Phase 1/2 Trial of a Novel AAVS3 Gene Therapy in Patients with Hemophilia B

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ABSTRACT

Background: FLT180a (verbrinacogene setparvovec) is a liver-directed adeno-associated virus (AAV) gene therapy that uses a synthetic capsid and a gain-of-function protein and aims to normalize factor IX (FIX) levels in patients with hemophilia B.

Methods: B-AMAZE was a multicenter, open-label, Phase 1/2 trial to assess safety and efficacy of varying doses of FLT180a in patients with hemophilia B (FIX ≤2%). All patients received glucocorticoids ± tacrolimus for immunosuppression to decrease the risk of vector-related immune responses. After 26 weeks, patients enrolled in a long-term follow-up study.

Results: Ten patients received one of four FLT180a doses: $3.84x10^{11}$, $6.4x10^{11}$, $8.32x10^{11}$, or $1.28x10^{12}$ vg/kg with dose-dependent increases in FIX levels. At a median follow up of 27.2 months (range, 19.1 to 42.4), sustained FIX activity was observed in all patients except one, who resumed FIX prophylaxis. As of a September 2021 data cut, five patients had normal FIX levels (range, 51 to 78%), three patients had levels from 23 to 43%, and one high-dose patient was at 260%. Approximately 10% and 24% of adverse events were related to FLT180a and immunosuppression, respectively. Increases in liver transaminases were the most common FLT180a-related adverse events. Late increases in transaminases occurred in patients who received prolonged tacrolimus beyond the steroid taper. A serious adverse event of arteriovenous fistula thrombosis occurred in the patient with high FIX levels.

Conclusions: Sustained FIX levels in the normal range were achieved with low FLT180a doses but required immunosuppression with glucocorticoids ± tacrolimus.

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Introduction

Hemophilia B is an X-linked, inherited bleeding disorder caused by mutations in the *F9* gene leading to decreased production of functional factor IX (FIX) protein.[1,2] Insufficient FIX results in a bleeding tendency that classically involves musculoskeletal tissues but can impact other tissues and critical organs. Severe hemophilia B is characterized by FIX levels of <1% of normal (<1 IU/dL). The standard of care for severe hemophilia B is lifelong FIX prophylaxis through regular intravenous (IV) infusions of FIX concentrate.[2] Extended half-life factor replacement therapies have been a major advance in the past decade, enabling less frequent dosing and maintenance of higher trough FIX levels.[2-4] However, factor prophylaxis remains an invasive, expensive, and burdensome life-long treatment approach that cannot completely prevent chronic complications.[5,6]

Adeno-associated virus (AAV) gene therapy is a promising treatment approach for hemophilia B, with multiple reports of FIX production in patients following a single vector infusion, albeit typically at levels below the normal range of 50-150%.[7-13] Remaining challenges for the development of AAV gene therapies in hemophilia B include generating sufficient FIX levels to normalize hemostasis and managing vector-related increases in liver transaminases that can lead to loss of transgene expression. Predictable and stable expression of FIX in the normal range would be expected to provide protection from bleeding even in situations that necessitate intensive FIX replacement, such as serious trauma and surgery.

FLT180a (verbrinacogene setparvovec) is a liver-directed AAV gene therapy in development for the treatment of hemophilia B. FLT180a consists of a synthetic capsid (AAVS3) constructed by rational design to transduce significantly more liver cells than other currently used natural serotypes (AAV5, AAV8).[14] The expression cassette encodes a FIX variant with a gain-of-function 'Padua' mutation (R338L) that has approximately eight-fold higher specific activity than wild-type FIX.[15-17] Here we report the safety and

efficacy of FLT180a in patients with severe or moderately severe hemophilia B from the first-in-human clinical trial (B-AMAZE; UCL 15/0552) and preliminary data from a long-term follow-up study. In addition to FLT180a, patients received prophylactic immunosuppression intended to mitigate vector-related immune responses, improve predictability of FIX expression, and preserve expression of transgenic FIX. The goal of this treatment approach is sustained FIX expression in the normal range.

METHODS

Study Conduct

B-AMAZE was approved by relevant regulatory authorities and ethics committees, registered at EudraCT (2017-000852-24) and www.clinicaltrials.gov (NCT03369444), and performed in accordance with Good Clinical Practice. Written informed consent was obtained from all participants before study-related activity. B-AMAZE was sponsored by University College, London, and was overseen by a trial steering committee, an independent data monitoring committee, and a trial management group. The long-term follow-up study (EudraCT no: 2017-005080-40; www.clinicaltrials.gov NCT03641703) is sponsored by Freeline Therapeutics.

The studies were designed by the sponsors with input from the authors. The clinical investigators, who are listed as authors, collected the data. The trial sponsors analyzed the data. All authors vouch for the data and analysis and vouch for adherence to the protocol. The lead and senior authors wrote the first draft of the manuscript with subsequent input from the other authors and editorial support provided by Freeline.

Study Patients

Men ≥18 years of age who had severe (FIX activity <1%) or moderately severe (FIX activity 1-2% with severe bleeding phenotype) hemophilia B with no evidence of inhibitors to FIX were eligible. Patients had to be negative for AAVS3 neutralizing antibodies and meet screening criteria. Study protocols are available at NEJM.org.

Study Design

B-AMAZE was a multicenter, open-label, single-dose Phase 1/2 clinical trial of FLT180a. Four dose levels were assessed in an ascending/descending adaptive design: $3.84x10^{11}$, $6.4x10^{11}$, $8.32\ x10^{11}$, and $1.28\ x10^{12}$ vector genomes per kilogram (vg/kg). These dose levels differ from prior reports of doses in this study based on a change in vector genome titer assay and reference standard (see Supplementary Methods). Prophylactic immunosuppression consisted of prednisolone ± tacrolimus. Vector-related increases in liver transaminases were treated with prednisolone, tacrolimus, and IV methylprednisolone. The dose and timing of immunosuppressive agents were iteratively adjusted throughout the study based on discussion with the Trial Management Group (see Supplementary Results). Patients were followed for 26 weeks in B-AMAZE before enrolling in ongoing long-term follow up. Per protocol, Study Week 1 of B-AMAZE began at Day 7.

Assessments

The primary endpoints in B-AMAZE were safety, as assessed by adverse events, and efficacy, as assessed by FIX levels at Week 26. Secondary endpoints included changes in annualized bleeding rates and FIX concentrate consumption, development of FIX inhibitors, and clearance of viral genomes. The objective of long-term follow up is to assess safety and durability for 15 years. FIX activity was measured using a one-stage clotting assay at a central laboratory (see Supplementary Appendix). Baseline annualized data for bleeding and FIX consumption are a retrospective collection from three years before treatment.

Annualized bleeding and FIX consumption after gene therapy are from Day 15 after gene therapy through last follow up.

Vector Design and Production

FLT180a (AAV2/S3-FRE1-Ti-FIXco1) is a single-stranded recombinant AAV vector. FLT180a consists of a rationally designed capsid (AAVS3) containing a transgene cassette comprising a liver-specific promoter (FRE1), and a partially codon-optimized FIX gene with a gain-of-function mutation ('Padua'; R338L) and a truncated intron in a natural position between FIX exons (E1 and E2) (Fig. S1 in the Supplementary Appendix). FLT180a was manufactured in an adherent mammalian cell production system by Children's GMP LLC (St. Jude Children's Research Hospital, Memphis, TN, USA) and all vector used in the study came from a single lot. Details of the vector manufacturing process are described in the Supplementary Appendix.

Statistical Analysis

The B-AMAZE study was ended early in October 2020 due to changes in the clinical development plan. The long-term follow-up study is ongoing. Preliminary data from the two studies were pooled based on a cut-off date of 20 September 2021, and descriptive statistics were produced for this article (see Supplementary Methods).

RESULTS

Characteristics of Study Participants

A total of 17 patients were screened and ten adult males with severe or moderately severe hemophilia B were treated with FLT180a (Fig. S2). The reasons patients were not treated included: a) liver dysfunction (n=1); b) presence or history of a FIX inhibitor (n=2); c) lack of a negative AAVS3 neutralizing antibody

screen (n=2); d) patient withdrawal (n=1); and e) early termination of the B-AMAZE study (n=2). One patient was not treated due to both b) and c) listed above. All ten patients completed the 26-week B-AMAZE study and enrolled in the long-term follow-up study. Patient characteristics are described in Table 1 and the representativeness of this patient sample for the broader hemophilia B population is described in Table S4.

Safety

No patients withdrew from the study due to toxicity and no deaths were noted. No infusion reactions and no discontinuations of infusions occurred. Inhibitors to FIX have not been detected. Treatment-emergent adverse events, serious adverse events, and adverse events related to FLT180a and immunosuppression are described in Table 2 and further detailed in the Supplementary Appendix. Approximately 10% and 24% of adverse events were related to FLT180a and immunosuppression, respectively. Of the 12 serious adverse events thought to be related to FLT180a, nine were increases in liver transaminases. All patients experienced at least one adverse event related to immunosuppression, and events were consistent with the known safety profiles of glucocorticoids and tacrolimus. Vector genome levels in plasma, urine, saliva, stool, and semen were typically below the limit of quantification within four weeks (Supplementary Results).

Treatment Response by Dose Level

The ascending/descending adaptive dosing design is shown in Figure 1. FIX levels at the Week 26 end-of-study visit for B-AMAZE, from Month 12 onward, and at latest follow-up for all patients are shown in Table 1. FIX levels, alanine aminotransferase (ALT), and immunosuppression over time are shown in Figure 2. Annualized bleeding rate and FIX consumption before and after gene therapy are in Table 1 and Figure 3.

Dose 1 - 3.84x10^{11} vg/kg

Patients 1 and 2 received the lowest dose of FLT180a. Prophylactic immunosuppression consisted of tapering courses of prednisolone starting at 60 mg daily from Week 6 to Week 12. Neither patient had increases in liver transaminases or adverse events related to FLT180a. Mean (±SD) FIX was 47.8% (3.95) in Patient 1 from Month 12-42 and 38.2% (3.63) in Patient 2 from Month 12-36. Neither patient received exogenous FIX after Day 5 following gene therapy. Two minor, traumatic bleeds in Patient 1 resolved without treatment.

Dose 2 – 1.28x10^{12} vg/kg

The highest dose was administered non-consecutively to Patients 3 and 6. FIX rose steadily in Patient 3, reaching 167% at Week 5. He started prophylactic prednisolone with 60 mg daily at Week 4. The patient was treated with IV methylprednisolone and tacrolimus for vector-related increases in ALT in Week 7. Mean (±SD) FIX from Month 12-30 was 80.1% (8.67). Patient 3 has had no bleeding since Day 3 after gene therapy and has not received exogenous FIX.

FIX levels in Patient 6 increased into the normal range approximately one week after infusion. He initiated prophylactic prednisolone at 90 mg daily in Week 3. As FIX levels continued to increase above the normal range, a thrombosis risk assessment was undertaken. In the context of advanced age, renal impairment, hypertension, a body mass index (BMI) of 32.1 and the use of glucocorticoids, prophylactic anticoagulation with apixaban was initiated in Week 3. At Week 4, his FIX level was 310% and his ALT increased to a peak of 69 IU/L. The ALT responded to IV methylprednisolone and tacrolimus and normalized within four days. Apixaban at doses of 2.5 and 5.0 mg twice daily was used for anticoagulation for approximately seven months. After a temporary interruption in apixaban due to suspected bleeding, he developed an arteriovenous (AV) fistula thrombosis in the right arm that required hospitalization and anticoagulation

with dalteparin. After discharge, the patient remains on apixaban prophylaxis at 2.5 mg twice a day. His FIX level has been stable above the normal range at a mean (±SD) of 279 (26.87) from Month 12-24 after gene therapy. Patient 6 also experienced four serious adverse events (epigastric pain, increased troponin and amylase, and chest sepsis) over an approximate two-week period that began ten weeks after FLT180a treatment. A narrative of these events is provided in the Supplementary Results. He reported one minor, spontaneous bleed after FLT180a treatment but has not received exogenous FIX.

Dose 3 – $6.4x10^{11}$ vg/kg

Patients 4 and 5 received an intermediate FLT180a dose. Patient 4 had an initial steady increase in FIX to 47% at Week 5. Prophylactic immunosuppression was initiated with 70 mg prednisolone daily in Week 4. After ALT increased from approximately 10 IU/L in Weeks 1-4 to 57 IU/L in Week 5, he was treated with IV methylprednisolone and tacrolimus. He also experienced an increase in ALT that occurred approximately 22 weeks after gene therapy while on 2.5 mg daily prednisolone. This increase in ALT was unanticipated due to the length of time since FLT180a infusion, leading to delayed recognition. Prednisolone was increased to 40 mg daily and tacrolimus was reinitiated. However, FIX levels declined to <2% at Month 11 and FIX prophylaxis was resumed during Month 13. The delayed recognition of the increased ALT in Patient 4 led to an extension of biweekly ALT monitoring in subsequent patients.

Patient 5 started prophylactic prednisolone with 80 mg daily at Week 3. He had a mild increase in ALT in Week 4 (39 IU/L) that was treated with IV methylprednisolone and tacrolimus. His mean (±SD) FIX level from Month 12-24 was 61% (4.24). Since receiving FLT180a, he reported one traumatic bleed and one minor, spontaneous bleed but has not received exogenous FIX.

Dose 4 – $8.32x10^{11}$ vg/kg

Given the FIX responses observed at dose levels 2 and 3, the next dose was selected to fall between $6.4x10^{11}$ and $1.28x10^{12}$ vg/kg. The immunosuppression regimen for Patients 7-10 was amended to include prophylactic tacrolimus beginning concurrently with glucocorticoids from Week 3.

FIX levels in Patient 7 reached 228% at Week 4. He had two episodes of increases in liver transaminases: an initial breakthrough case in Week 5 and another in Week 16 after tapering off glucocorticoids. His FIX levels declined following the second episode but subsequently reached steady levels with a mean (±SD) of 52.3 (2.91) for Months 12-22. Tacrolimus troughs in Patient 7 were difficult to get into the desired range (10-15 ng/ml), potentially due to a drug interaction with carbamazepine.[18]

Patients 8-10 all achieved FIX levels >150% by Week 4 or 5. Prophylactic immunosuppression with glucocorticoids and tacrolimus suppressed vector-related increases in liver transaminases, and high FIX levels were sustained throughout the 26 weeks of B-AMAZE. Notably, Patients 8-10 received 17- to 18-week courses of tacrolimus that extended beyond the glucocorticoid taper. After completion of tacrolimus, all three patients had increases in transaminases around Month 6. Immunosuppression was reinitiated, but FIX levels ultimately declined before stabilizing at levels consistent with mild hemophilia B or near the lower limit of normal (Table 1).

Across Patients 7-10 six minor traumatic bleeds were noted after FLT180a treatment. One traumatic bleed in Patient 8 was treated with FIX replacement based on physician choice despite endogenous FIX in the normal range.

Bleeding and exogenous FIX consumption

Across all ten patients the mean annualized bleeding rate at baseline was 2.93 events/year (range, 0 to 7.33) compared to a mean of 0.71 events/year (range, 0 to 1.70) after gene therapy, with the latter not distinguishing between treated and untreated bleeds. Annualized FIX consumption per patient declined from a baseline mean of 226,026 IU/year (range, 83,263 to 423,333) to a mean of 9,723 IU/year (range, 0 to 95,532) after gene therapy.

DISCUSSION

Achieving normal FIX levels (50-150%) is an important therapeutic goal for hemophilia B gene therapy. Normalization of hemostasis would be expected to not only protect against spontaneous bleeding, but also excessive bleeding in relation to trauma and surgery and damaging microbleeds.[19] Vector-related immune responses are a significant barrier to predictable and durable expression after liver-directed AAV gene therapy,[20-22] but maintenance of normal FIX levels after treatment is critical because retreatment with AAV vectors is unlikely to be successful due to persistence of capsid-specific neutralizing antibodies. To date, mean FIX levels following AAV gene therapy for hemophilia B have generally been below the normal range despite use of the FIX Padua variant.[9,12] While levels >50% have been reported on an occasional basis in some studies, it is in the context of wide variability across patients. In B-AMAZE, we selected patients who do not have neutralizing antibodies to AAVS3 and adopted a prophylactic immunosuppression regimen to improve the predictability of the dose response and to increase the chances that patients achieve and maintain normal FIX levels.

Our results confirm that FLT180a can achieve FIX levels in the normal range with relatively low vector doses. Initial FIX expression in patients treated with FLT180a in B-AMAZE was dose-dependent and exhibited a threshold effect with lower-dose patients plateauing near the lower limit of normal, whereas higher-dose patients reached levels >150% within a few weeks of treatment. Episodes of vector-related

increases in liver transaminases were the most common FLT180a-related adverse events and were associated with reductions in FIX activity in some cases. However, only one patient resumed FIX prophylaxis, and we believe the ineffectiveness of the immunosuppression regimen in this patient was due to delayed recognition of an immune response that occurred approximately 22 weeks after treatment. The remaining nine patients have reached steady levels with average FIX activity from Month 12 ranging from 28% to 279% and correspondingly low rates of bleeding and exogenous FIX consumption.

While nine of ten patients treated in B-AMAZE have sustained FIX expression, unexpected late episodes of increases in liver transaminases and declines in FIX levels were observed. The FIX activity patterns in patients 8-10 were particularly consistent. Increases in transaminases occurred upon initial withdrawal of an extended course of prophylactic tacrolimus. Immunosuppression was reinitiated, and FIX expression was initially steady, but subsequently declined when immunosuppression was stopped. These three patients have stabilized with FIX levels in the mild or normal range, but the similar trajectory of their FIX levels is notable.

The immune response to AAV gene therapy is complex and can be triggered by vector capsids, genomes, and protein products.[23] Immune responses to AAV are ubiquitous and have been seen across different disease states, routes of administration, and capsid serotypes. The CpG content of the transgene has been identified as one potential trigger for immunity. The transgene of FLT180a has only 5 CpG motifs as compared to 99 in the FIX transgene that was hypothesized to lead to an immune response in a prior hemophilia B clinical trial.[24] Therefore, we believe the immune response to FLT180a is unlikely to be due to CpG motifs.

The declines in FIX levels that occurred approximately 9-12 months after treatment only occurred in the patients who received prolonged courses of prophylactic tacrolimus beyond the glucocorticoid taper and developed late increases in transaminases. Tacrolimus has multiple attributes that make it a good candidate for investigation in AAV gene therapy. It potentiates glucocorticoid effects,[25,26] is effective in autoimmune hepatitis refractory to standard glucocorticoid-based approaches,[27] and is extensively used in solid organ transplantation based on its rapid action. Unlike immunosuppressive agents that disrupt the purine pathway,[28] tacrolimus is also not expected to interfere with second strand synthesis after AAV-mediated gene transfer. Furthermore, a recent publication of AAV gene therapy in the hemophilia A setting showed the potential utility of tacrolimus as a steroid-sparing agent.[29] Work is ongoing to refine the immunosuppression regimen with the goal of reducing vector-related immune responses in the early post-treatment period and enabling durable FIX expression without late declines.

Our results highlight important areas for consideration in AAV gene therapy. Emerging data from this study and the recent study in hemophilia A,[29] indicate that immune responses can occur later than previously expected and may coincide with withdrawal of immunosuppression. Consistent best practices for monitoring transaminases and deciding when ALT increases warrant intervention remain a critical topic for the field. We also observed, to our knowledge, the first case of thrombosis in a HB patient who achieved and maintained FIX levels above the normal range after gene therapy.

In conclusion, this study demonstrates that normal FIX levels can be achieved in patients with severe and moderately severe hemophilia B using relatively low vector doses of FLT180a. In all but one patient, gene therapy led to durable FIX expression, eliminated the need for FIX prophylaxis, and eliminated spontaneous bleeding requiring FIX replacement. Results of B-AMAZE support further evaluation of

FLT180a in clinical trials to confirm the dose and immunosuppressive regimen required to consistently achieve adequate hemostasis for patients with hemophilia B.

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Table 1. Characteristics of Patients at Screening and After Gene Transfer

	Patient									
	1	2	3	4	5	6	7	8	9	10
Dose, vg/kg	3.84×10^{11}	3.84×10^{11}	1.28×10^{12}	6.4×10^{11}	6.4×10^{11}	1.28×10^{12}	8.32×10^{11}	8.32×10^{11}	8.32×10^{11}	8.32×10^{11}
Characteristic										
Age, yr	32	25	27	48	29	67	39	48	32	25
Race	White	White	White	White	White	White	White	White	Asian	White
Weight, kg	68.0	71.5	97.4	72.5	82.9	89.6	100.3	88.0	83.0	78.8
BMI, kg/m^2	21.2	21.4	27.0	23.0	26.5	32.1	29.5	31.0	29.3	25.2
Hemophilia B severity	Severe	Severe	Severe	Severe	Severe	Severe	Moderately severe	Severe	Severe	Severe
F9 gene mutation	Hemizygous for C>676C>T	A to G substitution at position 1318 within exon 8 in the serine protease domain	C.1150C>T (P.ARG384*)	No variation from the normal sequence detected in coding region of the FIX gene	G.31260C>A	C.1150-1151INST, P. (ARG384FS) in exon 9	G31241G>T	C.1362DELT	Hemizygous for C.1150C> T (P.ARG384X) in exon 8	C.1303T>G (P.CYS435GLY)
Infection history										
Hepatitis B surface antigen	-	-	-	-	-	-	-	-	-	-
HIV antibody	-	-	-	-	-	-	-	-	-	-
Hepatitis C antibody	-	-	-	+	-	+	+	+	-	-
Baseline treatment regimen										
Treatment type	Prophylactic	On demand	Prophylactic	Prophylactic	Prophylactic	Prophylactic	Prophylactic	Prophylactic	Prophylactic	Prophylactic
FIX product	SHL	N/A	EHL	SHL	SHL	EHL	EHL	EHL	EHL	SHL

Dosing regimen	Every other day	N/A	Weekly	Twice weekly	Twice weekly	Weekly	Weekly	Weekly	Weekly	Three times weekly
Length of follow-up since vector infusion[a], months	41.2	42.4	36.0	30.2	30.4	24.2	21.9	21.7	20.0	19.1
FIX level										
Week 26, %	**44**	**46**	**71**	**7**	**64**	**280**	**53**	**180**	**190**	**143**
From Month 12 through last follow-up, mean % (SD)	47.8 (3.95)	38.2 (3.63)	80.1 (8.67)	10.53 (6.78)[d]	61.0 (4.24)[e]	279 (26.87)[e]	52.3 (2.91)	63.8 (19.67)	40.9 (4.16)	28.2 (5.34)
Last Follow up, % (month)	51 (M42)	43 (M36)	78 (M30)	9[d] (M30)	57 (M24)	260 (M24)	58 (M22)	59 (M21)	36 (M19)	23 (M18)
Annualized bleeding rate										
Pre-treatment,[b] events/yr	5.33	4.0	1.33	0	2.0	7.33	3.0	2.67	3.0	0.67
After gene therapy,[c] events/yr	0.59	0	0	1.61	0.80	0.51	0	1.70	0.61	1.29
Annualized FIX consumption										
Pre-treatment,[b] total IU/yr	238,000	96,333	389,333	326,000	319,333	423,333	83,263	164,667	91,000	129,000
After gene therapy,[c] total IU/yr	0	0	0	95,532[d]	0	0	0	1,698	0	0

BMI denotes body mass index, EHL extended half-life, FIX factor IX, HIV human immunodeficiency virus, SD standard deviation, SHL standard half-life, yr year

[a]Time in months from infusion to last visit date as of the data cut on 20 Sept 2021

[b]Annualized data are based on the retrospective collection, three years prior to treatment

[c]Annualized data are from ≥15 days post-infusion to the last follow up available as of the data cut, no distinction is made between treated and untreated bleeds

[d]Patient 4 resumed FIX prophylaxis in Month 13

[e]Less than 5 values available for calculation

Table 2: Treatment-Emergent Adverse Events by Dose Level in B-AMAZE and the Long-Term Follow-Up Study

	3.84 x 10^{11} vg/kg (n=2)		**1.28 x 10^{12} vg/kg (n=2)**		**6.4 x 10^{11} vg/kg (n=2)**		**8.32 x 10^{11} vg/kg (n=4)**	
	Incidence	**Number of Events**	**Incidence**	**Number of Events**	**Incidence**	**Number of Events**	**Incidence**	**Number of Events**
Any adverse event	**2 (100%)**	**29**	**2 (100%)**	**46**	**2 (100%)**	**41**	**4 (100%)**	**171**
Serious adverse events			**2 (100%)**	**7**	**2 (100%)**	**5**	**3 (75%)**	**5**
Transaminases increased[a]			2 (100%)	2	2 (100%)	3	3 (75%)	4
Abdominal pain upper			1 (50%)	1				
Amylase increased			1 (50%)	1				
Arteriovenous fistula thrombosis			1 (50%)	1				
Appendicitis					1 (50%)	1		
Coagulation FIX level decreased					1 (50%)	1		
Pulmonary sepsis			1 (50%)	1				
Toxicity to various agents (tacrolimus)							1 (25%)	1
Troponin increased			1 (50%)	1				
Adverse events related to FLT180a			**2 (100%)**	**14**	**2 (100%)**	**4**	**4 (100%)**	**11**
Transaminases increased[a]			2 (100%)	5	2 (100%)	3	4 (100%)	5
Fatigue / Malaise			1 (50%)	2			1 (25%)	2
Coagulation FIX level increased			2 (100%)	2				
Muscle spasms / Musculoskeletal pain / Myalgia							1 (25%)	3
Dyspepsia / Eructation			1 (50%)	2				
AV fistula thrombosis			1 (50%)	1				
Coagulation FIX level decreased					1 (50%)	1		
Headache							1 (25%)	1
Pulmonary sepsis			1 (50%)	1				
Somnolence			1 (50%)	1				
Adverse events related to glucocorticoids	**2 (100%)**	**2**	**2 (100%)**	**5**	**2 (100%)**	**11**	**3 (75%)**	**17**
Folliculitis / Rash maculo-papular / dermatitis acneiform	1 (50%)	1	1 (50%)	1	2 (100%)	3	1 (25%)	1
Insomnia / Sleep disorder					2 (100%)	3	2 (50%)	4
Fatigue / Malaise					1 (50%)	1	1 (25%)	2
Cushingoid					2 (100%)	2		

	3.84 x 10^{11} vg/kg (n=2)		1.28 x 10^{12} vg/kg (n=2)		6.4 x 10^{11} vg/kg (n=2)		8.32 x 10^{11} vg/kg (n=4)	
	Incidence	**Number of Events**	**Incidence**	**Number of Events**	**Incidence**	**Number of Events**	**Incidence**	**Number of Events**
Muscle spasms / Musculoskeletal pain / Myalgia							1 (25%)	3
Blood glucose increased							1 (25%)	2
Abdominal pain upper			1 (50%)	1				
Amylase increased			1 (50%)	1				
Arthralgia					1 (50%)	1		
Blood bilirubin increased	1 (50%)	1						
Coagulation factor IX level increased			1 (50%)	1				
Depression							1 (25%)	1
Dyspepsia / Eructation					1 (50%)	1		
Increased appetite							1 (25%)	1
Lower respiratory tract infection							1 (25%)	1
Nasopharyngitis							1 (25%)	1
Tremor							1 (25%)	1
Troponin increased			1 (50%)	1				
Adverse events related to tacrolimus					**1 (50%)**	**3**	**4 (100%)**	**33**
Diarrhea					1 (50%)	3	2 (50%)	6
Tremor							3 (75%)	5
Headache							2 (50%)	4
Paresthesia							2 (50%)	3
Dyspepsia / Eructation							1 (25%)	2
Nausea							1 (25%)	2
Tacrolimus toxicity							1 (25%)	2
Abdominal pain							1 (25%)	1
Alopecia							1 (25%)	1
Decreased appetite							1 (25%)	1
Depression							1 (25%)	1
Drug level increased							1 (25%)	1
Fatigue / Malaise							1 (25%)	1
Feeling hot							1 (25%)	1
Hypomagnesaemia							1 (25%)	1
Insomnia / Sleep disorder							1 (25%)	1

AV denotes arteriovenous; FIX Factor IX

Coded data collected up to data cut on 20 Sept 2021. Follow up varies by dose level.

a. This category includes events of transaminases increased, alanine aminotransferase increase, and aspartate aminotransferase increased.

Figure 1. Ascending/Descending Adaptive Dosing in B-AMAZE. Patients 1 and 2 received the initial, lowest FLT180a dose of $3.84x10^{11}$ vg/kg. FIX levels increased to near the lower limit of the normal range in these patients leading to a protocol-driven dose increase to $1.28x10^{12}$ vg/kg. Patient 3 had a rapid increase in FIX to 167% by Week 5, but then experienced an increase in ALT that led to a decline in FIX that subsequently stabilized in the normal range following treatment with IV methylprednisolone and tacrolimus. The high initial FIX level and immune response observed in Patient 3 led to a decrease in dose to an intermediate level of $6.4x10^{11}$ vg/kg for Patients 4 and 5. In these patients, FIX also increased to near the lower limit of normal. The FIX response at the $6.4x10^{11}$ vg/kg level prompted a dose increase back to $1.28x10^{12}$ vg/kg for Patient 6. However, after his FIX level exceeded the normal range, the dose was reduced to $8.32x10^{11}$ vg/kg for Patients 7-10.

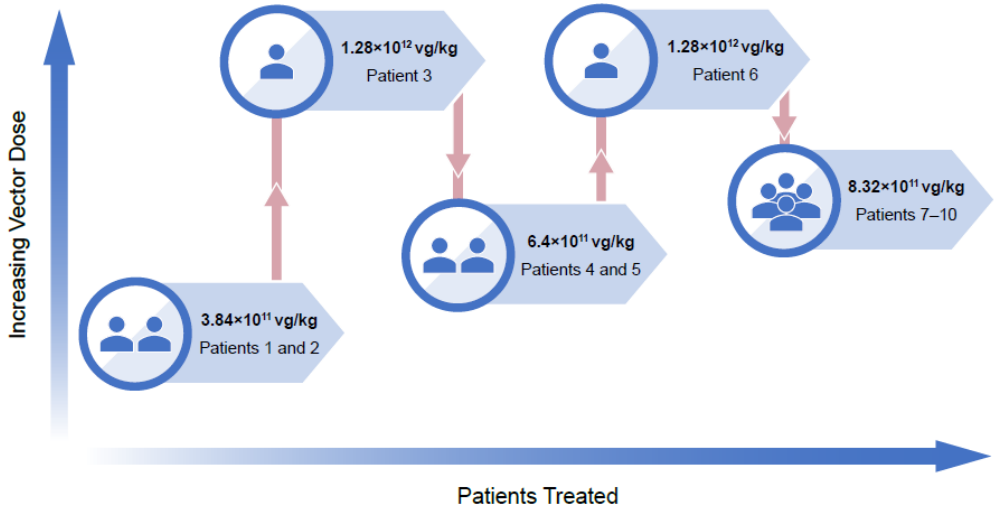
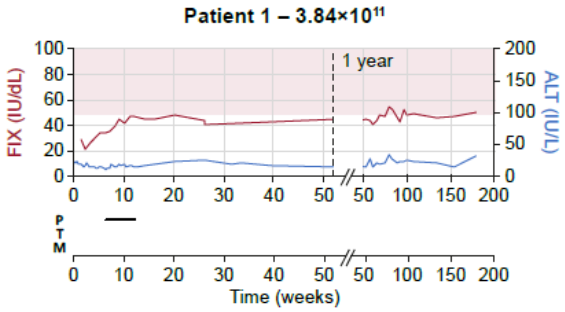
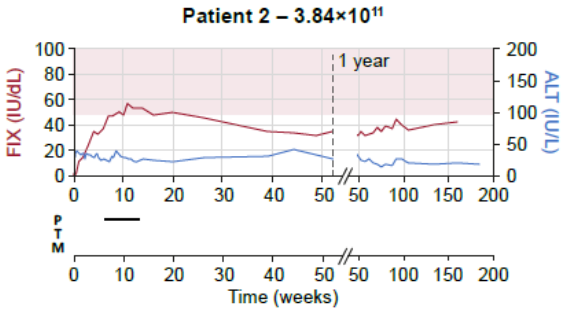
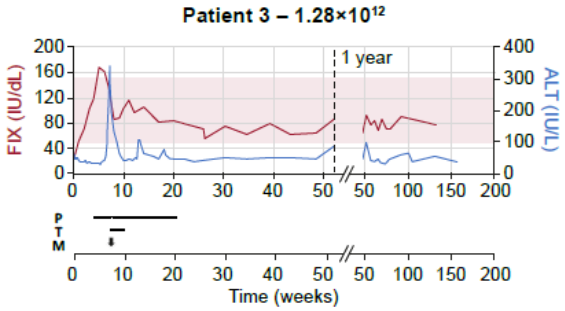
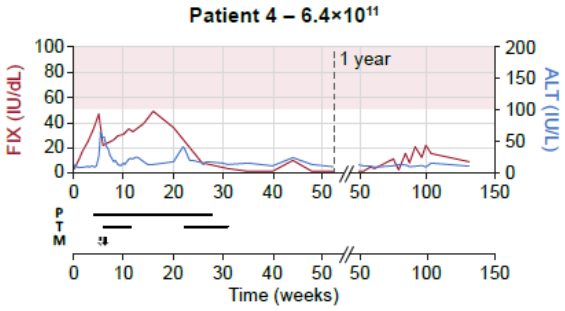


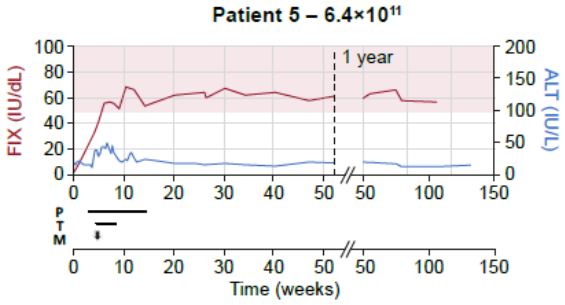
Figure 2. Factor IX Levels, Alanine Aminotransferase Levels, and Immunosuppression Following Gene Therapy. FIX levels (left axes, red) and serum alanine aminotransferase levels (right axes, blue) over time are shown for each patient throughout B-AMAZE and LTFU. Vector dose in vector genomes per kilogram is indicated in the title for each patient. The normal range of FIX (50-150 IU/dL; 50-150%) is indicated in red shading on each plot. Note that the axes have been rescaled and/or split by patient for clarity. The immunosuppression regimen for each patient is displayed and shows the timing of prednisolone (P) and tacrolimus (T) (black bars), and methylprednisolone (M) (black arrows). Declines in FIX level occurred in response to increases in liver transaminases and withdrawal of corticosteroids. Patient 4 resumed FIX prophylaxis in Month 13 so FIX values beyond that point include exogenous FIX. FIX activity is from a central laboratory using a one-stage clotting assay and ALT values are local laboratory values. Doses and tapering of the immunosuppressive agents by patient are available in Supplementary Results.

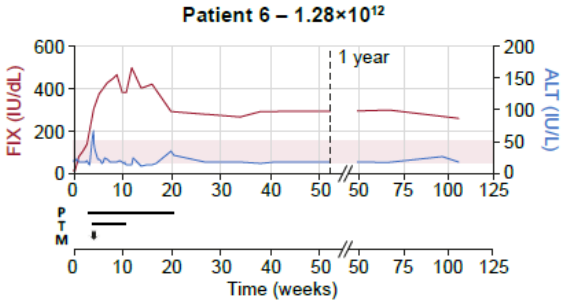

Patient 1 – 3.84×10^11
FIX (IU/dL)
ALT (IU/L)
1 year
P
T
M
Time (weeks)

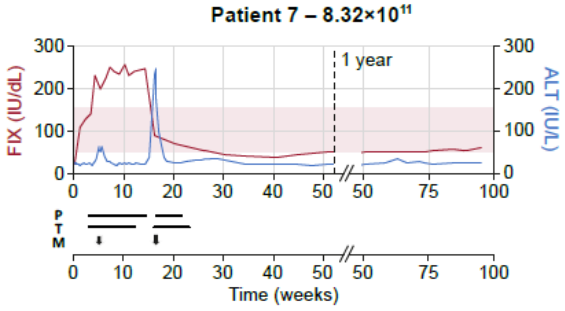

Patient 2 – 3.84×10^11
FIX (IU/dL)
ALT (IU/L)
1 year
P
T
M
Time (weeks)

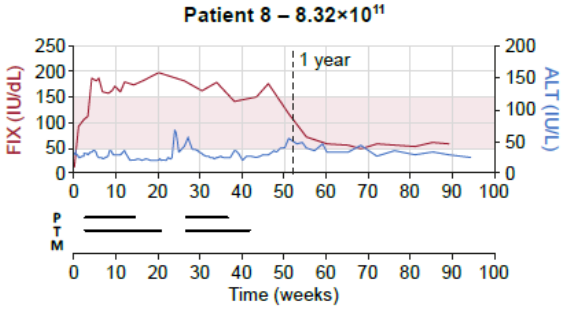

Patient 3 – 1.28×10^12
FIX (IU/dL)
ALT (IU/L)
1 year
P
T
M
Time (weeks)

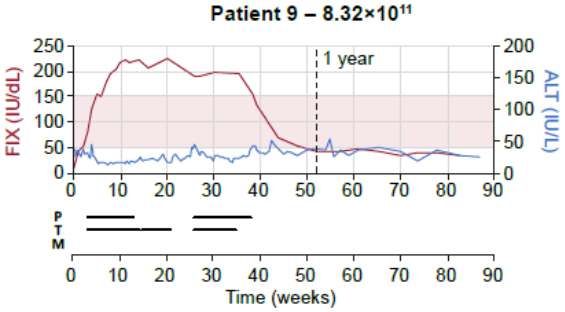

Patient 4 – 6.4×10^11
FIX (IU/dL)
ALT (IU/L)
1 year
P
T
M
Time (weeks)


Patient 5 – 6.4×10^11
FIX (IU/dL)
ALT (IU/L)
1 year
P
T
M
Time (weeks)


Patient 6 – 1.28×10^12
FIX (IU/dL)
ALT (IU/L)
1 year
P
T
M
Time (weeks)


Patient 7 – 8.32×10^11
FIX (IU/dL)
ALT (IU/L)
1 year
P
T
M
Time (weeks)


Patient 8 – 8.32×10^11
FIX (IU/dL)
ALT (IU/L)
1 year
P
T
M
Time (weeks)


Patient 9 – 8.32×10^11
FIX (IU/dL)
ALT (IU/L)
1 year
P
T
M
Time (weeks)

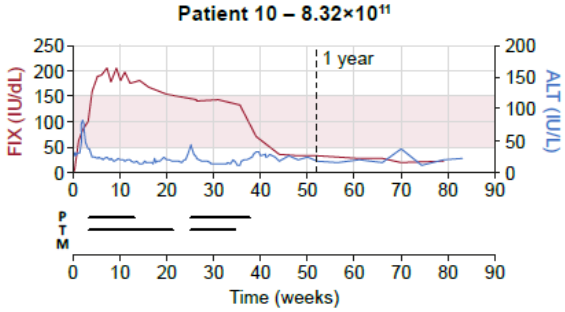

Patient 10 – 8.32×10^11
FIX (IU/dL)
ALT (IU/L)
1 year
P
T
M
Time (weeks)

Figure 3. Effect of Gene Therapy on Annualized Bleeding Rate and Total FIX Consumption. Bar charts of annualized bleeding rate (A) and annualized total FIX consumption (B) for each patient are shown. Pre-treatment data is a retrospective collection over three years prior to patients receiving gene therapy. Post-treatment data only includes bleeding events and FIX consumption from Day 15 after gene therapy to latest follow up as of the data cut date. Patient 4 indicated with an asterisk resumed FIX prophylaxis. Outside of Patient 4, there were two spontaneous bleeds: one each in Patients 5 and 6. All other bleeds were traumatic. Only Patients 4 and 8 received exogenous FIX after treatment. Patient 8 had a single infusion to treat a traumatic bleed and therefore his annualized total FIX consumption was <2,000 IU/year.