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ORIGINAL ARTICLE

Long-term safety and efficacy outcomes of valoctocogene roxaparvovec gene transfer up to 6 years post-treatment

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Abstract

Introduction: Valoctocogene roxaparvovec uses an adeno-associated virus serotype 5 (AAV5) vector to transfer a factor VIII (FVIII) coding sequence to individuals with severe haemophilia A, providing bleeding protection.

Aim: To assess safety and efficacy of valoctocogene roxaparvovec 5–6 years post-treatment.

Methods: In a phase 1/2 trial, adult male participants with severe haemophilia A (FVIII $\leq$1 IU/dL) without FVIII inhibitors or anti-AAV5 antibodies received valoctocogene roxaparvovec and were followed for 6 (6×10^{13} vg/kg; $n = 7$) and 5 (4×10^{13} vg/kg; $n = 6$) years. Safety, including investigation of potential associations between a malignancy and gene therapy, and efficacy are reported.

Results: No new treatment-related safety signals emerged. During year 6, a participant in the 6×10^{13} vg/kg cohort was diagnosed with grade 2 parotid gland acinar cell carcinoma; definitive treatment was uncomplicated parotidectomy with lymph node dissection. Target enrichment sequencing of tumour and adjacent healthy tissue revealed low vector integration (8.25×10^{-5} per diploid cell). Integrations were not elevated in tumour samples, no insertions appeared to drive tumorigenesis, and no clonal expansion of integration-containing cells occurred. During all follow-ups, >90% decreases from baseline in annualised treated bleeds and FVIII infusion rates were maintained. At the end of years 6 and 5, mean FVIII activity (chromogenic assay) was 9.8 IU/dL (median, 5.6 IU/dL) and 7.6 IU/dL (median, 7.1 IU/dL) for the 6×10^{13} and 4×10^{13} vg/kg cohorts, respectively, representing proportionally smaller year-over-year declines than earlier timepoints.

Conclusions: Valoctocogene roxaparvovec safety and efficacy profiles remain largely unchanged; genomic investigations showed no association with a parotid tumour.

KEYWORDS
acinar cell carcinoma, adeno-associated virus, clinical trial, gene therapy, haemophilia, phase 2

1 | INTRODUCTION

Standard of care for severe haemophilia A, a bleeding disorder caused by a deficiency in functional clotting protein factor VIII (FVIII), is regular prophylaxis with exogenous FVIII or the FVIIIa-mimicking antibody emicizumab to prevent bleeding.[1–3] However, bleeding may still occur despite adherence to regular prophylactic therapy.[1,4–9]

Valoctocogene roxaparvovec is a gene therapy for severe haemophilia A using an adeno-associated virus serotype 5 (AAV5) capsid to deliver a B-domain-deleted (BDD) FVIII coding sequence controlled by a hepatocyte-selective promoter through a single infusion; it was granted conditional marketing authorisation by the European Medicines Agency and approval by the US Food and Drug Administration.[10–17] In phase 1/2 and phase 3 clinical trials in adult participants with severe haemophilia A, valoctocogene roxaparvovec provided transgene expression sufficient for haemostasis in most participants, and all participants experienced significantly lower rates of bleeding compared with baseline on FVIII prophylaxis.[12–15,18] Across both studies, the most common adverse events were alanine aminotransferase (ALT) elevations, which occurred early on in the studies and were manageable with immunosuppressants; in the phase 3 trial, infusion-related reactions and steroid-related adverse events were also common.[12–15,18]

As the follow-up from valoctocogene roxaparvovec clinical trials grows, we continue to monitor long-term safety and efficacy. Such data augment risk/benefit evaluations for haemophilia A gene therapy, including malignancy risk. Here, we report updated safety data from the phase 1/2 trial, including the diagnosis and investigation of a serious adverse event of acinar cell carcinoma (AcCC) of the parotid gland that occurred in one 6×10^{13} vg/kg cohort participant during year 6, to determine whether vector integration drove tumorigenesis. Additionally, we examine FVIII expression durability, bleeding rates, and FVIII utilisation in years 6 and 5 from the 6×10^{13} and 4×10^{13} vg/kg cohorts, respectively, representing the longest follow-up from any haemophilia A gene therapy trial.

2 | MATERIALS AND METHODS

2.1 | Study design

Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) is a replication-incompetent AAV5 vector containing an approximately 5-kb genome that includes a BDD FVIII coding sequence controlled by a hybrid human liver-selective promoter with a synthetic polyadenylation sequence and inverted terminal repeats on the 3′ and 5′ ends.[10] Design of this open-label, phase 1/2, dose-escalation study was described previously.[12–14] Eligible participants were males aged ≥18 years with severe haemophilia A (FVIII ≤1 IU/dL) and no history of FVIII inhibitors or anti-AAV5 antibodies. Exclusion criteria included significant liver dysfunction, significant liver fibrosis, and liver cirrhosis. Participants received one intravenous infusion of valoctocogene roxaparvovec: participant 1 received 6×10^{12} vg/kg, participant 2 received 2×10^{13} vg/kg, participants 3–9 received 6×10^{13} vg/kg, and participants 10–15 received 4×10^{13} vg/kg. The protocol has been previously published.[13]

2.2 | Assessments

Safety was assessed for all participants through physical examinations, laboratory assessments, and adverse events. A liver ultrasound was performed at screening, at year-end visits starting year 5, and at the investigator's discretion. Vector DNA shedding in biological fluids was assessed as described previously using validated quantitative polymerase chain reaction until ≥3 consecutive results were negative or below the limit of quantitation (BLQ).[12] For the participant with the AcCC, additional genomic analyses investigating causality were performed. Surgically removed tissues preserved as formalin-fixed paraffin-embedded (FFPE) tissue blocks were sectioned, and DNA was extracted from two sets of physically separated sections in one FFPE block containing visible tumour tissue and another FFPE block containing adjacent healthy tissue with zero to minimal observable tumour content. Haematoxylin and eosin staining and histological review of nearby slide sections confirmed the presence (2%–3% tumour area) or absence (<0.05%) of tumour in each location (Supplemental Methods). Target enrichment sequencing (TES) was performed by ProtaGene CGT GmbH (formerly GeneWerk GmbH) to identify valoctocogene roxaparvovec vector integrations in tumour-containing and healthy tissue. To facilitate repeated detection of clonally enriched integration sites, two independently pooled tumour DNA samples from anatomically separate portions of the tumour and two independently pooled healthy DNA samples were generated. For TES analysis, the DNA from each of the two tumour-containing and two healthy samples was divided and analysed in three technical replicates of 1 μg each; the results were combined and reported for each sample. Additional details are in the Supporting Information.

Annualised bleeding rates (ABRs) and annualised FVIII infusion rates were calculated as described previously; baseline rates were derived from the 12 months prior to enrolment for participants who were using regular FVIII prophylaxis.[12,13] Problem joints were identified at baseline by investigators as those with chronic joint pain, chronic synovitis, haemophilic arthropathy, limited motion, or recurrent bleeding. FVIII activity was assessed using the chromogenic substrate assay (CSA) and one-stage assay (OSA) at a central laboratory as described previously.[12–14] Data were summarised with

TABLE 1 Summary of incidence of AEs in each year by cohort.

	6×10^{13} vg/kg cohort ($n = 7$)						4×10^{13} vg/kg cohort ($n = 6$)				
	Y1	Y2	Y3	Y4	Y5	Y6	Y1	Y2	Y3	Y4	Y5
Any AE	7 (100)	6 (85.7)	7 (100)	7 (100)	7 (100)	4 (57.1)	6 (100)	5 (83.3)	5 (83.3)	4 (66.7)	6 (100)
Any SAE	0	1 (14.3)	1 (14.3)	1 (14.3)	0	1 (14.3)	1 (16.7)	0	1 (16.7)	1 (16.7)	1 (16.7)
Any treatment-related AE	6 (85.7)	1 (14.3)	1 (14.3)	2 (28.6)	0	0	6 (100)	0	0	0	0
Any treatment-related SAE	0	0	0	0	0	0	1 (16.7)[a]	0	0	0	0
AEs of special interest											
ALT elevation[b]	6 (85.7)	0	0	1 (14.3)	1 (14.3)	0	4 (66.7)	0	1 (16.7)	0	0
AEs of liver dysfunction[c]	6 (85.7)	1 (14.3)	0	1 (14.3)	1 (14.3)	0	5 (83.3)	0	1 (16.7)	0	0
Potential Hy's law case	0	0	0	0	0	0	0	0	0	0	0
Infusion-related reactions	3 (42.9)	0	0	0	0	0	4 (66.7)	0	0	0	0
Systemic hypersensitivity	0	0	0	0	0	0	0	0	0	0	0
Anaphylactic or anaphylactoid reactions	0	0	0	0	0	0	0	0	0	0	0
Thromboembolic events	0	0	0	0	0	0	0	0	0	0	0

Note: Data are presented as *n* (%).
Abbreviations: AE, adverse event; ALT, alanine aminotransferase; MedDRA, Medical Dictionary for Regulatory Activities; SAE, serious AE; ULN, upper limit of normal; Y, year.
[a]Pyrexia on study day 2.
[b]Defined as ALT ≥1.5× ULN or ALT ≥1.5× baseline.
[c]Identified with a MedDRA search strategy using the high-level term "liver function analyses."

descriptive statistics; missing data were not imputed. Yearly rate of change in FVIII activity was determined using a linear regression model (FVIII = intercept + slope*week, with random intercept and slope).

3 | RESULTS

3.1 | Participants

All seven participants in the 6×10^{13} vg/kg cohort and six participants in the 4×10^{13} vg/kg cohort remained on study through the 6 and 5 years of follow-up. Baseline characteristics were described previously (>80% of participants in both cohorts were White); all participants were previously using regular FVIII prophylaxis, except 1 in the 6×10^{13} vg/kg cohort who used on-demand FVIII.[12–14]

3.2 | Safety

No new treatment-related safety signals were observed in the past year (Table 1); no treatment-related serious adverse events occurred after year 1. No participant developed FVIII inhibitors. No transient ALT elevations occurred in the year prior to this report, and no participants experienced any long-term sequelae from previous corticosteroid treatment (summarised in Table S1). Two serious adverse events unrelated to valoctocogene roxaparvovec were reported in the past year: an AcCC of the parotid gland in a participant in the 6×10^{13} vg/kg cohort (described below) and exacerbation of existing Crohn's disease in a 4×10^{13} vg/kg cohort participant (Supplemental Results).

A participant in his forties reported an asymptomatic lump on the right side of his neck that had been present for about a year (Figure 1A). He had medical history of haemophilia A, bilateral ankle arthropathy, and hepatitis C virus with no known AcCC risk factors. An adenoma in the tail of the parotid gland was diagnosed by ultrasound. Fine needle aspirate cytology of the parotid gland was consistent with AcCC (Grade 2). The participant underwent right parotidectomy with lymph node dissection in December 2021. There were no FVIII activity assessments near the time of the procedure; the participant received 3000 IU of exogenous FVIII prior to the procedure and 1000–2500 IU per day for 3 days post-procedure. Pathology confirmed the AcCC diagnosis and confirmed clear surgical margins (Figure 1B). Surgery was uncomplicated and considered the definitive treatment. As of the data cutoff date in April 2022, the participant continues to be clinically well.

As part of routine vector shedding monitoring, saliva samples from this participant were analysed for vector DNA. His saliva shedding profile was consistent with other participants in the 6×10^{13} vg/kg cohort (Table S2). The first of three consecutive BLQ samples was observed at 5 weeks, compared with a cohort median (minimum [min], maximum [max]) of 7 (4, 12) weeks. The first of three consecutive negative samples and the third negative sample were observed at 32 and 40 weeks, compared to a group median (min, max) of 36 (27, 44) weeks and 44 (40, 52) weeks, respectively.

Genomic analyses using TES were conducted on DNA extracted from tumour-containing tissue sections and nearby healthy parotid tissue (Figure 1C) to determine if the event was related to valoctoco-

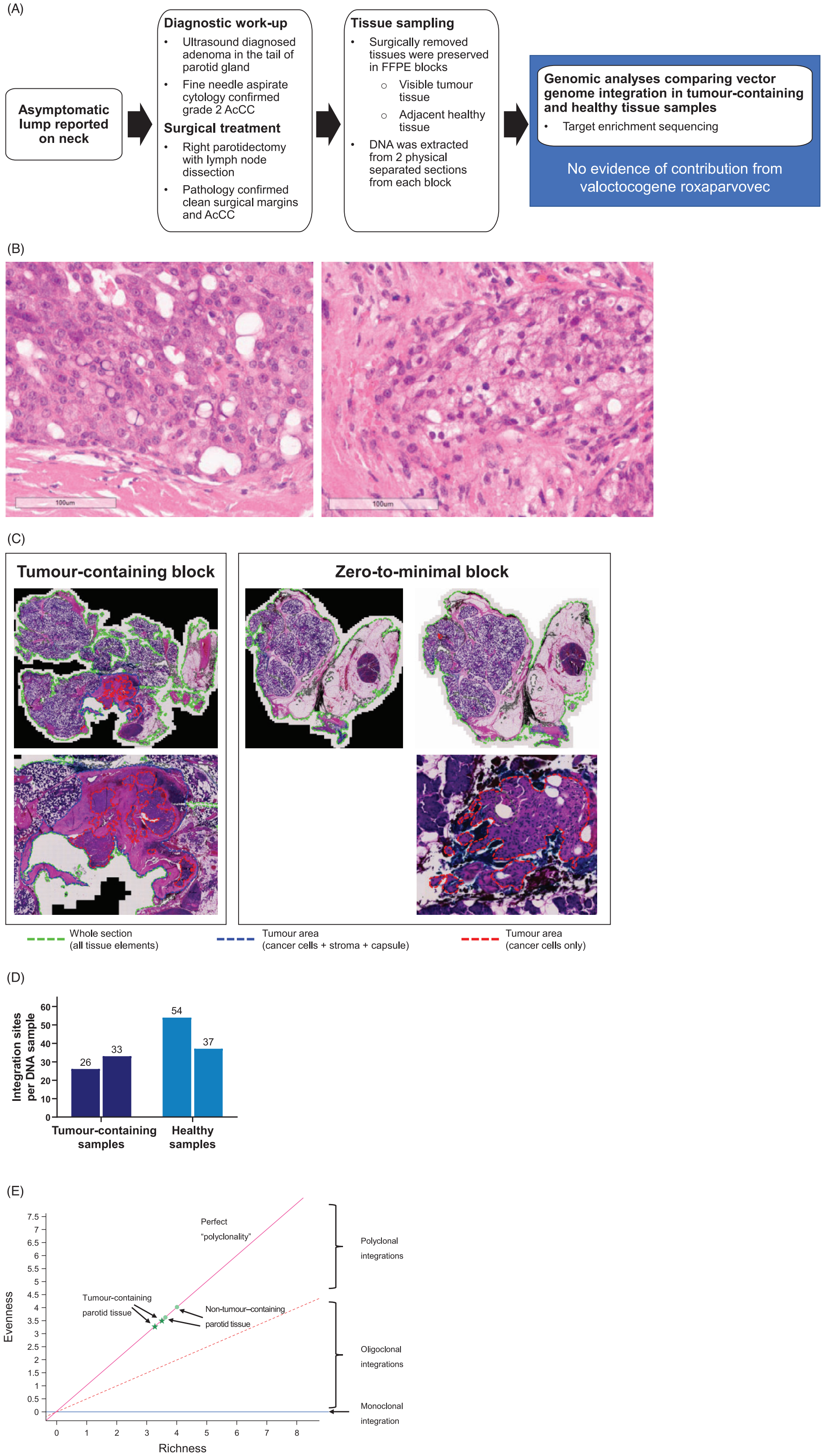


FIGURE 1 (A) Diagnosis of acinar cell carcinoma and investigation workflow. (B) Histopathological features of the approximately 9 × 18 mm nodular tumour of the superficial parotid gland. (C) Representative section from the tumour-containing tissue block and the comparator block of adjacent healthy tissue. (D) Number of integration sites in samples taken from tumour-containing and healthy-adjacent parotid tissue. (E) Richness

gene roxaparvovec integration into human chromosomal DNA. The 2 tumour-containing DNA samples had slightly fewer detected vector integration sites (26 and 33) compared with two healthy parotid DNA samples (37 and 54), indicating no increased integrations in tumour-containing samples (Figure 1D). Using human subgenomic control sequences, average frequency of detected integration sites across all parotid samples was calculated as 8.25×10^{-5} per diploid cell (6.49×10^{-5} across tumour-containing samples; 1.00×10^{-4} across healthy samples). No vector insertions were identical across both tumour-containing samples, which would be expected if the vector had integrated in a tumour-initiating cell that subsequently hyperproliferated. Two integration sites (corresponding to 1.34% of all detected integrations), one each in a cancer-containing and healthy sample, were within 10 kb of the transcription start site of cancer-annotated genes (COSMIC): one at position 6592333 of chromosome 12 within an intron of *CDH4* and another at position 167022891 of chromosome 6 in an intron of *FGFR1OP*.[19] Neither gene is a known driver of AcCC or insertional tumorigenesis in retro- or lentiviral gene therapies.

Clonality of cells harbouring vector integrations in parotid samples was examined graphically using Richness/Evenness plots of Rényi indices (Figure 1E), wherein an increased number of integration sites increases the richness of the data, and evenness describes the relative frequency distribution of sequencing reads for individual integration sites.[20] Any increase in sequencing reads for a single insertion site decreases the corresponding evenness from the 45-degree line of perfect polyclonality, while samples within the lower shaded area would represent oligoclonal samples with one or more hyper-proliferative clones. In the parotid samples, all data fell on the line of perfect polyclonality, indicating no evidence of malignant or pre-malignant outgrowth of cells containing valoctocogene roxaparvovec integrations or of clonally expanded integration sites in either tumour-containing or healthy tissue.

3.3 | Efficacy

Through 5 or 6 years, a sustained reduction from baseline in annualised rates of treated bleeds and FVIII utilisation was maintained. In the 6×10^{13} vg/kg cohort, mean ABR over the entire study period was 0.7 treated bleeds/year (median, 0.0), representing a 96% reduction in ABR from baseline for all seven participants (Figure 2A). Over the entire study period, the mean FVIII infusion rate was 4.5 infusions/year (median, 1.5), representing a 96% reduction from baseline. Participant 6, who had the lowest year 6 FVIII activity in this cohort (1.9 IU/dL), had the highest FVIII use. During year 6, mean ABR and FVIII infusion rates were 0.7 (median, 0.0) treated bleeds/year and 4.5 (median, 3.0) infusions/year. In the past year, three participants reported treated bleeds: participant 4 reported one spontaneous joint bleed in his right ankle (a problem joint); participant 6 reported one spontaneous bleed in his right ankle (a problem joint); and participant 3 reported three traumatic bleeds in problem joints (two in his right ankle and one in his left ankle) that all occurred during the same week while he was working. Investigators and the medical monitor from the sponsoring company discussed returning to prophylaxis with all participants with low FVIII activity levels; to date, all seven participants in this cohort have chosen to remain off prophylaxis.

In the 4×10^{13} vg/kg cohort, mean ABR over the entire study period was 1.1 treated bleeds/year (median, 0.6), a 91% reduction in ABR from baseline for all six participants (Figure 2B). Over the entire study period, the mean FVIII infusion rate was 9.6 (median, 4.2) infusions/year, a 93% reduction from baseline. During year 5, mean ABR was 0.7 (median, 1.0) treated bleeds/year, similar to previous years, and mean FVIII infusion rate increased from previous years to 22.5 (median, 18.9) infusions/year. In the past year, four participants reported treated bleeds: participant 15 reported a spontaneous bleed in his right ankle (problem joint); participant 11 reported a spontaneous bleed in his right ankle (problem joint); participant 12 reported spontaneous stool bleeding related to Crohn's disease; and participant 14 reported a traumatic bleed in his toe. As with the 6×10^{13} vg/kg cohort, return to prophylaxis was discussed with all participants with low FVIII levels. Participant 15, who had the lowest FVIII expression in this cohort, temporarily returned to prophylaxis on week 238 after bleeds in his ankle; however, he found adherence to a regular regime difficult and switched to on-demand or targeted prophylaxis after 1 month. In the 20 weeks between his switch to on-demand FVIII and the data cutoff date, he had no need for FVIII infusions. To date, the five other participants have chosen to remain off prophylaxis. The observed increased FVIII infusion rate in year 5 was driven primarily by the participant diagnosed with Crohn's disease in year 4, who was frequently using intermittent prophylaxis, and the participant who temporarily resumed prophylaxis.

and evenness of vector integrations to assess potential clonal expansion. Microscopically, the tumour was surrounded and dissected by a thick, paucicellular fibrotic capsule and stroma, which dissected between generally solid or microcystic islands, and strands of neoplastic cells, which were infiltrative to the surrounding stroma. Neoplastic cells displayed a typical acinar morphology with a finely granular, eosinophilic cytoplasm with some areas of cytoplasmic clearing, round to ovoid nuclei, stippled chromatin and small nucleoli. Mitotic figures were infrequent with a low proliferative index (1%–2% based on MIB-1/Ki-67 immunohistochemistry). Lymphovascular, perineural, or extra-parotid invasion was not evident. For each of the two samples from tumour-containing or healthy tissue, data were pooled from three technical replicates of 1 μg each. For all four samples, the total vector copy number per cell was 0.5. In panel C, the total section area is outlined in green, and magnified portions are shown below when applicable. In the tumour block, the surrounding fibrous capsule is outlined in blue, and the tumour cell area is outlined in red; the tumour cell area represents 2.6% of the section area in this block. The two comparator sections, sandwiching the comparator DNA sections, have either no visible tumour (left) or approximately 0.04% of the section area as tumour without obvious fibrous capsule (right). In panel (E), richness is a measure of how many unique integrations were detected, and evenness is a measure for the relative frequency distribution of sequencing reads for detected integrations. AcCC, acinar cell carcinoma; FFPE, formalin-fixed paraffin-embedded.

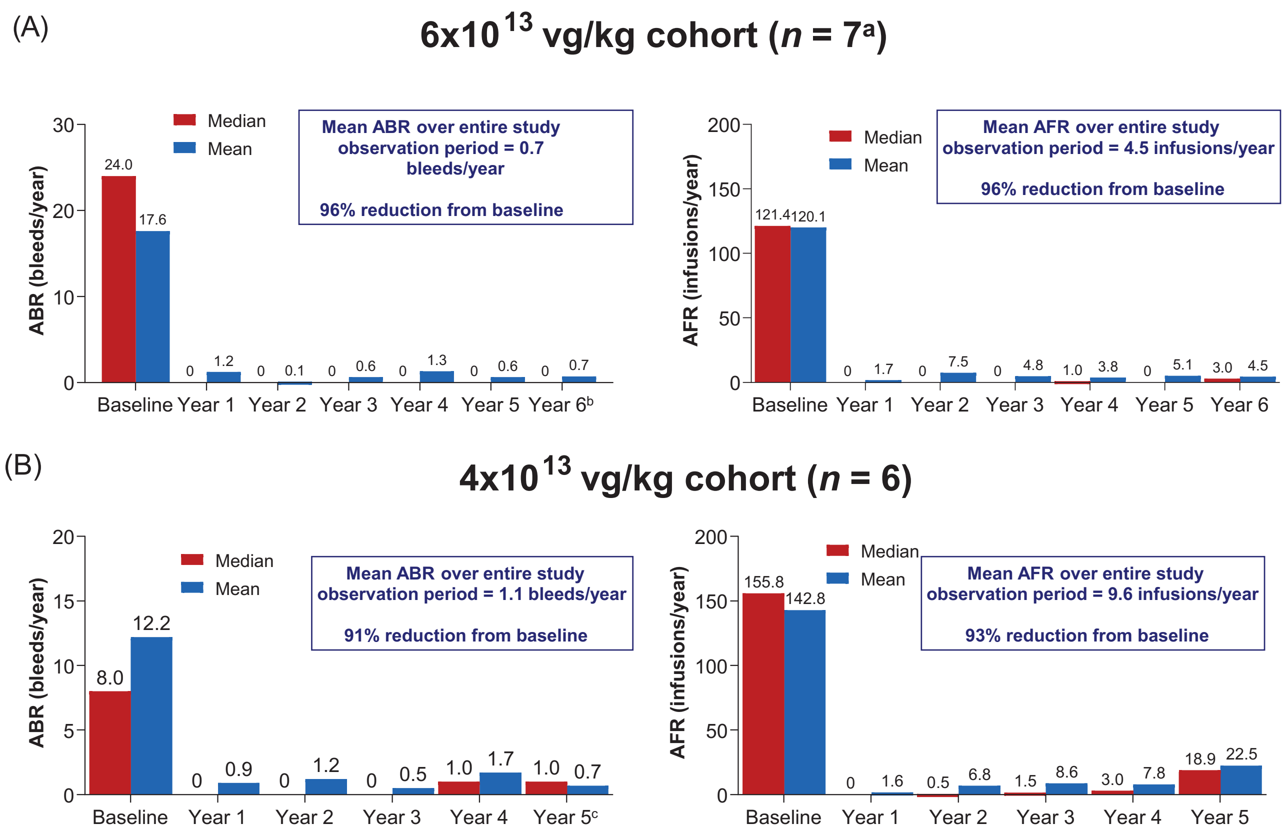


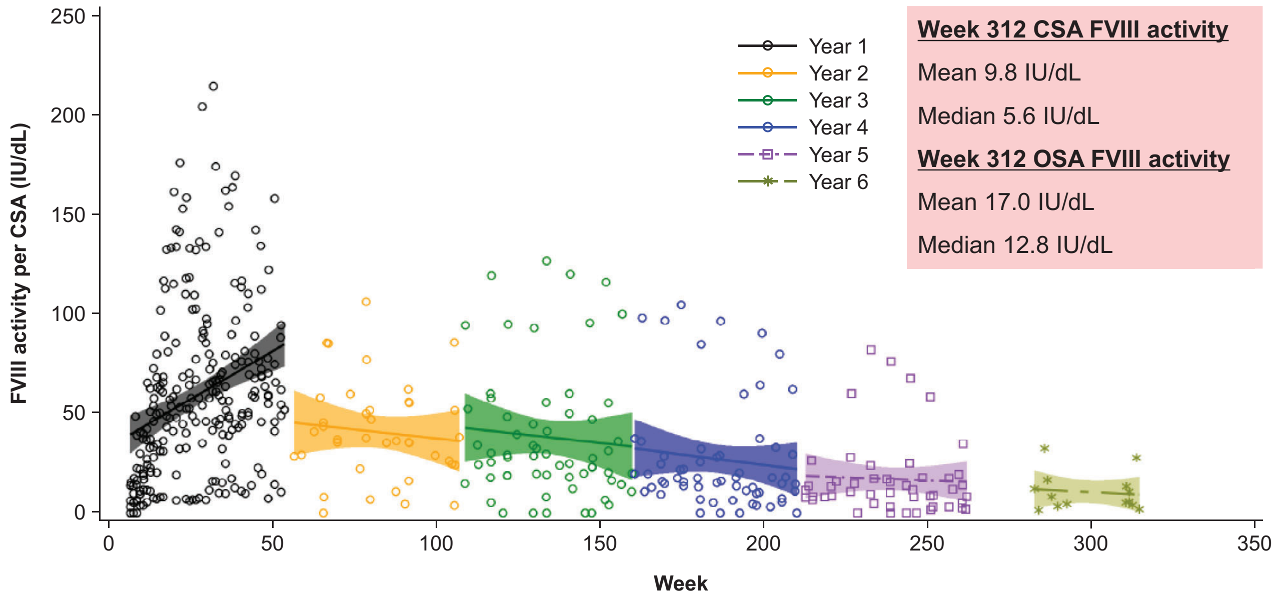
FIGURE 2 Annualised rates of treated bleeding and FVIII infusion per year of follow-up for the (A) 6×10^{13} vg/kg cohort and (B) 4×10^{13} vg/kg cohort. [a]When excluding the participant who was not receiving prophylaxis at baseline ($n = 6$), baseline ABR mean and median were 16.3 and 16.5 bleeds/year, and the mean ABR over the entire study was 0.8 bleeds/year, representing a 95% decrease from baseline. For these six participants, mean and median AFR at baseline were 135.6 infusions/year and 136.5 infusions/year, respectively, and the mean AFR over the entire study period was 5.0 infusions/year, representing a 96% reduction from baseline. [b]In year 6, three participants reported treated bleeds: participant 4 reported one spontaneous bleed in a problem joint (right ankle joint; nearest FVIII measurement to the time of the bleed was 4 IU/dL per CSA, 7.1 IU/dL per OSA); participant 6 reported one spontaneous bleed in a problem joint (right ankle joint; FVIII, 1.5 IU/dL per CSA, 2.6 IU/dL per OSA); participant 3 reported three traumatic bleeds in problem joints (two in right ankle joint, one in left ankle joint; from moving boxes and standing at work; all occurred during the same week; FVIII, 3.4 IU/dL per CSA, 5.6 IU/dL per OSA). [c]In year 5, four participants reported treated bleeds: participant 15 reported a spontaneous bleed in a problem joint (right ankle joint; closest FVIII activity measurement to the time of the bleed was 1.6 IU/dL per CSA, 2.6 IU/dL per OSA using last observation carried forward imputation); participant 11 reported a spontaneous bleed in a problem joint (right ankle joint; FVIII, 9 IU/dL per CSA, 15.2 IU/dL per OSA); participant 12 reported a spontaneous bleed (bleeding in stool related to Crohn's disease; FVIII, 5 IU/dL per CSA, 6.8 IU/dL per OSA); participant 14 reported a traumatic bleed (toe; from hitting it on the bath and breaking it; FVIII, 18.0 IU/dL per CSA, 26.7 IU/dL per OSA). Problem joints were identified at baseline by investigators as those with chronic joint pain, chronic synovitis, haemophilic arthropathy, limited motion, or recurrent bleeding. FVIII activity is the closest reported valid result to the time of the bleed. ABR, annualised bleeding rate; AFR, annualised FVIII infusion rate; CSA, chromogenic substrate assay; FVIII, factor VIII; OSA, one-stage assay.

Mean and median FVIII activity were maintained at levels consistent with mild haemophilia (Figure 3): for the 6×10^{13} vg/kg cohort, mean FVIII activity (per CSA) at the end of year 6 was 9.8 IU/dL (median, 5.6 IU/dL), and for the 4×10^{13} vg/kg cohort, mean FVIII activity (by CSA) at the end of year 5 was 7.6 IU/dL (median, 7.1 IU/dL). In both cohorts, the yearly rate of decline of FVIII was slower than observed previously; estimated change in FVIII activity per CSA was −0.06 IU/dL/week for the 6×10^{13} vg/kg cohort in year 6 and −0.01 IU/dL/week for the 4×10^{13} vg/kg cohort in year 5. A similar pattern of slowing decline was observed in individual FVIII trajectories (Figure 4). At the end of the most recent year of follow-up, five of seven participants in the 6×10^{13} vg/kg cohort and three of six participants in the 4×10^{13} vg/kg cohort had FVIII activity per CSA in the mild haemophilia range (>5–40 IU/dL), two participants in each cohort had FVIII activity in the moderate haemophilia range (1.5 [lower limit of quantitation]–5 IU/dL), and one participant in the 4×10^{13} vg/kg cohort had FVIII <1.5 IU/dL (Figure S1).

4 | DISCUSSION

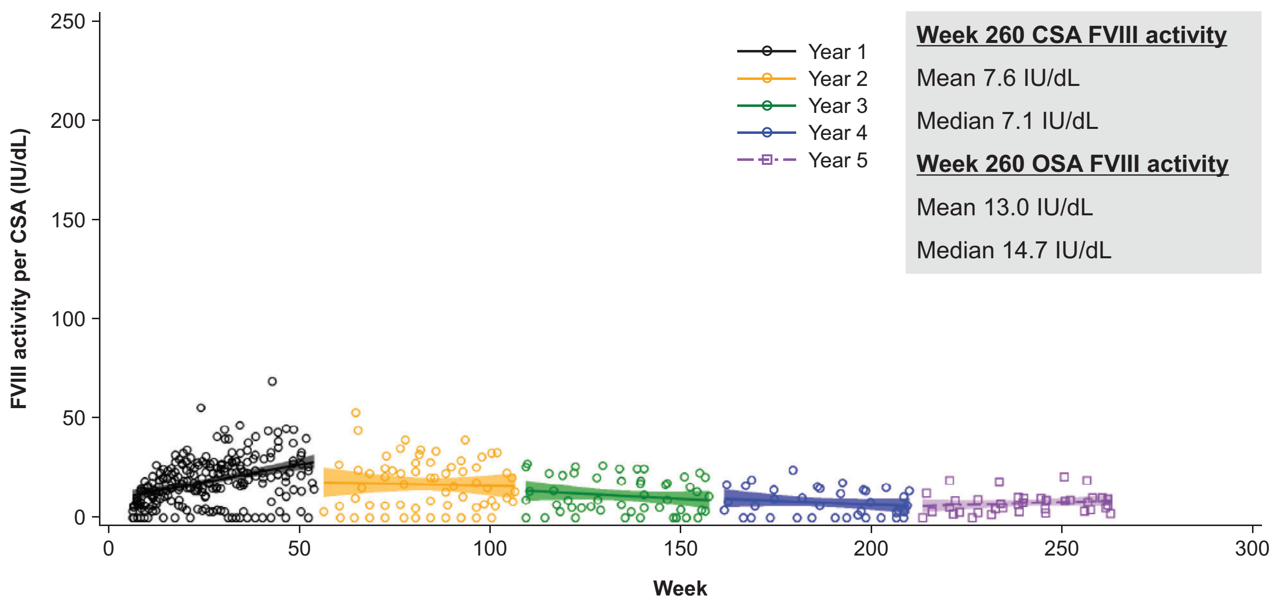
These 6-year data from seven participants in the 6×10^{13} vg/kg cohort are the longest follow-up from any gene therapy trial for haemophilia A, further augmented by 5-year data from the 4×10^{13} vg/kg cohort.

(A)



	Y1	Y2	Y3	Y4	Y5	Y6
Slope, IU/dL/wk (95% CI)	1.01 (−0.04, 2.06)	−0.24 (−0.68, 0.21)	−0.15 (−0.47, 0.16)	−0.26 (−0.49, −0.03)	−0.13 (−0.31, 0.04)	−0.06 (−0.20, 0.08)

(B)



	Y1	Y2	Y3	Y4	Y5
Slope, IU/dL/wk (95% CI)	0.35 (−0.01, 0.71)	−0.15 (−0.37, 0.07)	−0.08 (−0.17, 0.02)	−0.05 (−0.13, 0.02)	−0.01 (−0.08, 0.07)

FIGURE 3 FVIII activity over (A) 6 years for the 6×10^{13} vg/kg cohort ($n = 7$) and (B) 5 years for the 4×10^{13} vg/kg cohort ($n = 6$). Missing data were not imputed. Slope (95% CI) are for FVIII activity per CSA. CI, confidence interval; CSA, chromogenic substrate assay; FVIII, factor VIII; OSA, one-stage assay; Y, year.

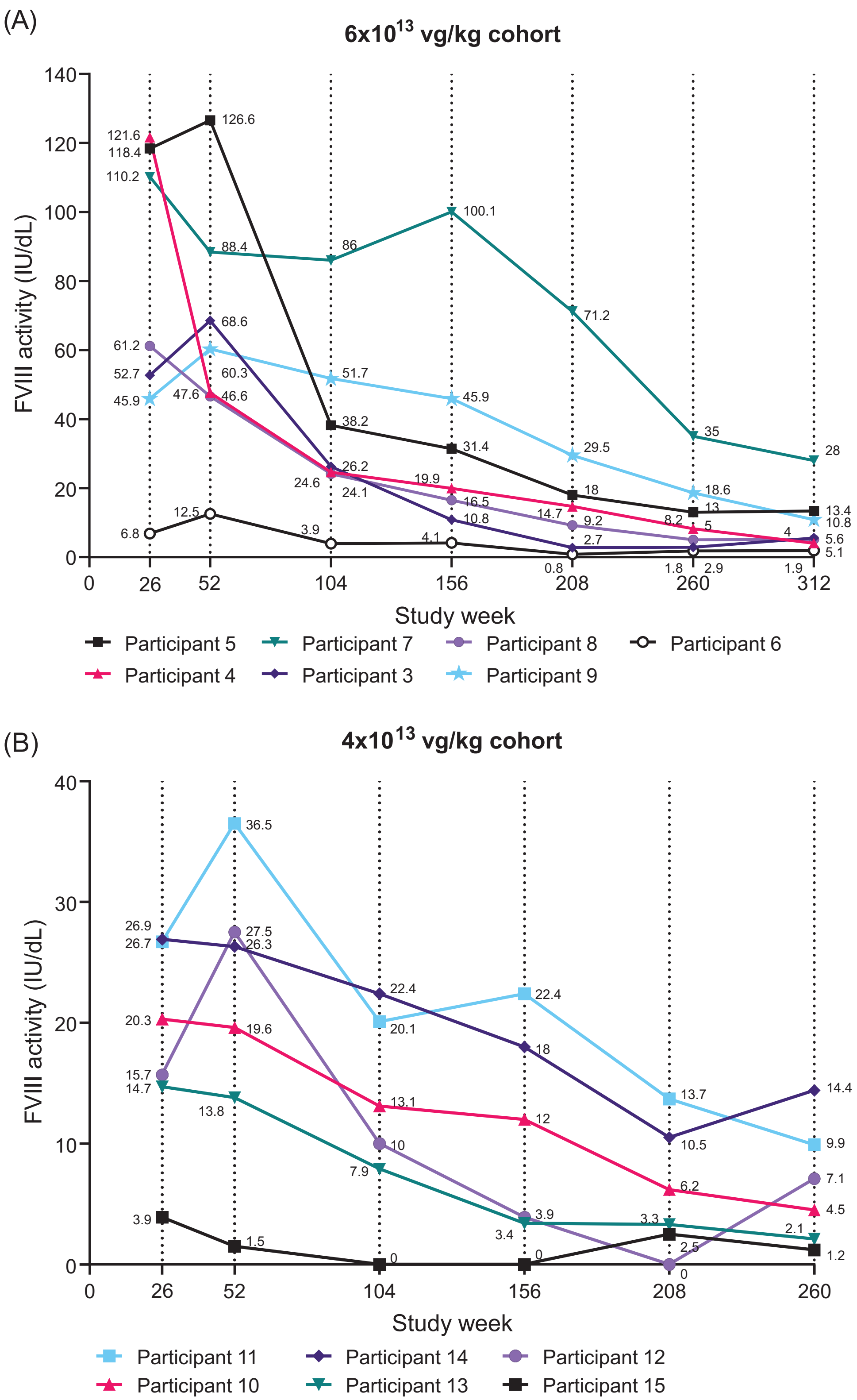


FIGURE 4 Individual FVIII activity per CSA for each participant over (A) 6 years for the 6×10^{13} vg/kg cohort ($n = 7$) and (B) 5 years for the 4×10^{13} vg/kg cohort ($n = 6$). CSA, chromogenic substrate assay; FVIII, factor VIII.

The valoctocogene roxaparvovec safety profile remains tolerable and unchanged from previous reports; genomic investigation determined the AcCC event was unrelated to valoctocogene roxaparvovec. No participants developed FVIII inhibitors through 6 years. All seven participants in the 6×10^{13} vg/kg cohort remain off prophylaxis, suggesting haemostatic efficacy is largely maintained, even for those with low levels of expressed FVIII. In the 4×10^{13} vg/kg cohort, one participant resumed prophylaxis for a 4.5-week period. The rate of decline in FVIII expression was more attenuated than observed in prior years. Mechanisms contributing to FVIII decline are under investigation; sustained hepatocytic transduction suggests involvement of various intracellular or immune responses.[11,21] However, lower expressed FVIII levels appear to provide haemostatic benefit consistent with moderate or mild haemophilia, as observed in the phase 3 trial.[15,22] Resuming prophylaxis was discussed with participants with low FVIII levels and psychological counselling was offered when appropriate.

In the most recent year of follow-up, no ALT elevations occurred. The most common adverse event in this study remains mild, transient, asymptomatic ALT elevations that occurred in year 1 shortly after valoctocogene roxaparvovec treatment.[12–14] In response to these ALT elevations, tapering regimens of corticosteroids were used as previously described.[12–14] Although small sample size precluded formal analysis, transient year 1 ALT elevations did not appear to impact long-term FVIII expression. No long-term sequelae or corticosteroid dependency resulted.

This is the first detailed description of processes employed following report of tumourigenesis in a patient receiving AAV gene therapy for haemophilia A. While the methodological strategy (detection of vector integration sites by TES) and data analyses used here were suitable for biopsy samples with a relatively low tumour content (2%–3%), they may be adapted for future adverse events. For example, future samples may allow enrichment of tumour cells to higher purity, which could facilitate non-targeted whole genome sequencing and/or diagnostic deep sequencing of cancer genes to identify vector integration sites and/or alternative causes of tumorigenesis independent of gene therapy treatment. For this AcCC parotid gland case, genomic analyses of resected parotid tissue did not establish any causal association with valoctocogene roxaparvovec vector integrations. We note that AcCC does not currently have a profile of established genetic mutations that can be specifically evaluated.[23]

The average number of integration sites per diploid cell across all parotid samples was >1 log lower than livers of mice and dogs treated with similar AAV5 vectors, as expected given the parotid gland is a non-target tissue with generally lower vector tropism.[21,24] Furthermore, the liver-selective valoctocogene roxaparvovec promoter is unlikely to ectopically activate proto-oncogenes in parotid tissue. Various AAV gene therapies have been administered to >3000 patients in >100 clinical trials for >20 years, in addition to patients on commercial therapy (e.g., >3000 patients treated with onasemnogene abeparvovec), with no cancers attributable to treatment to date.[25–27] A participant treated with etranacogene dezaparvovec for haemophilia B was diagnosed with hepatocellular carcinoma, which was not found to be associated with treatment.[28] Integration has been observed in humans, non-human primates, and dogs with no reports of insertional oncogenesis, although sample sizes of humans and large animals are low with short follow-ups.[24,29,30] Conversely, several studies have observed insertional oncogenesis in mice, although findings are inconsistent.[31–35] No cancers related to valoctocogene roxaparvovec have occurred in any clinical or non-clinical studies.[10,12–15,18,36,37] No clinical hold was imposed on the valoctocogene roxaparvovec clinical development programme due to the AcCC event.

Endoplasmic reticulum and cellular stress induction can be associated with high BDD FVIII expression driven by strong promoters.[38,39] Recently, it was suggested that misfolded BDD FVIII protein produced from transgenes in hepatocytes of mice on high-fat diets can cause hepatocellular carcinoma, independent of vector integration.[40] Mouse and human liver biopsies showed no valoctocogene roxaparvovec–dependent hepatocytic endoplasmic reticulum stress markers, possibly due to moderate expression compared to other vectors.[11,38]

Given elevated cancer rates in people with haemophilia relative to the general population, close monitoring of individuals with haemophilia following AAV gene therapy will undoubtedly lead to future reports of cancers.[41–43] A clear protocol to diagnose and investigate the causality of such cancers will be important. While we have yet to fully identify a comprehensive, time-, and cost-efficient approach for analysing tumours that develop after gene therapy, we report our initial experience for transparency. As valoctocogene roxaparvovec and other haemophilia AAV gene therapies are commercialised, it is important and responsible to provide timely updates as data evolve and mature. Long-term follow-up and monitoring of people with haemophilia who have received gene therapy (e.g., in the World Federation of Haemophilia Gene Therapy Registry) will also be critical.[44]

5 | CONCLUSION

The haemostatic efficacy and safety profile of valoctocogene roxaparvovec was largely maintained; the AcCC event was likely unrelated to treatment.

AUTHOR CONTRIBUTIONS

Emily Symington, Savita Rangarajan, Will Lester, Bella Madan, Priyanka Raheja, and Michael Laffan carried out the clinical study. Glenn F. Pierce and Wing Yen Wong contributed to the conception and design of the study. Tara M. Robinson and Dane Osmond oversaw conduct of the study; Tara M. Robinson was the medical monitor. Chris B. Russell, Christian Vettermann, and Suresh K. Agarwal analysed and interpreted data. Mingjin Li performed statistical analyses. All authors critically reviewed the manuscript, provided input on data interpretation, and approved the final draft for submission.

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CONFLICT OF INTEREST STATEMENT

Emily Symington received funding from CSL Behring and Novo Nordisk to attend conferences. Savita Rangarajan received travel support from Reliance Life Sciences and Shire/Takeda; consulting payments from Reliance Life Sciences; and has attended advisory boards for Pfizer, Sanofi, and Shire/Takeda. Will Lester received grants from BioMarin Pharmaceutical Inc.; personal fees from Bayer, LFB Biopharmaceuticals, Novo Nordisk, Sobi, and Takeda; and travel support from Takeda and CSL. Glenn F. Pierce received consulting payments from BioMarin Pharmaceutical Inc., Decibel Therapeutics, Frontera, Generation Bio, Regeneron, Spark, and Third Rock Ventures; and is a board member of the World Federation of Hemophilia, Voyager Therapeutics, VermX, and the Medical and Scientific Advisory Council of the US National Hemophilia Foundation. Priyanka Raheja has received grant/travel support from CSL Behring, Sobi and Takeda and advisory honoraria from Idogen, Sigilon, Sobi, and Pfizer. Tara M. Robinson, Dane Osmond, Chris B. Russell, Christian Vettermann, Suresh K. Agarwal, Mingjin Li, and Wing Yen Wong are employees and shareholders of BioMarin Pharmaceutical Inc. Michael Laffan received grants from BioMarin Pharmaceutical Inc.; personal fees from Bayer, LEO Pharma, LFB Biopharmaceuticals, Pfizer, Roche, Shire, and Sobi; and travel support from Bayer, LFB Biopharmaceuticals, and Sobi. Bella Madan has no conflicts to disclose.

DATA AVAILABILITY STATEMENT

The de-identified 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 non-commercial 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 programme 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.

ETHICS STATEMENT

Procedures were performed in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines; participants provided written informed consent.

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REFERENCES

1. Srivastava A, Santagostino E, Dougall A, et al. WFH guidelines for the management of hemophilia. *Haemophilia*. 2020;26(suppl 6):1-158.
2. van Vulpen LFD, Holstein K, Martinoli C. Joint disease in haemophilia: pathophysiology, pain and imaging. *Haemophilia*. 2018;24(suppl 6):44-49.
3. Witkop M, Guelcher C, Forsyth A, et al. Treatment outcomes, quality of life, and impact of hemophilia on young adults (aged 18–30 years) with hemophilia. *Am J Hematol*. 2015;90(suppl 2):S3-10.
4. Mahlangu J, Oldenburg J, Paz-Priel I, et al. Emicizumab prophylaxis in patients who have hemophilia A without inhibitors. *N Engl J Med*. 2018;379(9):811-822.
5. Callaghan MU, Negrier C, Paz-Priel I, et al. Long-term outcomes with emicizumab prophylaxis for hemophilia A with or without FVIII inhibitors from the HAVEN 1–4 studies. *Blood*. 2021;137(16):2231-2242.
6. Kenet G, Chen YC, Lowe G, et al. Real-world rates of bleeding, factor VIII use, and quality of life in individuals with severe haemophilia A receiving prophylaxis in a prospective, noninterventional study. *J Clin Med*. 2021;10(24):5959.
7. Furlan R, Krishnan S, Vietri J. Patient and parent preferences for characteristics of prophylactic treatment in hemophilia. *Patient Prefer Adherence*. 2015;9:1687-1694.
8. Park YS, Hwang TJ, Cho GJ, et al. Patients' and parents' satisfaction with, and preference for, haemophilia A treatments: a cross-sectional, multicentre, observational study. *Haemophilia*. 2021;27(4):563-573.
9. van Balen EC, Wesselo ML, Baker BL, et al. Patient perspectives on novel treatments in haemophilia: a qualitative study. *Patient*. 2020;13(2):201-210.
10. Bunting S, Zhang L, Xie L, et al. Gene therapy with BMN 270 results in therapeutic levels of FVIII in mice and primates and normalization of bleeding in hemophilic mice. *Mol Ther*. 2018;26(2):496-509.
11. Fong S, Yates B, Sihn CR, et al. Interindividual variability in transgene mRNA and protein production following adeno-associated virus gene therapy for hemophilia A. *Nat Med*. 2022;28(4):789-797.
12. Rangarajan S, Walsh L, Lester W, et al. AAV5-factor VIII gene transfer in severe hemophilia A. *N Engl J Med*. 2017;377(26):2519-2530.
13. Pasi KJ, Rangarajan S, Mitchell N, et al. Multiyear follow-up of AAV5-hFVIII-SQ gene therapy for hemophilia A. *N Engl J Med*. 2020;382(1):29-40.
14. Pasi KJ, Laffan M, Rangarajan S, et al. Persistence of haemostatic response following gene therapy with valoctocogene roxaparvovec in severe haemophilia A. *Haemophilia*. 2021;27(6):947-956.
15. Ozelo MC, Mahlangu J, Pasi KJ, et al. Valoctocogene roxaparvovec gene therapy for hemophilia A. *N Engl J Med*. 2022;386(11):1013-1025.

16. *First gene therapy for adults with severe hemophilia A, BioMarin's ROCTAVIAN™ (valoctocogene roxaparvovec), approved by European Commission (EC) [press release]*. CA: BioMarin Pharmaceutical Inc.; 2022. https://investors.biomarin.com/2022-08-24-First-Gene-Therapy-for-Adults-with-Severe-Hemophilia-A,-BioMarins-ROCTAVIAN-TM-valoctocogene-roxaparvovec-,-Approved-by-European-Commission-EC
17. *FDA Approves BioMarin's Gene Therapy for a Genetic Bleeding Disorder [press release]*. BioMarin Pharmaceutical Inc.; 2023. https://www.biomarin.com/blog/fda-approves-biomarins-gene-therapy-for-a-genetic-bleeding-disorder/
18. Mahlangu J, Kaczmarek R, von Drygalski A, et al. Two-year outcomes of valoctocogene roxaparvovec therapy in hemophilia A. *N Engl J Med*. 2022;388:694-705.
19. Tate JG, Bamford S, Jubb HC, et al. COSMIC: the catalogue of somatic mutations in cancer. *Nucl Acids Res*. 2019;47(D1):D941-D947.
20. Afzal S, Gil-Farina I, Gabriel R, et al. Systematic comparative study of computational methods for T-cell receptor sequencing data analysis. *Brief Bioinform*. 2019;20(1):222-234.
21. Handyside B, Ismail AM, Zhang L, et al. Vector genome loss and epigenetic modifications mediate decline in transgene expression of AAV5 vectors produced in mammalian and insect cells. *Mol Ther*. 2022;30(12):3570-3586.
22. Mahlangu J, Kaczmarek R, von Drygalski A, et al. Two-year outcomes of valoctocogene roxaparvovec therapy for hemophilia A. *N Engl J Med*. 2023;388(8):694-705.
23. Grunewald I, Vollbrecht C, Meinrath J, et al. Targeted next generation sequencing of parotid gland cancer uncovers genetic heterogeneity. *Oncotarget*. 2015;6(20):18224-18237.
24. Batty P, Fong S, Franco M, et al. Frequency, location and nature of AAV vector insertions after long-term follow up of FVIII transgene delivery in a hemophilia A dog model. *Res Pract Thomb Haemost*. 2020;4(suppl 1):550.
25. Kuzmin DA, Shutova MV, Johnston NR, et al. The clinical landscape for AAV gene therapies. *Nat Rev Drug Discov*. 2021;20(3):173-174.
26. Mullard A. Gene therapy community grapples with toxicity issues, as pipeline matures. *Nat Rev Drug Discov*. 2021;20(11):804-805.
27. Novartis. Novartis shares Zolgensma long-term data demonstrating sustained durability up to 7.5 years post-dosing; 100% achievement of all assessed milestones in children treated prior to SMA symptom onset. 2023; Accessed June 15, 2023 https://www.novartis.com/news/media-releases/novartis-shares-zolgensma-long-term-data-demonstrating-sustained-durability-75-years-post-dosing-100-achievement-all-assessed-milestones-children-treated-prior-sma-symptom-onset
28. Schmidt M, Foster GR, Coppens M, et al. Molecular evaluation and vector integration analysis of HCC complicating AAV gene therapy for hemophilia B. *Blood Adv*. 2023;7(17):4966-4969.
29. Gil-Farina I, Fronza R, Kaeppel C, et al. Recombinant AAV integration is not associated with hepatic genotoxicity in nonhuman primates and patients. *Mol Ther*. 2016;24(6):1100-1105.
30. Nguyen GN, Everett JK, Kafle S, et al. A long-term study of AAV gene therapy in dogs with hemophilia A identifies clonal expansions of transduced liver cells. *Nat Biotechnol*. 2021;39(1):47-55.
31. Bell P, Moscioni AD, McCarter RJ, et al. Analysis of tumors arising in male B6C3F1 mice with and without AAV vector delivery to liver. *Mol Ther*. 2006;14(1):34-44.
32. Bell P, Wang L, Lebherz C, et al. No evidence for tumorigenesis of AAV vectors in a large-scale study in mice. *Mol Ther*. 2005;12(2):299-306.
33. Donsante A, Miller DG, Li Y, et al. AAV vector integration sites in mouse hepatocellular carcinoma. *Science*. 2007;317(5837):477.
34. Donsante A, Vogler C, Muzyczka N, et al. Observed incidence of tumorigenesis in long-term rodent studies of rAAV vectors. *Gene Ther*. 2001;8(17):1343-1346.
35. Chandler RJ, Sands MS, Venditti CP. Recombinant adeno-associated viral integration and genotoxicity: insights from animal models. *Hum Gene Ther*. 2017;28(4):314-322.
36. Sihn CR, Handyside B, Liu S, et al. Molecular analysis of AAV5-hFVIII-SQ vector-genome-processing kinetics in transduced mouse and nonhuman primate livers. *Mol Ther Methods Clin Dev*. 2022;24:142-153.
37. Zhang L, Yates B, Murphy R, et al. Young mice administered adult doses of AAV5-hFVIII-SQ achieve therapeutic factor VIII expression into adulthood. *Mol Ther Methods Clin Dev*. 2022;26:519-531.
38. Fong S, Handyside B, Sihn CR, et al. Induction of ER stress by an AAV5 BDD FVIII construct is dependent on the strength of the hepatic-specific promoter. *Mol Ther Methods Clin Dev*. 2020;18:620-630.
39. Malhotra JD, Miao H, Zhang K, et al. Antioxidants reduce endoplasmic reticulum stress and improve protein secretion. *Proc Natl Acad Sci USA*. 2008;105(47):18525-18530.
40. Kapelanski-Lamoureux A, Chen Z, Gao ZH, et al. Ectopic clotting factor VIII expression and misfolding in hepatocytes as a cause for hepatocellular carcinoma. *Mol Ther*. 2022;30(12):3542-3551.
41. Dunn AL, Austin H, Soucie JM. Prevalence of malignancies among U.S. male patients with haemophilia: a review of the Haemophilia Surveillance System. *Haemophilia*. 2012;18(4):532-539.
42. Liu CJ, Yu YB, Teng CJ, et al. Increased cancer risk in patients with haemophilia A: a nationwide population-based 14-year study in Taiwan. *Haemophilia*. 2014;20(6):741-746.
43. Lovdahl S, Henriksson KM, Baghaei F, Holmstrom M, Berntorp E, Astermark J. Malignancies in Swedish persons with haemophilia: a longitudinal registry study. *Blood Coagul Fibrinolysis*. 2016;27(6):631-636.
44. Konkle B, Pierce G, Coffin D, et al. Core data set on safety, efficacy, and durability of hemophilia gene therapy for a global registry: communication from the SSC of the ISTH. *J Thromb Haemost*. 2020;18(11):3074-3077.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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