapy for severe HA [21,22]. While guidelines for target FVIII activity from exogenous FVIII prophylaxis have been established, an understanding of the relationship between endogenously produced transgene-derived FVIII, bleeding frequency, acute FVIII product use, and HRQOL is essential for patients and physicians. Here, we use 3-year follow-up data from the phase 3 GENEr8-1 trial to investigate these topics.

Mean and median FVIII activity at week 156 were consistent with values predicted by previously published pharmacokinetic modeling using 2-year data, confirming the adequacy of the model even without updated data from year 3 [20]. Comparison with previous data cuts shows that year-on-year decreases in FVIII activity are becoming smaller [19,20]. Considering the subset of 17 participants who have been followed up for more than 4 years and the previously published pharmacokinetic model as predictors, FVIII expression appears to approach stability over time. The pattern of an initial peak in the first year after infusion and a trajectory of decline similar to a plateau is evident for all FVIII activity levels when stratified at week 208. This observation provides a modicum of insight into how individual FVIII expression may evolve over time based on the peak FVIII activity reached in year 1, but further follow-up will be required for confirmation.

Consistent with previous data cuts and findings from the phase 1/2 trial [19,20,36], superior hemostatic efficacy compared with FVIII prophylaxis was maintained over 3 years, even for some participants with FVIII activity between 3 and 5 IU/dL. Modeling analyses using 2-year data from GENEr8-1 suggest a rate of 1 treated joint bleed/y at FVIII activity of 5 IU/dL per CSA [20]. Therefore, it is possible that

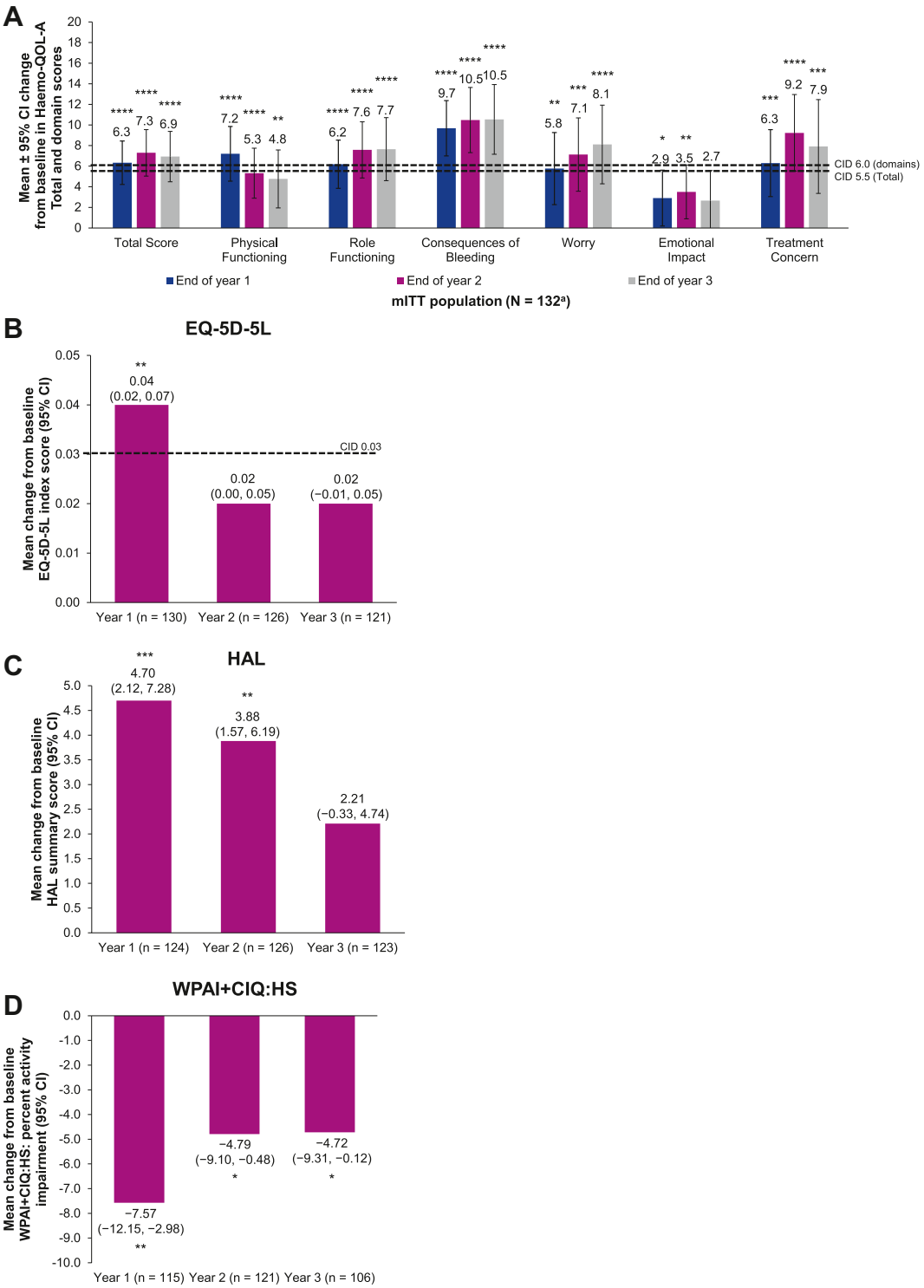


FIGURE 4 Change from baseline in patient-reported outcomes in the modified intent-to-treat (mITT, *N* = 132) population. (A) Haemophilia-Specific Quality of Life Questionnaire for Adults (Haemo-QOL-A) Total Score and domain scores. (B) EQ-5D-5L Utility Index Score. (C) Haemophilia Activities List (HAL) summary score. (D) Work Productivity and Impairment plus Classroom Impairment Questions: Hemophilia Specific (WPAI + CIQ:HS). **P* < .05; ***P* < .01; ****P* < .001; *****P* < .0001 based on a 2-tailed *t*-test against the null hypothesis that there was no change. Error bars are 95% CIs in panel A; data are presented as mean (95% CI) in panels B to D. Missing data were not imputed. [a]Change from baseline in Haemo-QOL-A Total Score and domain score results are based on available data at each time point, which may differ from the given *N*. Participants who resumed prophylaxis were excluded. CID, clinically important difference; EQ-5D-5L, EuroQol 5 Dimension 5 Level.

TABLE 1 Adverse events in the intent-to-treat population.

AE (*N* = 134)	Year 1, *n* (%)	Year 2, *n* (%)	Year 3, *n* (%)	All follow-up, *n* (%)
Any AE	134 (100)	113 (84.3)	104 (78.8)	134 (100)
AEs occurring in ≥30%				
ALT increased	-	-	-	121 (90.3)
Arthralgia	-	-	-	60 (44.8)
Headache	-	-	-	58 (43.3)
Nausea	-	-	-	52 (38.8)
AST increased	-	-	-	49 (36.6)
Fatigue	-	-	-	41 (30.6)
Any SAE	21 (15.7)	6 (4.5)	9 (6.8)	32 (23.9)
Any AE grade ≥3	31 (23.1)	13 (9.7)	12 (9.1)	50 (37.3)
Any fatal AE	0	1 (0.7)	0	1 (0.7)
Valoctocogene roxaparvovec–related				
AEs	123 (91.8)	28 (20.9)	13 (9.8)	123 (91.8)
SAEs	5 (3.7)	0	0	5 (3.7)
Glucocorticoid-related				
AEs	80 (59.7)	9 (6.7)	1 (0.8)	81 (60.4)
SAEs	3 (2.2)	0	0	3 (2.2)
Nonsteroidal immunosuppressant–related				
AEs	12 (9.0)	2 (1.5)	2 (1.5)	16 (11.9)
SAEs	1 (0.7)	0	0	1 (0.7)
AEs of special interest				
ALT elevation[a]	114 (85.1)	40 (29.9)	31 (23.5)	121 (90.3)
ALT elevation grade ≥3	11 (8.2)	1 (0.7)	0	11 (8.2)
AEs related to liver function	116 (86.6)	40 (29.9)	32 (24.2)	121 (90.3)
Potential Hy's law case[b]	0	0	0	0
Infusion-related reactions[c]	12 (9.0)	0	0	12 (9.0)
Infusion-associated reactions[d]	50 (37.3)	0	0	50 (37.3)
Systemic hypersensitivity	7 (5.2)	0	0	7 (5.2)
Anaphylactic or anaphylactoid reactions	3 (2.2)	0	0	3 (2.2)
Thromboembolic events	0	0	0	0
Anti-FVIII neutralizing antibodies	0	0	0	0
Malignancy (except NMSC)	0	0	1 (0.8)	1 (0.7)

AEs were coded using Medical Dictionary for Regulatory Activities v24.0 and graded for severity using Common Terminology Criteria for AEs v4.03. Relationship to study drug was determined by the investigator. Percentages were calculated using the total number of participants (*N*) in each analysis population as the denominator. Participants with more than 1 AE of the same category were counted only once for that category.

AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FVIII, factor VIII; NMSC, nonmelanoma skin cancer; SAE, serious adverse event.

[a] The threshold for an AE of special interest of ALT elevation evolved through the trial. First, the threshold was defined as ALT ≥1.5× the upper limit of normal (ULN, 43 U/L), then amended to include elevations greater than ULN when ALT was >2× baseline, and then amended again to include elevations greater than ULN or ≥1.5× baseline.

[b] Hy's law cases have 3 components: 1) ALT or AST elevation >3× ULN, often much greater (>5× or >10× ULN); 2) total bilirubin elevations >2× ULN, without findings of obstruction (such as elevated alkaline phosphatase), malignancy, or impaired glucuronidation capacity; and 3) no other explanation can be found for the combination of increased ALT/AST and total bilirubin (eg, viral hepatitis and preexisting liver disease).

[c] Infusion-related reactions were defined as AEs occurring during infusion or within 6 hours after infusion, irrespective of causal association with valoctocogene roxaparvovec.

[d] Infusion-associated reactions were defined as AEs occurring within 48 hours after infusion, irrespective of causal association with valoctocogene roxaparvovec.

TABLE 2 Immunosuppressant use in the intent-to-treat population (*N* = 134).

Variable	Year 1	Year 2	Year 3 (*N* = 131)	Overall
With postbaseline ALT >ULN, *n* (%)	105 (78.4)	39 (29.1)	20 (15.3)	109 (81.3)
With postbaseline ALT >1.5× baseline, *n* (%)[a]	120 (89.6)	78 (58.2)	46 (35.1)	126 (94.0)
Used corticosteroids for any purpose, *n* (%)	108 (80.6)	47 (35.1)	4 (3.1)	109 (81.3)
Total duration (wk), median (min, max)	32.0 (0.1, 50.1)	6.7 (0.1, 49.9)	1.1 (0.7, 33.0)	32.9 (0.1, 120.1)
Total dose (mg), median (min, max)	6178.8 (40, 25 110)	735.0 (1, 9040)	225.0 (200, 1515)	6310 (40, 31 760)
Used corticosteroids for ALT elevation, *n* (%)	105 (78.4)	45 (33.6)	1 (0.8)[b]	106 (79.1)
Total duration (wk), median (min, max)	32.9 (3.4, 50.1)	7.0 (0.1, 49.9)	33.0 (33.0, 33.0)	32.9 (3.1, 120.1)
Total dose (mg), median (min, max)	6310.0 (960, 25 110)	735.0 (1, 9040)	1515.0 (1515, 1515)	6420 (960, 31 760)

ALT, alanine aminotransferase; max, maximum; min, minimum; ULN, upper limit of normal.
[a] Not all events qualified as an adverse event.
[b] Participant initiated use for ALT elevation at the start of year 2 and continues use despite return to normal ALT levels prior to year 3.

steady endogenous FVIII production provides better bleeding protection than FVIII prophylaxis due to fewer fluctuations in FVIII activity. However, the reduction in ABR for treated bleeds is confounded by the inclusion of 17 of 134 ITT participants who resumed prophylaxis (as defined by the protocol) before the cutoff. While this may have artificially deflated bleeding rates, participants who returned to prophylaxis tended to have bleeding rates that were consistently higher through all years of follow-up (ie, before and after returning to prophylaxis) compared with the overall mITT population. Thus, the decision to RTP appears to have negligibly affected the overall bleeding rates reported here. Based on the totality of parameters evaluated in these participants, the decision to RTP is impacted by the confluence of factors such as FVIII activity, bleeding frequency, required or desired physical activity, and participant-investigator shared decision making. Generally, participants with FVIII activity <3 IU/dL or bleed rates above their baseline value are most likely to decide to RTP.

HRQOL measures are helpful for assessing long-term outcomes after gene therapy, particularly when real-world studies are designed to generate high-quality data relevant to patients. The Haemo-QOL-A has been deemed fit for the purpose of assessing HRQOL outcomes in HA, and results over 3 years in GENEr8-1 indicate that the tool is sensitive to changes in HRQOL among people who have received gene therapy [28]. Consistent with year 2 results [37], participants reported improvements in HRQOL at year 3 compared with baseline, suggesting real-world benefits related to the ability to perform daily activities, role functioning, worry about bleeds, and more. However, the change in Physical Functioning domain score from baseline did not reach the CID threshold at 3 years [37]. Additionally, the impact of lifelong coping (ie, disability paradox) may have contributed to higher-than-anticipated ratings on HRQOL assessments (ie, EQ-5D-5L), limiting opportunities to observe improvements over time [37,38].

At year 3, the safety profile remains unchanged, with ALT elevation remaining the most common AE. However, given the ALT elevation threshold of ALT ≥1.5× baseline or ALT >ULN, it is expected for some instances of ALT elevation to be unrelated to valoctocogene roxaparvovec treatment. For example, a systematic review found the incidence of elevated ALT (ALT > ULN) in study populations to range from 2.8% to 39.4% [39]. Furthermore, ALT elevations do not consistently predict long-term liver damage or disease [39,40]. While their relationship was not formally tested here, a review of FVIII activity before and after year 3 ALT elevation did not show any clear patterns, in contrast to results from early gene therapy trials for hemophilia B that led to the hypothesis that cellular immune responses to transduced hepatocytes could lead to the loss of transgene-derived protein [41,42]. Furthermore, based on immunogenicity monitoring during the GENEr8-1 trial that indicates ALT elevations that occur beyond ~6 months after valoctocogene roxaparvovec infusion are less likely to be related to adaptive immune responses, the benefit of corticosteroid treatment of later ALT elevations should be considered carefully [Brian R. Long, 2024, manuscript in review].

One participant experienced an SAE of B-ALL, which was determined as unlikely to be caused by valoctocogene roxaparvovec treatment by an independent Data Monitoring Committee based on extensive genomic analysis. Studies of genomic vector integrations in rodent models are conflicting, with several reports suggesting integration-driven tumorigenesis and others finding no relationship [43–45]. One study in HA canines observed AAV integrations proximal to genes related to cell growth and evidence of clonal expansion of cells with vector integration, although these observations were not associated with tumorigenesis [46]. Furthermore, low integration rates were observed in nonhuman primates and patients treated with an AAV-based gene therapy for acute intermittent porphyria, with no integration clusters associated with hepatocellular carcinomas [47]. Given the conflicting literature related to insertional mutagenesis with AAV-based gene therapies and the need to further elucidate possible genotoxic risk, we continue to monitor and characterize all instances of tumorigenesis in valoctocogene roxaparvovec–treated individuals [48]. An instance of acinic cell carcinoma in year 6 of the phase 1/2 270-201 trial of valoctocogene roxaparvovec was also deemed to be unrelated to vector integration [36].

This study is limited by participant reporting and categorization of bleeding events, which were not clinically adjudicated but reflect participant experiences. Lifestyle factors such as physically strenuous activities, which may have potentially increased after receiving gene therapy and/or contributed to bleeding or RTP, were not systematically assessed. Joint health was not scored, and problem joints were only identified at baseline, preventing assessment of their change or contribution to RTP or HRQOL, though we note instances of exercise-related bleeds in participants who resumed prophylaxis. Other GENEr8-1 study limitations are discussed elsewhere [19,20]. Post-marketing phase 4 trials will be performed to confirm real-world applicability of these results.

Valoctocogene roxaparvovec provides hemostatic efficacy with unchanged safety after 3 years. Outcomes will be assessed for up to 15 years after treatment.

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ETHICS STATEMENT

The Institutional Review Board or independent Ethics Committees of participating sites approved the protocol; participants provided written informed consent. Analysis was performed by authors who are employees of the sponsor; all authors had access to data.

AUTHOR CONTRIBUTIONS

B.M., M.C.O., P.R., E.S., D.V.Q., A.D.L., S.W.P., G.L., G.K., M.T.R., J. Mason, M.W., A.v.D., R.K., S.S., H.C., A.L.D., J.O., S.-C.C., F.P., C.M.M., and J. Mahlangu were clinical investigators who performed study procedures. D.O. and T.M.R. oversaw study conduct; H.Y. oversaw statistical analysis; and E.D.-A. oversaw health-related quality of life analysis interpretations. All authors critically reviewed the manuscript and provided substantive input during drafting of the manuscript.

DECLARATION OF COMPETING INTERESTS

M.C.O. has participated in advisory boards for Bayer, BioMarin Pharmaceutical Inc, Pfizer, Sanofi, and Takeda and received honoraria from BioMarin Pharmaceutical Inc, Biotest, Novo Nordisk, Pfizer, Roche, Takeda, and Sanofi. P.R. has received grant/travel support from CSL Behring, Sobi, and Takeda and advisory honoraria from Idogen, Pfizer, Sigilon, and Sobi. E.S. has received travel grants from CSL Behring and Novo Nordisk. D.V.Q. has served on advisory boards and speakers bureaus or as a consultant for Bayer, BioMarin Pharmaceutical Inc, Genentech, Novo Nordisk, Octapharma, Sanofi, Takeda, and uniQure. A.D.L. has served as an investigator for BioMarin Pharmaceutical Inc and Pfizer. S.W.P. has served as a consultant for ApcinteX, Bayer, BioMarin Pharmaceutical Inc, CSL Behring, Equilibra Bioscience, GeneVentiv, HEMA Biologics, LFB, Novo Nordisk, Pfizer, Regeneron, Roche, Sanofi, Siemens, Spark, Takeda, and uniQure. G.L. has received honoraria for participating in educational events from Alexion, LEO Pharma, Novartis, Novo Nordisk, Sanofi, Sobi, and Takeda and consulting fees from UCB. G.K. has received grant funding from Genentech and Pfizer and served on advisory boards or as a consultant for Bayer, BioMarin Pharmaceutical Inc, Genentech, Novo Nordisk, Sanofi, Sobi, Spark, and Takeda. M.T.R. has served as an investigator for Bayer and BioMarin Pharmaceutical Inc and served on advisory boards or speakers bureaus for Bayer, CSL Behring, Genentech, HEMA Biologics, Novo Nordisk, Sanofi, and Takeda. M.W. has served as a consultant for Bayer, BioMarin Pharmaceutical Inc, Bioverativ, Catalyst, CSL Behring, Genentech, HEMA Biologics, and Novo Nordisk. A.v.D. has received research funding from Pfizer and Sanofi, is a cofounder and board member of Hematherix Inc, and served as a consultant for ASC Therapeutics, BioMarin Pharmaceutical Inc, CSL Behring, Genentech, Regeneron, Sanofi, Takeda, and uniQure. R.K. has received grants from Bayer, CSL Behring, and LEO Pharma; consulting fees from Bayer, BioMarin Pharmaceutical Inc, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Roche/Chugai, Sanofi, Sobi, and Takeda; and payment of honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from Bayer, BioMarin Pharmaceutical Inc, Biotest, CSL Behring, Daiichi Sankyo, Grifols, LEO Pharma, Novo Nordisk, Octapharma, Pfizer, Roche/Chugai, Sanofi, Sobi, Shire/Takeda, and uniQure. S.S. has received speaking, educational, or travel honoraria from CSL Behring, Pfizer, Roche, Sobi, and Takeda. H.C. has served as a consultant for BioMarin Pharmaceutical Inc, Novo Nordisk, Roche, and Sobi. A.L.D has received research funding from BioMarin Pharmaceutical Inc, Novo Nordisk, Sanofi, and Takeda; served as a consultant for CSL Behring, Roche, and uniQure; and is a board member of the National Hemophilia Foundation and World Federation of Hemophilia USA. J.O. has received research funding from Bayer, Biotest, CSL Behring, Octapharma, Pfizer, Swedish Orphan Biovitrum, and Takeda and consultancy, speakers bureau, honoraria, scientific advisory board, and travel expenses from Bayer, Biogen Idec, BioMarin Pharmaceutical Inc, Biotest, Chugai, CSL Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Spark Therapeutics, Swedish Orphan Biovitrum, and Takeda. F.P. has served as a consultant for BioMarin Pharmaceutical Inc, Grifols, Roche, Sanofi, Sobi, and Takeda. C.M.M. has received research funding from CSL Behring, Grifols, and Takeda and has served as a speaker or consultant for CSL Behring, LFB Pharma, Novo Nordisk, Octapharma, Takeda, and Sobi. D.O., H.Y., E.D.-A., and T.M.R. are employees and shareholders of BioMarin Pharmaceutical Inc. J.Mahlangu has received research funding from BioMarin Pharmaceutical Inc, Catalyst, Roche, Novo Nordisk, Pfizer, Sandoz, Sanofi, and Spark Therapeutics. B.M., J. Mason, and S.-C.C. have no conflicts to declare.

DATA AVAILABILITY

The deidentified individual participant data that underlie the results reported in this article (including text, tables, figures, and appendices) will be made available together with the research protocol and data dictionaries for noncommercial academic purposes. Additional supporting documents may be available upon request. Investigators will be able to request access to these data and supporting documents via a data-sharing portal beginning 6 months and ending 2 years after publication. Data associated with any ongoing development program will be made available within 6 months after approval of relevant product. Requests must include a research proposal clarifying how the data will be used, including proposed analysis methodology. Research proposals will be evaluated relative to publicly available criteria available at www.BioMarin.com/patients/publication-data-request/ to determine if access will be given, contingent upon execution of a data access agreement with BioMarin Pharmaceutical Inc.

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SUPPLEMENTARY MATERIAL

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