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Etranacogene dezaparvovec in participants with hemophilia B and without adeno-associated virus serotype 5 neutralizing antibodies: A 4-year subgroup analysis (HOPE-B)

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Etranacogene dezaparvovec in participants with hemophilia B and without adeno-associated virus serotype 5 neutralizing antibodies: A 4-year subgroup analysis (HOPE-B)

Treatment for hemophilia B has until recently been lifelong regular infusions of factor replacement therapies, which is burdensome for patients

Gene therapy offers the potential of a single-dose treatment for long-term disease correction of hemophilia B

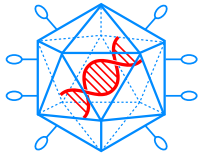
How does a subgroup of participants with no AAV5 NAbs respond to etranacogene dezaparvovec for hemophilia B?

Assessing outcomes post-etranacogene dezaparvovec in participants with hemophilia B and who are AAV5 NAb-negative

The pivotal HOPE-B trial (N=54) included 33 participants who were AAV5 NAb-negative

Lead-in period (≥6 months; regular FIX prophylaxis)

A single dose of the AAV5 gene therapy etranacogene dezaparvovec was infused



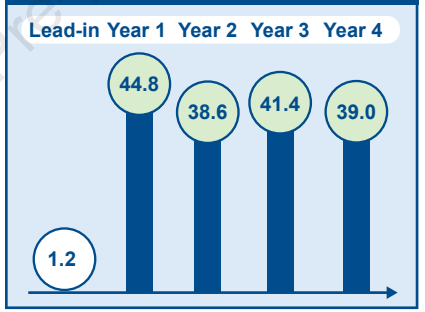
4 years

Efficacy | Safety

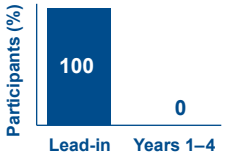
4-year post-hoc subgroup analysis results

Annualized bleeding rate	Lead in	Months 7–48
All bleeds		–85%
Spontaneous bleeds		–89%
Joint bleeds		–94%

Mean endogenous FIX expression (IU/dL)



Receipt of continuous regular FIX prophylaxis



	Year 1	Years 2–4
Treatment-related AEs (n)	56	0
Treatment-related serious AEs (n)	0	0

Participants with severe or moderately severe hemophilia B who were NAb-negative experienced substantial benefits over 4 years of follow-up after a single infusion of etranacogene dezaparvovec

Etranacogene dezaparvovec in participants with hemophilia B and without adeno-associated virus serotype 5 neutralizing antibodies: A 4-year subgroup analysis of HOPE-B

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KEYWORDS
adeno-associated virus (AAV), factor IX (FIX), gene therapy, hemophilia B, neutralizing antibody (NAb)

Etranacogene dezaparvovec in participants with hemophilia B and without adeno-associated virus serotype 5 neutralizing antibodies: A 4-year subgroup analysis (HOPE-B)

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Social media post

Focus on patients without AAV5 neutralizing antibodies from the HOPE-B trial reveals lasting bleeding reduction, stable near-normal FIX activity, and 100% freedom from FIX prophylaxis over 4 years posttreatment with etranacogene dezaparvovec.

#AAV #genetherapy; #hemophilia #hemophiliaB; #etranacogenedezaparvovec; #HOPE-B; #neutralizing antibody-negative

Abstract

Background

In the phase 3 HOPE-B trial, a single dose of etranacogene dezaparvovec was administered to participants with severe or moderately severe hemophilia B following a lead-in period (≥6 months) in which they received factor IX (FIX) prophylaxis. Participants were enrolled regardless of adeno-associated virus serotype 5 (AAV5) neutralizing antibody (NAb) status at screening.

Objectives

To determine efficacy, pharmacokinetic, and safety outcomes over 4 years post-gene therapy in HOPE-B participants who were NAb-negative (NAb−).

Methods

Participants provided serum samples for AAV5 NAb determination using an in vitro AAV5 transduction inhibition assay prior to etranacogene dezaparvovec infusion. Participants who were AAV5 NAb− at this time point were examined in this post hoc subgroup analysis.

Results

In NAb− participants (n=33), mean adjusted ABR was significantly reduced between Months 7–48 post-etranacogene dezaparvovec versus lead-in (0.57 vs 3.80; p<0.0001). At 1, 2, 3, and 4 years, ABRs were 0.99, 0.72, 0.41, and 0.41, respectively (p<0.0001 versus lead-in; n=33 throughout). Mean (standard deviation) endogenous FIX activity was 40.6 (18.6) IU/dL at Month 6 post-infusion (n=33), remained stable, and was 39.0 (16.8) IU/dL at Year 4 (n=33). Exogenous FIX consumption decreased by 99% during Months 7–48 versus the lead-in period, and no NAb− participant returned to continuous FIX prophylaxis over 4 years post-infusion. No treatment-related oncogenic events or persistent late hepatotoxicity was observed.

Conclusions

Etranacogene dezaparvovec proved highly effective, superior to FIX prophylaxis for bleeding protection, and safe over 4 years post-infusion in NAb− participants with severe or moderately severe hemophilia B (NCT03569891).

Keywords: Gene Therapy, Hemophilia B, Neutralizing Antibody, Adeno-Associated Virus, Factor IX

Essentials

- HOPE-B tested etranacogene dezaparvovec in hemophilia B people with or without vector antibodies
- This post-hoc study reports 4-year results post-gene therapy in antibody-negative patients
- Etranacogene dezaparvovec was safe and reduced bleeding events, compared with standard therapy
- Around half of patients produced stable FIX at the same level as people without hemophilia

Introduction

Treatment for hemophilia B, an X-linked bleeding disorder resulting in deficient factor IX (FIX) activity, commonly involves FIX protein replacement therapies. However, despite advances associated with newer FIX products, the lifelong need for regular infusions is burdensome for people with hemophilia B.[1] Infusion-related treatment burdens include time taken to prepare and administer treatment, pain during and/or after injections, and the need to store medication and supplies.[2] Such burdens may cause a delay in treatment, missed infusions, or a complete stopping of prophylactic treatment, resulting in a deterioration in health outcomes for people with hemophilia B.[3]

The recent development of gene therapy for hemophilia B offers the potential of a single-dose infusion, resulting in durable FIX expression, substantial reduction of treatment burden, and improved patient quality of life.[1, 4] The most common method used to deliver the FIX coding sequence into cells utilizes the adeno-associated virus (AAV), a non-replicative single-stranded DNA parvovirus. Adeno-associated viruses offer several advantages for *in vivo* gene therapy, including the absence of known pathogenicity in humans. Wild-type AAVs demonstrate preferential tropism for specific target organs, and typically persist as episomal circular DNA within the nucleus of host cells, with low genomic DNA integration rates.[5] However, when recombinant AAV vectors are utilized in clinical trials, the dosing regimens may lead to higher absolute integration rates in targeted tissues, warranting careful consideration and long-term monitoring.[6] Several AAV serotypes that vary in capsid amino acid sequence homology and other features have been used in gene therapy.[1, 7-10] Wild-type AAVs occur naturally in the environment, and therefore people who are exposed to a wild-type AAV can develop neutralizing antibodies to the viral capsid[11-13] that are cross-reactive with recombinant AAVs of the same or different serotypes; cross-neutralization has the potential to inhibit transduction of the target tissue during gene therapy.[14] Consequently, people with pre-existing AAV neutralizing antibodies have generally been excluded from clinical trials of AAV-based gene therapies. For example, a phase 3 trial of a

gene therapy that utilized a recombinant AAV serotype rh74 capsid excluded participants based on the presence of AAV rh74 neutralizing antibodies; of 316 men screened, 188 (60%) were ineligible to enter the trial on this basis.[15]

Etranacogene dezaparvovec is an AAV serotype 5 (AAV5)-based gene therapy with a codon-optimized gene expression cassette encoding the naturally occurring human FIX Padua (R338L) variant.[16, 17] The primary analysis of the pivotal phase 3 HOPE-B trial (ClinicalTrials.gov identifier: NCT03569891) of etranacogene dezaparvovec (CSL222, HEMGENIX®) demonstrated significantly improved bleeding outcomes in participants with hemophilia B (FIX ≤2 IU/dL) who had previously been receiving standard-of-care continuous FIX prophylaxis.[16, 17] Data from the previous phase 2b trial of etranacogene dezaparvovec showed that FIX expression was maintained for at least 5 years in participants.[18] In contrast to most AAV-based gene therapy clinical trials, HOPE-B enrolled participants with and without AAV5 neutralizing antibodies.[17] However, few data on long-term outcomes following gene therapy for hemophilia B in patients according to neutralizing antibody status are available. In the post-hoc analysis of the HOPE-B study reported here, long-term efficacy and tolerability outcomes were assessed in the subgroup of participants without AAV5 neutralizing antibodies prior to etranacogene dezaparvovec infusion. Screening determination of AAV5 neutralizing antibody status is available not only in the clinical trial setting, but also for individuals with hemophilia B considering etranacogene dezaparvovec therapy in the real-world setting. Importantly, individuals without AAV5 neutralizing antibodies constitute the majority, representing approximately 55–60% of the global population.[11, 19, 20]

This post hoc analysis provides the longest-term follow-up to date from a phase 3 study of systemically-delivered, liver-directed AAV-based gene therapy in hemophilia B. Focusing on the most prevalent population, individuals without AAV5 neutralizing antibodies, these results inform clinical decision-making for these specific individuals and facilitate more

126 accurate indirect comparisons with other gene therapy trials that restricted enrollment to
127 antibody-negative patients.

128

Methods

Study participants

The HOPE-B study enrolled adult males with severe (FIX activity <1 IU/dL) or moderately severe (FIX activity between 1–≤2 IU/dL) hemophilia B. Participants were required to have been receiving stable, continuous FIX prophylaxis for ≥2 months prior to screening, with the specific dose and product determined by their physician. Informed consent was another inclusion criterion. Following screening, participants then continued to receive their continuous FIX prophylaxis regimen during the lead-in period of 6 months or longer. Key exclusion criteria included a history of FIX inhibitors, active hepatitis B or C viral infection, and known severe infection or another significant concurrent uncontrolled medical condition. Neutralizing antibody positivity was not an exclusion criterion. Full eligibility criteria have been reported previously.[17] Participants eligible for this post hoc analysis of the HOPE-B study were AAV5 neutralizing antibody-negative on the day of dosing, prior to etranacogene dezaparvovec infusion.

Study design

HOPE-B was a phase 3, open-label, multinational study in which participants received a single intravenous dose of etranacogene dezaparvovec at $2x10^{13}$ genome copies per kg body weight and were planned to be followed for 5 years post-gene therapy. The trial was conducted in accordance with the International Council for Harmonisation Good Clinical Practice guidelines and the ethical principles stated in the Declaration of Helsinki. The study protocol was approved by independent ethics committees and institutional review boards at each study site.

The primary endpoint of HOPE-B was the annualized bleeding rate during a 52-week period from Months 7–18 post-gene therapy. Secondary endpoints included endogenous FIX activity (measured by a one-stage assay) at 26 and 52 weeks after steady-state FIX activity was reached as well as factor replacement use, frequency and severity of adverse

events, and reactive use of corticosteroids. We report here a post-hoc subgroup analysis of efficacy, pharmacokinetic, and safety outcomes over 4 years in participants who were neutralizing antibody-negative on the day of dosing prior to etranacogene dezaparvovec infusion.

Analysis of adeno-associated virus neutralizing antibodies

Serum samples for AAV5 neutralizing antibody determination were obtained from participants during the screening period, lead-in period (at 8 weeks and 4 weeks prior to etranacogene dezaparvovec infusion), and on the day of etranacogene dezaparvovec infusion. A central laboratory assessed AAV5 neutralizing antibody levels (Precision for Medicine, Frederick, USA). This cell-based neutralizing antibody assay assessed the potential for participant serum to inhibit the *in vitro* transduction of mammalian cells by AAV5 reporter vector expressing luciferase. Descriptions of antibody determination methodology have been reported previously.[16, 17]

Molecular analysis to assess neoplasm transformation

Molecular analyses were conducted for the detection of vector integration sites by ProtaGene CGT GmbH (Heidelberg, Germany), independently from the sponsor, on DNA samples extracted from neoplasm tissue and blood. A detailed description of the analyses can be found in the **Supplementary materials**.

Briefly, DNA was extracted using the QIAamp DNA Mini Kit (Qiagen). A polymerase chain reaction (PCR) with vector-specific primers (hFIXco_FW and hFIXco_RV) was performed on 10 ng DNA per sample with vector-containing plasmids as positive control. Whole genome sequencing data were analyzed for the detection of integration sites (IS) and to perform somatic variant calling.

Statistical analysis

Demographic and baseline characteristics were summarized descriptively using sample size (n), mean, standard deviation (SD), minimum, maximum, median, and interquartile

range (IQR) for continuous measurements, and frequency and percentages (%) for categorical variables. Adjusted annualized bleeding rates and comparison of annualized bleeding rates between lead-in and post-gene therapy period are estimated from a repeated-measures regression model with a negative binomial distribution and using generalized estimating equations, with an offset term accounting for the paired design and the differential collection periods. One-stage activated partial thromboplastin time-based (SynthaSIL®) FIX activity measurements (expressed as IU/dL) from the central laboratory were summarized descriptively. Post-gene therapy FIX samples were considered contaminated and were excluded from the analysis if drawn within 5 half-lives of FIX concentrate administration, on the basis of the reported half-life of each product. Annualized FIX consumption, excluding FIX replacement for invasive procedures, was computed for each period by dividing the total consumption by the time under observation (in years) and compared between the post-gene therapy and lead-in phase using a two-sided paired t test (using the pair of values from each participant). The analyses reported here describe a retrospective post-hoc examination of data collected prospectively during the lead-in phase and 4 years of follow-up for all participants treated in the HOPE-B phase 3 trial which was not specifically powered to detect significant differences or associations. All analyses were performed in SAS 9.4; figures were generated using GraphPad®.

Data availability statement

Individual participant data will not be shared. CSL Behring can provide scientific researchers access to deidentified participant data collected in clinical trials to improve participant care to support the advancement of medical science. Any data requests should be sent to this email address: Office.CMO@cslbehring.com

Results

Participants

The study began on June 27, 2018, and this 4-year post-hoc analysis includes data up to June 03, 2024. Overall, 33 of 54 participants in HOPE-B were AAV5 neutralizing antibody-negative on the day of dosing, prior to etranacogene dezaparvovec infusion. Baseline demographics for these participants are shown in **Table 1.** Most participants (85%) had a severe hemophilia B diagnosis (FIX <1 IU/dL) and around half (52%) had experienced a prior hepatitis C viral infection. All 33 participants completed 4 years of follow-up after etranacogene dezaparvovec infusion.

Annualized bleeding rates

In the neutralizing antibody-negative participants (n=33), the mean adjusted annualized bleeding rate (all bleeds) was reduced by 85% (two-sided Wald confidence interval [CI]: 75–91; p<0.0001), from 3.80 during lead-in to 0.57 between Months 7–48 post-etranacogene dezaparvovec infusion (**Figure 1A**). Mean adjusted annualized bleeding rates for all bleeds at 1, 2, 3, and 4 years post-etranacogene dezaparvovec infusion were 0.99, 0.72, 0.41, and 0.41, respectively (all p<0.0001 vs lead-in; n=33 at all time points; **Figure 1B**).

Compared with the lead-in period, the mean adjusted annualized bleeding rate for spontaneous bleeds was reduced by 89% (two-sided Wald 95% CI: 64–96; p<0.0001), from 1.04 during lead-in to 0.12 over Months 7–48 (**Figure 1A**). Mean adjusted spontaneous annualized bleeding rates were 0.20, 0.06, 0.19 and 0.13 at Years 1, 2, 3, and 4 post-etranacogene dezaparvovec infusion, respectively (p<0.001, p<0.0001, p<0.01, and p<0.01, respectively, vs lead-in; **Figure 1B**).

Similarly, the mean adjusted ABR for joint bleeds was reduced by 94% (two-sided Wald 95% CI: 88–97; p<0.0001), from 1.75 during lead-in to 0.10 during Months 7–48 (**Figure 1A**). At Years 1, 2, 3, and 4, mean adjusted joint annualized bleeding rates were 0.20, 0.09,

0.09, and 0.06 post-etranacogene dezaparvovec infusion, respectively (all p<0.0001 vs lead-in; **Figure 1B**).

These reductions in ABR and the associated p-values met the statistical thresholds for both non-inferiority and superiority when compared with the lead-in standard-of-care treatment.

Endogenous factor IX activity

All 33 participants who were neutralizing antibody-negative expressed endogenous transgene-derived FIX post-gene therapy (**Figure 2**). Mean (SD) endogenous FIX activity was 40.6 (18.6) IU/dL at Month 6 (n=33), remained stable over 4 years post-gene therapy, and was 39.0 (16.8) IU/dL at Year 4 (n=33). Median (range) FIX activity at Year 4 was 35.7 (4.7–80.1) IU/dL.

Use of exogenous factor IX

Over the 4-year time period reported here, no neutralizing antibody-negative participant returned to continuous exogenous FIX prophylaxis following etranacogene dezaparvovec infusion. In each year post-gene therapy, approximately 80% of neutralizing antibody-negative participants did not require any exogenous FIX infusions (**Figure 3**). During the lead-in period, bleeds requiring FIX treatment comprised 82% of total bleeds; post-gene therapy, 37% of all bleeds over 4 years required exogenous FIX treatment. Exogenous FIX consumption, excluding invasive procedures, decreased by 99%, from a mean (SD) of 264,888 (153,545) IU/year during the lead-in period to a mean (SD) of 1,878 (3337) IU/year during Months 7–48 post-gene therapy (mean [standard error] reduction of 263,010 [26615] IU/year; p<0.0001; n=33).

Safety

Of 455 treatment-emergent adverse events reported in neutralizing antibody-negative participants (**Figure 4**) during Years 1–4 post-etranacogene dezaparvovec infusion, 78% were mild, 19% moderate, and 3% severe. Overall, 22 of 33 participants experienced treatment-related adverse events during the first 3 months following etranacogene

dezaparvovec infusion; no treatment-related adverse events were reported from 3 to 42 months of follow up and one participant reported three treatment-related adverse events during Months 43–48. The most frequent treatment-related adverse event was transient alanine transaminase elevation in six (18%) participants (**Figure S1**). These elevations occurred between 22 and 71 days post-etranacogene dezaparvovec infusion. The peak alanine aminotransferase level for one participant was 2-fold the upper limit of normal, for three participants, peak alanine aminotransferase elevations were between 1–2-fold the upper limit of normal, while for two participants peak alanine aminotransferase elevations were approximately 2-fold the value of the participants' pre-gene therapy baseline alanine aminotransferase levels; however, these elevations remained within normal limits. Five out of 6 participants with treatment-related alanine aminotransferase elevations and one participant with non-treatment related alanine aminotransferase elevations received a reactive course of corticosteroid treatment, with the mean (SD) total duration of corticosteroid use for these participants being 79.5 (30.3) days. Time to receipt of corticosteroid treatment following alanine aminotransferase elevation ranged from 0 to 21 days. Mean (SD) endogenous FIX activity at or near the time of corticosteroid initiation was 20.8 (10.3) IU/dL (n=6). Mean (SD) endogenous FIX activity remained stable over 4 years post-gene therapy and was 20.5 (13.5) IU/dL (n=6) at Year 4. Median (range) FIX activity at Year 4 was 18.5 (4.7–37.6) IU/dL (n=6) (**Figure S1**).

No persistent late hepatotoxicity was observed, including in participants who experienced early liver inflammation and those with a history of chronic viral hepatitis. No serious adverse events considered related to treatment, development of inhibitors, or thrombotic events were reported. No oncogenic events considered related to treatment were reported. During Year 4, one serious adverse event of glossopharyngeal schwannoma was observed in one neutralizing antibody-negative participant and explored by molecular analysis for vector integration. No evidence of AAV5 vector DNA in tumor or control sample was detected using polymerase chain (PCR), no integration events were identified in affected tissue using whole

genome sequencing, while premalignant signatures of somatic *NF2* defects consistent with the development of a schwannoma were found; consequently, this serious adverse event was considered unrelated to treatment. A detailed description of the patient narrative, molecular analyses for the detection of integration site, and identification of relevant genetic signatures and corresponding findings are provided in the Supplementary Materials, including **Figure S2** and **Figure S3**.

Discussion

The present manuscript provides 4-year follow-up data from a phase 3 gene therapy trial, representing the longest duration of post-treatment observation in such a setting to date, supporting the sustained efficacy and durability of AAV-based gene therapy for hemophilia B. Additionally, the post-hoc analysis includes detailed outcomes for the participants who tested negative for AAV5 neutralizing antibodies prior to etranacogene dezaparvovec. This subgroup not only represented the largest subset within the trial but also reflects the expectation that most individuals with hemophilia B do not have pre-existing neutralizing antibodies to AAV5, conversely to other AAV serotypes. [11, 19, 20]

Neutralizing antibody-negative participants demonstrated that they had stable endogenous FIX activity over 4 years of follow-up post-gene therapy, accompanied by durable bleed protection and limited treatment-related adverse events, with no treatment-related adverse events reported after Month 3 post-gene therapy. While it has been previously reported that 2 participants with pre-existing AAV5 neutralizing antibodies did not express endogenous transgene-derived FIX Padua protein following treatment with etranacogene dezaparvovec,[16, 17] all treated neutralizing antibody-negative participants expressed stable endogenous transgene-derived FIX throughout the 4-year analysis period, with the median value of one-stage FIX activity levels at 4 years follow-up being 35.7 IU/dL; the median value for the intent-to-treat population (N=54), ie. including neutralizing antibody-positive participants, at 4 years was 34.6 IU/dL (data on file). Approximately half of the neutralizing antibody-negative participants had endogenous FIX activity that was in the non-hemophilia range. However, the response was variable, with endogenous FIX values ranging from 4.7 through to 80.1 IU/dL at Year 4. The impact of early alanine aminotransferase elevation on FIX expression was one important contributor to the observed wide variation in response – three participants with alanine aminotransferase elevations also had the lowest endogenous FIX values (<15 IU/dL) at Years 1, 2, 3, and 4. Of note, these participants already had the lowest FIX expression prior to the occurrence of

alanine aminotransferase elevation. With the exception of these three individuals, all neutralizing antibody-negative participants maintained >20 IU/dL FIX activity through Months 7–48. Transient liver function abnormalities treated with corticosteroids during the first 6 months after gene therapy were not associated with subsequent instability or decreases in endogenous FIX activity during the months 7–48 follow-up; FIX expression that was preserved at discontinuation of corticosteroids was in general maintained at stable levels through the remainder of the follow-up period. Moreover, all endogenous FIX levels, including the lower values, allowed discontinuation of continuous FIX prophylaxis in the first weeks after gene therapy, and all neutralizing antibody-negative participants remained free of continuous FIX prophylaxis during the 4-year analysis period.

Focusing exclusively on the subset of HOPE-B participants with undetectable pre-existing AAV5 neutralizing antibodies is valuable, as it enables a meaningful indirect comparison with other AAV-based gene therapy trials for both hemophilia A and hemophilia B. This is because most of these trials [21] have excluded participants who were baseline AAV antibody-positive to their respective AAV vectors, primarily due to concerns that AAV neutralizing antibodies would prevent transduction of target cells.

Moreover, few studies have reported long-term pharmacokinetic and efficacy data for hemophilia gene therapies. Long-term data are essential to determine the durability and safety of this recently developed therapeutic modality, and to guide development of future gene therapies. Long-term maintenance of therapeutic levels of endogenous factor VIII expression has been challenging in trials of gene therapy for participants with hemophilia A.[12, 22] In a 5-year analysis of the phase 3 factor VIII gene therapy GENEr8-1 trial of valoctocogene roxaparvovec (N=134; all of whom were AAV5 immunoglobulin G- binding antibody-negative pre-gene therapy) mean (standard error) chromogenic assay-assessed endogenous factor VIII activity was 13.7 (2.1) IU/dL (mean one stage assay-assessed factor VIII: 24.0 IU/dL) at Year 5; mean annualized bleeding rate for treated bleeds was 0.6 and 78% of patients had 0 bleeds during Year 5.[23] However, despite these relatively

positive outcomes, 19% of patients required re-initiation of factor VIII replacement treatment within 5 years post-gene therapy.[22, 23]

Through use of the gain-of-function Padua FIX variant in hemophilia B gene therapy, long-term stable expression of FIX at protective levels has been observed, although the extent of protection appears to vary according to gene therapy.[15] Three- and five-year follow up data from the initial phase 2b trial of etranacogene dezaparvovec (N=3; all participants were neutralizing antibody-positive) also showed sustained endogenous factor IX activity (36.9 and 45.7 IU/dL at Year 3 and 5 post-gene therapy, respectively), a significant reduction in bleeding events and a significant decrease in requirement for exogenous FIX, supporting the longer term therapeutic benefit of FIX Padua-based gene therapy.[24, 25] The HOPE-B post-hoc analysis reported herein found a mean adjusted all-bleed annualized bleeding rate for all bleeds of 0.57 between Months 7–48 post-etranacogene dezaparvovec infusion and importantly, showing superior results compared to other vectors in late stage development Indeed, another phase 3 study (N=45) of hemophilia B gene therapy fidanacogene elaparvovec, also utilizing FIX Padua, reported mean endogenous one-stage FIX activity of 26.9 IU/dL at month 15 post-gene therapy in participants with hemophilia B, all of whom were AAV neutralizing antibody-negative (for the AAVrh74 serotype used in that trial.[15] This resulted in an annualized bleeding rate for all bleeds of 1.28 at Month 15 post-gene therapy. Although longer-term follow up of this trial is not available yet, it is notable that already 6 out of 45 participants returned to continuous FIX prophylaxis within fewer than 15 months after fidanacogene elaparvovec administration. In contrast, it is remarkable that no neutralizing antibody-negative-participants returned to continuous FIX prophylaxis over the 4-year period post-etranacogene dezaparvovec reported here. The mechanisms underlying the observed superior outcomes observed with etranacogene dezaparvovec in neutralizing antibody-negative participants compared to other AAV vectors remain unknown. A range of factors, including capsid-specific immune responses, transduction efficiency and dosing, vector genome attributes (such as CpG content),

manufacturing process, and the recipient's hepatic function and immunological profile, can collectively influence both the durability and extent of transgene expression, as well as the potential for related hepatotoxicity. Adverse events considered related to etranacogene dezaparvovec, which was administered at a dose of $2x10^{13}$ genome copies per kg body weight, occurred in 67% of neutralizing antibody-negative participants, all within the first 3 months after gene therapy administration. The most common treatment-related adverse event was transiently increased alanine aminotransferase levels, occurring in 18% of participants, which were successfully managed using corticosteroid therapy, with stable endogenous FIX activity achieved and none of these participants requiring a return to continuous exogenous FIX prophylaxis. However, due to potential effects on hepatocyte-derived FIX Padua expression, as previously discussed, it is important to closely monitor liver transaminases in the first few months after gene delivery. This allows for immediate supportive care with corticosteroids to minimize impact on treatment efficacy.

In contrast, in BENEGENE-2, a phase 3 trial in which participants received a single dose of fidanacogene elaparvovec of $5x10^{11}$ genome copies per kg body weight,[15] 24/45 (53%) participants experienced increased transaminase levels. Of the six participants who resumed continuous exogenous FIX prophylaxis due to low FIX activity, all had received at least one course of glucocorticoids (2 of these participants received 2 courses of steroids for increased transaminase levels). A recent phase 1 study (N=10) of BBM-H901, an AAV vector expressing Padua FIX, reported 1 (10%) participant with treatment-related alanine aminotransferase elevation which was associated with a decrease in FIX activity. In this study, participants were excluded if they had a hepatitis B or C virus infection, alanine aminotransferase levels higher than 2-fold the upper limit of normal or liver conditions such as liver fibrosis stage ≥3; all participants received per-protocol prophylactic glucocorticoids from day 7 prior to BBM-H901 infusion, and for approximately 7–9 weeks afterwards.[26] This suggests that prophylactic corticosteroids neither fully prevent post-gene therapy alanine aminotransferase elevations nor support long-term stability of FIX activity. Notably,

etranacogene dezaparvovec was associated with infrequent and mild alanine aminotransferase elevations, all effectively managed with short, reactive corticosteroid courses, and was not associated with decreases in FIX levels after support with corticosteroids was initiated, underscoring that timely corticosteroid initiation at the first sign of alanine aminotransferase elevation is essential for maintaining stable FIX levels.

Regarding long-term safety, the case of the participant who developed a schwannoma was comprehensively evaluated using molecular analyses, including tests for vector integration. There was no evidence of vector DNA in the analyzed tissues, and no vector integration was detected within schwannoma sample, so that vector involvement could be excluded. The results were concordant with the established preferential hepatic tropism of the AAV5 serotype, and align with the low integration frequency characteristic of recombinant AAV vectors as described in previous clinical reports.[27, 28] In more than two decades of clinical use, AAV-based gene therapy for hemophilia has not resulted in any confirmed cases of AAV-related cancer, despite concerns about potential insertional mutagenesis.[22] While ongoing long-term follow-up studies continue to characterize the safety profile and address any latent risks, current evidence increasingly supports the benign nature of AAV vector integration in the clinical setting. However, an estimated 0.1–3% of liver-targeted recombinant AAV vector may integrate into hepatocyte DNA, which potentially equates to many million hepatic integration events at the dose of 2×10^{13} genome copies per kg bodyweight. Therefore, continued monitoring with special focus on hepatic neoplasms, including long-term registry follow ups, remains scientifically valuable, especially to reinforce confidence in the safety of AAV-based therapies and guide evidence-based risk-benefit assessments and post-marketing strategies.[29]

Limitations of this post-hoc subgroup analysis include the fact that it was not prespecified and the relatively low participant numbers. However, given the low number of people with hemophilia B in the general population, we believe that the insights into the efficacy and

safety of etranacogene dezaparvovec in neutralizing antibody-negative participants generated by this analysis have high clinical value.

Conclusions

All participants with severe to moderately severe hemophilia B in the HOPE-B trial who tested negative for AAV5 neutralizing antibodies prior to etranacogene dezaparvovec infusion expressed endogenous FIX at therapeutic levels, and durable bleed protection was achieved over a 4-year period, with no participants returning to continuous FIX prophylaxis during this time frame. No treatment-related adverse events occurred after the first 3 months following gene therapy; importantly, no events of AAV5-associated genotoxicity and no events of persistent late hepatotoxicity were observed. These data provide important information that will allow physicians and individuals with hemophilia B considering etranacogene dezaparvovec gene therapy to assess and understand potential outcomes, allowing informed decision making.

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Author contributions

Priyanka Raheja participated as a principal investigator and recruited and treated patients in this clinical trial, and contributed to analysis and interpretation of the data, the drafting and review of the content, the approval of the final draft, and agrees to be accountable for all aspects of the work.

Niamh O'Connell participated as a principal investigator and recruited and treated patients in this clinical trial and contributed to analysis and interpretation of the data, the drafting and review of the content, the approval of the final draft, and agrees to be accountable for all aspects of the work.

Peter Verhamme participated as a principal investigator and recruited and treated patients in this clinical trial, and contributed to analysis and interpretation of the data, the drafting and review of the content, the approval of the final draft, and agrees to be accountable for all aspects of the work.

Peter Kampmann participated as a principal investigator and recruited and treated patients in this clinical trial, and contributed to analysis and interpretation of the data, the drafting and review of the content, the approval of the final draft, and agrees to be accountable for all aspects of the work.

Richard S. Lemons participated as a principal investigator and recruited and treated patients in this clinical trial, and contributed to analysis and interpretation of the data, the drafting and

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Frank W. G. Leebeek participated as a principal investigator and recruited and treated patients in this clinical trial contributed to the drafting and review of the content, the approval of the final draft, and agrees to be accountable for all aspects of the work.

Fei Wang performed statistical analyses of the data and contributed to the drafting and review of the content, the approval of the final draft, and agrees to be accountable for all aspects of the work.

Sean Gill contributed to the analysis and interpretation of the data, the drafting and review of the content, the approval of the final draft, and agrees to be accountable for all aspects of the work.

Paul E. Monahan contributed to the conception and design of the research, the analysis and interpretation of the data, the drafting and review of the content, the approval of the final draft,and agrees to be accountable for all aspects of the work.

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References

1 Pierce GF, Fong S, Long BR, Kaczmarek R. Deciphering conundrums of adeno-associated virus liver-directed gene therapy: focus on hemophilia. *J Thromb Haemost*. 2024; **22**: 1263–89. 10.1016/j.jtha.2023.12.005.

2 Jiménez-Yuste V, Percier C, Shridhar N, Reynolds N, Rajkovic-Hooley O, Dewar T, Castaman G. Burden of Treatment on People with Hemophilia: Global Real-World Data. *Blood*. 2024; **144**: 5077–. 10.1182/blood-2024-200008.

3 Thornburg CD, Duncan NA. Treatment adherence in hemophilia. *Patient Prefer Adherence*. 2017; **11**: 1677–86. 10.2147/ppa.S139851.

4 Lewandowska M, Nasr S, Shapiro AD. Emerging Therapies in Hemophilia: Improving Equitable Access to Care. *J Blood Med*. 2025; **16**: 95–115. 10.2147/jbm.S490588.

5 Martins KM, Breton C, Zheng Q, Zhang Z, Latshaw C, Greig JA, Wilson JM. Prevalent and Disseminated Recombinant and Wild-Type Adeno-Associated Virus Integration in Macaques and Humans. *Hum Gene Ther*. 2023; **34**: 1081–94. 10.1089/hum.2023.134.

6 Miesbach W, Peyvandi F, Pierce GF. AAV Gene Therapy in Severe Hemophilia B. *N Engl J Med*. 2025; **393**: 829–30. 10.1056/NEJMc2509703.

7 Mendell JR, Connolly AM, Lehman KJ, Griffin DA, Khan SZ, Dharia SD, Quintana-Gallardo L, Rodino-Klapac LR. Testing preexisting antibodies prior to AAV gene transfer therapy: rationale, lessons and future considerations. *Mol Ther Methods Clin Dev*. 2022; **25**: 74–83. 10.1016/j.omtm.2022.02.011.

8 Bantel-Schaal U, Delius H, Schmidt R, zur Hausen H. Human adeno-associated virus type 5 is only distantly related to other known primate helper-dependent parvoviruses. *J Virol*. 1999; **73**: 939–47. 10.1128/JVI.73.2.939-947.1999.

9 Govindasamy L, DiMattia MA, Gurda BL, Halder S, McKenna R, Chiorini JA, Muzyczka N, Zolotukhin S, Agbandje-McKenna M. Structural insights into adeno-associated virus serotype 5. *J Virol*. 2013; **87**: 11187–99. 10.1128/JVI.00867-13.

10 Mietzsch M, Jose A, Chipman P, Bhattacharya N, Daneshparvar N, McKenna R, Agbandje-McKenna M. Completion of the AAV Structural Atlas: Serotype Capsid Structures Reveals Clade-Specific Features. *Viruses*. 2021; **13**. 10.3390/v13010101.

11 Klamroth R, Hayes G, Andreeva T, Gregg K, Suzuki T, Mitha IH, Hardesty B, Shima M, Pollock T, Slev P, Oldenburg J, Ozelo MC, Stieltjes N, Castet SM, Mahlangu J, Peyvandi F, Kazmi R, Schved JF, Leavitt AD, Callaghan M, Pan-Petesch B, Quon DV, Andrews J, Trinh A, Li M, Wong WY. Global Seroprevalence of Pre-existing Immunity Against AAV5 and Other AAV Serotypes in People with Hemophilia A. *Hum Gene Ther*. 2022; **33**: 432–41. 10.1089/hum.2021.287.

12 Li C, Narkbunnam N, Samulski RJ, Asokan A, Hu G, Jacobson LJ, Manco-Johnson MJ, Monahan PE. Neutralizing antibodies against adeno-associated virus examined prospectively in pediatric patients with hemophilia. *Gene Ther*. 2012; **19**: 288–94. 10.1038/gt.2011.90.

13 Kruzik A, Fetahagic D, Hartlieb B, Dorn S, Koppensteiner H, Horling FM, Scheiflinger F, Reipert BM, de la Rosa M. Prevalence of Anti-Adeno-Associated Virus Immune Responses in International Cohorts of Healthy Donors. *Mol Ther Methods Clin Dev*. 2019; **14**: 126–33. 10.1016/j.omtm.2019.05.014.

14 Gao G, Lu Y, Calcedo R, Grant RL, Bell P, Wang L, Figueredo J, Lock M, Wilson JM. Biology of AAV serotype vectors in liver-directed gene transfer to nonhuman primates. *Mol Ther*. 2006; **13**: 77–87. 10.1016/j.ymthe.2005.08.017.

15 Cuker A, Kavakli K, Frenzel L, Wang JD, Astermark J, Cerqueira MH, Iorio A, Katsarou-Fasouli O, Klamroth R, Shapiro AD, Hermans C, Ishiguro A, Leavitt AD, Oldenburg JB, Ozelo MC, Teitel J, Biondo F, Fang A, Fuiman J, McKay J, Sun P, Rasko JEJ, Rupon J, Investigators B-T. Gene Therapy with Fidanacogene Elaparvovec in Adults with Hemophilia B. *N Engl J Med*. 2024; **391**: 1108–18. 10.1056/NEJMoa2302982.

16 Coppens M, Pipe SW, Miesbach W, Astermark J, Recht M, van der Valk P, Ewenstein B, Pinachyan K, Galante N, Le Quellec S, Monahan PE, Leebeek FWG, Investigators H-B. Etranacogene dezaparvovec gene therapy for haemophilia B (HOPE-B): 24-month

post-hoc efficacy and safety data from a single-arm, multicentre, phase 3 trial. *Lancet Haematol*. 2024; **11**: e265–e75. 10.1016/S2352-3026(24)00006-1.

17 Pipe SW, Leebeek FWG, Recht M, Key NS, Castaman G, Miesbach W, Lattimore S, Peerlinck K, Van der Valk P, Coppens M, Kampmann P, Meijer K, O'Connell N, Pasi KJ, Hart DP, Kazmi R, Astermark J, Hermans C, Klamroth R, Lemons R, Visweshwar N, von Drygalski A, Young G, Crary SE, Escobar M, Gomez E, Kruse-Jarres R, Quon DV, Symington E, Wang M, Wheeler AP, Gut R, Liu YP, Dolmetsch RE, Cooper DL, Li Y, Goldstein B, Monahan PE. Gene Therapy with Etranacogene Dezaparvovec for Hemophilia B. *N Engl J Med*. 2023; **388**: 706–18. 10.1056/NEJMoa2211644.

18 von Drygalski A, Gomez E, Giermasz A, Castaman G, Key NS, Lattimore SU, Leebeek FWG, Miesbach WA, Recht M, Monahan PE, Le Quellec S, Pipe SW. Completion of phase 2b trial of etranacogene dezaparvovec gene therapy in patients with hemophilia B over 5 years. *Blood Adv*. 2025; **9**: 3543–52. 10.1182/bloodadvances.2024015291.

19 Klamroth R, Recht M, Key NS, Miesbach W, Pipe SW, Kaczmarek R, Drelich D, Salazar B, Le Quellec S, Monahan PE, Galante N, van der Valk P, Tarrant J. Natural history of preexisting AAV5 antibodies in adults with hemophilia B during the lead-in of the etranacogene dezaparvovec phase 3 study. *Molecular Therapy Methods & Clinical Development*. 2025; **33**: 101568. https://doi.org/10.1016/j.omtm.2025.101568.

20 Pabinger I, Ayash-Rashkovsky M, Escobar M, Konkle BA, Mingot-Castellano ME, Mullins ES, Negrier C, Pan L, Rajavel K, Yan B, Chapin J. Multicenter assessment and longitudinal study of the prevalence of antibodies and related adaptive immune responses to AAV in adult males with hemophilia. *Gene Ther*. 2024; **31**: 273–84. 10.1038/s41434-024-00441-5.

21 Deshpande SR, Joseph K, Tong J, Chen Y, Pishko A, Cuker A. Adeno-associated virus-based gene therapy for hemophilia A and B: a systematic review and meta-analysis. *Blood Adv*. 2024; **8**: 5957–74. 10.1182/bloodadvances.2024014111.

22 Herzog RW, Kaczmarek R, High KA. Gene therapy for hemophilia – From basic science to first approvals of “one-and-done” therapies. *Molecular Therapy*. 2025; **33**: 2015–34. https://doi.org/10.1016/j.ymthe.2025.03.043.

23 Leavitt AD, Mahlangu J, Raheja P, Symington E, Quon DV, Giermasz A, Kenet G, Lowe G, Key NS, Millar CM, Pipe SW, Chou S-C, Klamroth R, Mason J, Chambost H, Peyvandi F, Majerus E, Pepperell D, Chavele KM, Ozelo MC, for the GENEr8-1 Trial Group. Final GENEr8-1 results confirm enduring efficacy, safety, and quality of life improvements 5 years after valoctocogene roxaparvovec gene transfer_PB0804. *33rd Congress of the International Society on Thrombosis and Haemostasis (ISTH)*. Washington DC, 2025, PB0804.

24 von Drygalski A, Gomez E, Giermasz A, Castaman G, Key NS, Lattimore SU, Leebeek FWG, Miesbach WA, Recht M, Gut R, Dolmetsch R, Monahan PE, Le Quellec S, Pipe SW. Stable and durable factor IX levels in patients with hemophilia B over 3 years after etranacogene dezaparvovec gene therapy. *Blood Advances*. 2023; **7**: 5671–9. 10.1182/bloodadvances.2022008886.

25 von Drygalski AM, Gomez E, Giermasz A, Castaman G, Key NS, Lattimore SU, Leebeek FWG, Miesbach WA, Recht M, Monahan PM, Le Quellec S Md P, Pipe SM. Completion of Phase 2b trial of etranacogene dezaparvovec gene therapy in patients with hemophilia B over 5 years. *Blood Adv*. 2025. 10.1182/bloodadvances.2024015291.

26 Xue F, Li H, Wu X, Liu W, Zhang F, Tang D, Chen Y, Wang W, Chi Y, Zheng J, Du Z, Jiang W, Zhong C, Wei J, Zhu P, Fu R, Liu X, Chen L, Pei X, Sun J, Cheng T, Yang R, Xiao X, Zhang L. Safety and activity of an engineered, liver-tropic adeno-associated virus vector expressing a hyperactive Padua factor IX administered with prophylactic glucocorticoids in patients with haemophilia B: a single-centre, single-arm, phase 1, pilot trial. *Lancet Haematol*. 2022; **9**: e504–e13. 10.1016/s2352-3026(22)00113-2.

27 Schmidt M, Foster GR, Coppens M, Thomsen H, Dolmetsch R, Heijink L, Monahan PE, Pipe SW. Molecular evaluation and vector integration analysis of HCC complicating AAV

gene therapy for hemophilia B. *Blood Advances*. 2023; **7**: 4966–9. 10.1182/bloodadvances.2023009876.

28 Reiss UM, Davidoff AM, Tuddenham EGD, Chowdary P, McIntosh J, Muczynski V, Journou M, Simini G, Ireland L, Mohamed S, Riddell A, Pie AJ, Hall A, Quaglia A, Mangles S, Mahlangu J, Haley K, Recht M, Shen YM, Halka KG, Fortner G, Morton CL, Gu Z, Hayden RT, Neufeld EJ, Okhomina VI, Kang G, Nathwani AC. Sustained Clinical Benefit of AAV Gene Therapy in Severe Hemophilia B. *N Engl J Med*. 2025; **392**: 2226–34. 10.1056/NEJMoa2414783.

29 Pierce GF, Ozelo MC, Mahlangu J, Dunn AL, Beijlevelt M, Bender R, Carcao M, Coffin D, Grazzi EF, Foster GR, Frantsve-Hawley J, Iorio A, Jackson S, Kaczmarek R, Kaeser GE, Konkle BA, Lewandowska M, Miesbach W, O'Mahony B, Pipe S, Reiss UM, Wilson AK, Gouider E. The WFH Guidelines for the Management of Haemophilia: AAV Gene Therapy, 2025. *Haemophilia*. 2025. 10.1111/hae.70113.

Tables and figures

Tables

TABLE 1. Baseline demographics and clinical characteristics.

Characteristic	Neutralizing antibody-negative participants (n=33)
Age, mean (SD, min–max), years	39.5 (14.5, 21–73)
Race/ethnicity, n (%)	
White	28 (85)
Hispanic or Latino	2 (6)
Other	1 (3)
Missing	2 (6)
Positive HIV status, n (%)	2 (6)
Prior hepatitis B, n (%)	4 (12)
Prior or ongoing hepatitis C, n (%)[a]	17 (52)
Severity of hemophilia B at diagnosis, n (%)	
Severe (factor IX <1 IU/dL)	28 (85)
Moderately severe (factor IX ≥1 IU/dL and ≤2 IU/dL)	5 (15)
Pre-screening factor IX treatment, n (%)	
Extended half-life	17 (52)
Standard half-life	16 (48)
Participants with zero reported bleeds during the lead-in period, n (%)	11 (33)

[a]Most participants had experienced prior hepatitis C infections (n=16); one participant was undergoing eradication of hepatitis C at the time of screening and had evidence of hepatitis C virus eradication at the time of etranacogene dezaparvovec infusion.

HIV, human immunodeficiency virus; max, maximum; min, minimum; SD, standard deviation.

Figures

FIGURE 1. Comparison of annualized bleeding rates between lead in and Months 7–48 (A) and Years 1–4 post-gene therapy (B) (n=33).

*p<0.01 vs lead-in; **p<0.001 vs lead-in; ***p<0.0001 vs lead-in. Error bars in Figure 1B show the 95% confidence interval.

Figure 2. Endogenous factor IX activity levels at Years 1–4 post-treatment (n=33)

[a]Assessed by one-stage activated partial thromboplastin time FIX activity assay. Only uncontaminated samples were included in this analysis (i.e., blood sampling did not occur within 5 half-lives of exogenous FIX use).
FIX, factor IX; Q1–Q3, interquartile range.

FIGURE 3. Proportion of neutralizing antibody-negative participants who required exogenous factor IX infusions by year (n=33)[a].

[a]Factor IX infusions for the management of invasive procedures were excluded from this analysis.

663 **FIGURE 4**. Number of treatment-related and non-treatment-related adverse events by year
664 post-gene therapy (n=33).

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666 AEs, adverse events.

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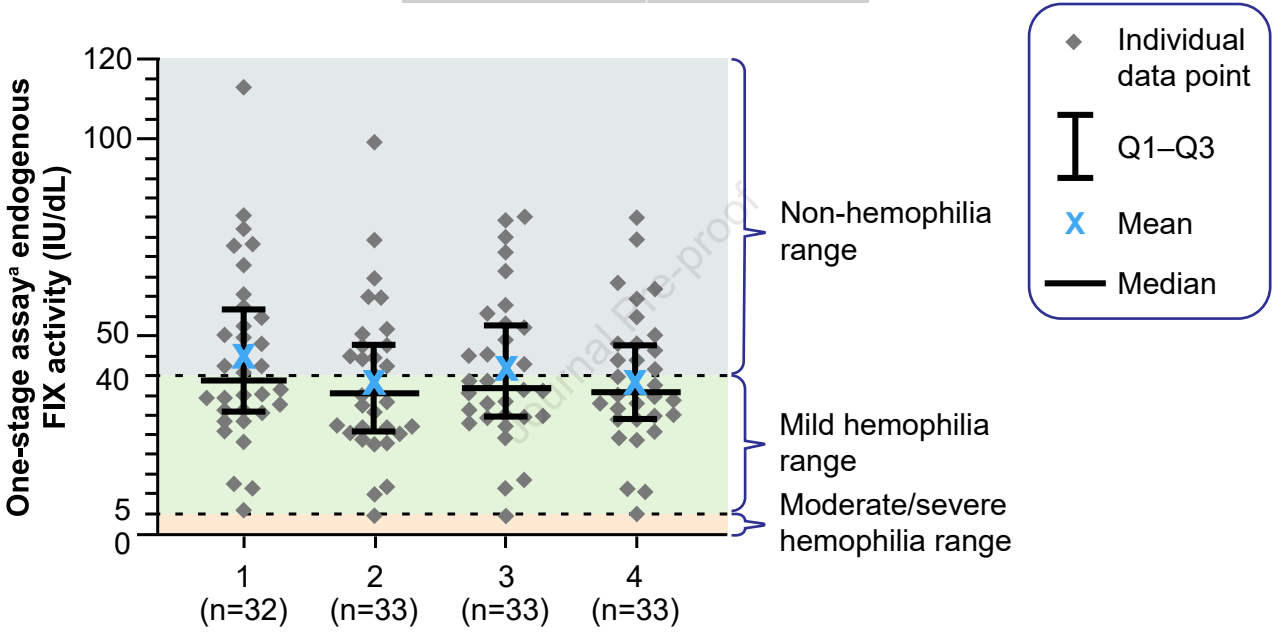

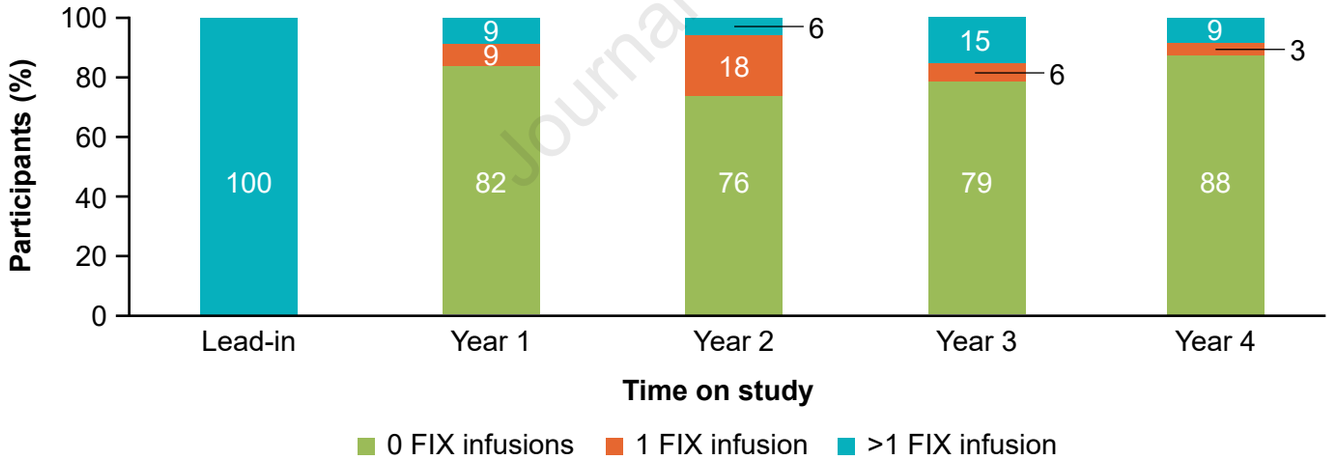

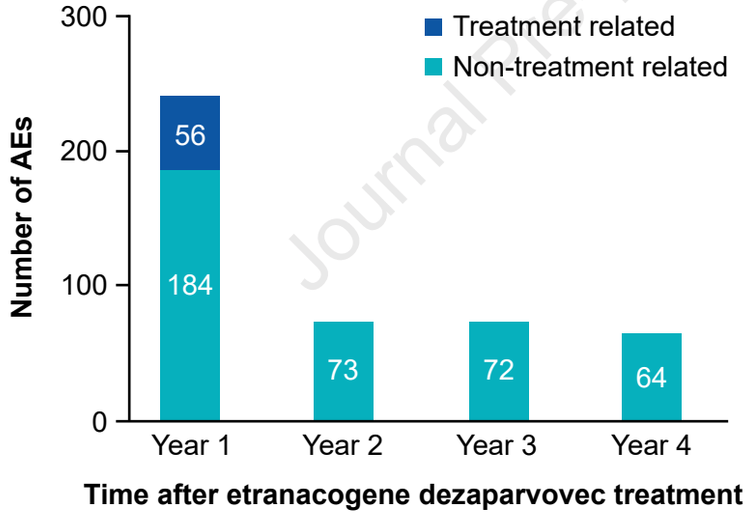

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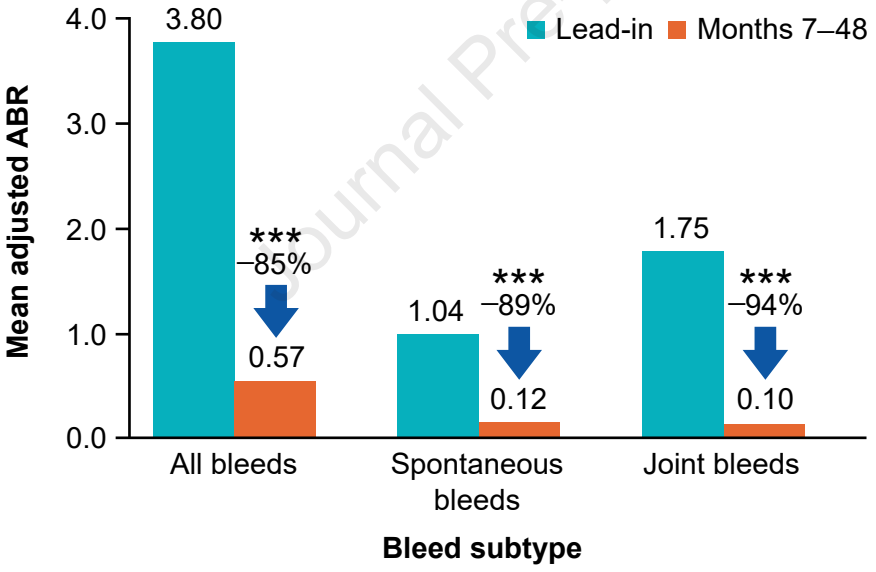

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