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ORIGINAL ARTICLE

Phase 1–2 Trial of 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 to normalize factor IX levels in patients with hemophilia B.

METHODS

In this multicenter, open-label, phase 1–2 trial, we assessed the safety and efficacy of varying doses of FLT180a in patients with severe or moderately severe hemophilia B (factor IX level, ≤2% of normal value). All the patients received glucocorticoids with or without tacrolimus for immunosuppression to decrease the risk of vector-related immune responses. After 26 weeks, patients were enrolled in a long-term follow-up study. The primary end points were safety and efficacy, as assessed by factor IX levels at week 26.

RESULTS

Ten patients received one of four FLT180a doses of vector genomes (vg) per kilogram of body weight: 3.84×10^{11} vg, 6.40×10^{11} vg, 8.32×10^{11} vg, or 1.28×10^{12} vg. After receiving the infusion, all the patients had dose-dependent increases in factor IX levels. At a median follow-up of 27.2 months (range, 19.1 to 42.4), sustained factor IX activity was observed in all the patients except one, who resumed factor IX prophylaxis. As of the data-cutoff date (September 20, 2021), five patients had normal factor IX levels (range, 51 to 78%), three patients had levels from 23 to 43%, and one had a level of 260%. Of the reported adverse events, approximately 10% were related to FLT180a and 24% to immunosuppression. Increases in liver aminotransferase levels were the most common FLT180a-related adverse events. Late increases in aminotransferase levels occurred in patients who had received prolonged tacrolimus beyond the glucocorticoid taper. A serious adverse event of arteriovenous fistula thrombosis occurred in the patient with high factor IX levels.

CONCLUSIONS

Sustained factor IX levels in the normal range were observed with low doses of FLT180a but necessitated immunosuppression with glucocorticoids with or without tacrolimus. (Funded by Freeline Therapeutics; ClinicalTrials.gov numbers, NCT03369444 and NCT03641703; EudraCT numbers, 2017-000852-24 and 2017-005080-40.)

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Hemophilia B is an X-linked, inherited bleeding disorder caused by mutations in the gene encoding coagulation factor IX (*F9*) that lead to decreased production of the protein.[1,2] Insufficient coagulation factor IX results in a bleeding tendency that classically involves musculoskeletal tissues but can affect other tissues and critical organs. Severe hemophilia B is characterized by coagulation factor IX levels of less than 1% of the normal value (<1 IU per deciliter). The standard care for severe hemophilia B is lifelong prophylaxis by means of regular intravenous infusions of factor IX concentrate.[2] In the past decade, the use of factor-replacement therapies with an extended half-life has been a major advance by enabling less frequent administration and maintenance of higher factor trough levels.[2-4] However, factor prophylaxis remains an invasive, expensive, and burdensome lifelong treatment approach that cannot completely prevent long-term complications.[5,6]

Adeno-associated virus (AAV) gene therapy is a promising treatment approach for hemophilia B, with multiple reports of factor production in patients after a single vector infusion, albeit typically at levels below the normal range of 50 to 150 IU per deciliter (50 to 150% of the normal value).[7-13] Remaining challenges for the development of AAV gene therapies in hemophilia B include generating sufficient factor levels to normalize hemostasis and managing vector-related increases in liver aminotransferase levels, which can lead to loss of transgene expression. Predictable and stable factor expression in the normal range would be expected to provide protection from bleeding, even in situations that necessitate intensive factor replacement, such as serious trauma or 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 substantially more liver cells than other currently used natural serotypes (AAV5 and AAV8).[14] The expression cassette encodes a variant of factor IX with a gain-of-function Padua mutation (R338L) that has approximately eight times the specific activity as wild-type factor IX.[15-17] Here, we report the safety and efficacy of FLT180a in patients with severe or moderately severe hemophilia B from a clinical trial (B-AMAZE) 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 the predictability of factor expression, and preserve the expression of transgenic factor. The goal of this treatment approach was sustained expression of factor IX in the normal range.

METHODS

TRIAL OVERSIGHT

B-AMAZE was approved by the relevant regulatory authority and ethics committee at each trial site and was performed in accordance with the Good Clinical Practice guidelines of the International Council for Harmonisation. Written informed consent was obtained from all the patients before trial-related activity.

The trial, which was funded by Freeline Therapeutics, was sponsored by University College, London, and was overseen by a trial steering committee, an independent data and safety monitoring committee, and a trial management group. The ongoing long-term follow-up study is also funded by Freeline Therapeutics.

The trial was designed by University College London and Freeline Therapeutics with input from the authors. The data were collected by the clinical investigators, who are listed among the authors, and were analyzed by the trial sponsors. The first and last authors wrote the first draft of the manuscript, with subsequent input from the other authors and editorial support provided by Freeline Therapeutics. The authors vouch for the completeness and accuracy of the data and for the fidelity of the trial to the protocol, which is available with the full text of this article at NEJM.org.

PATIENTS

Adult men (≥18 years of age) who had hemophilia B that was categorized as severe (factor IX level, <1%) or moderately severe (factor IX level, 1 to 2%, with a severe bleeding phenotype) with no evidence of inhibitors to factor IX were eligible to participate in the trial. All the patients had no evidence of AAVS3-neutralizing antibodies and met the screening criteria, as described in the protocol.

TRIAL DESIGN

B-AMAZE was a multicenter, open-label, single-dose, phase 1–2 clinical trial of FLT180a. FLT180a was assessed with the use of an ascending–descending adaptive design in which patients received one of four doses in vector genomes (vg) per kilogram of body weight: 3.84×10^{11} vg, 6.40×10^{11} vg, 8.32×10^{11} vg, and 1.28×10^{12} vg. These dose levels differ from doses that were described in previous reports of this trial owing to a change in the vector genome titer assay and reference standard. (Details regarding these changes are provided in the Methods section in the Supplementary Appendix, available at NEJM.org.)

Prophylactic immunosuppression consisted of prednisolone with or without tacrolimus. Vector-related increases in liver aminotransferase levels were treated with various combinations of oral prednisolone, tacrolimus, and intravenous methylprednisolone. The dose and timing of immunosuppressive agents were iteratively adjusted throughout the trial on the basis of discussion with the trial management group. Patients were followed for 26 weeks before enrollment in the ongoing long-term follow-up study. According to the protocol, trial week 1 of B-AMAZE began on day 7 after treatment.

ASSESSMENTS

The primary end points were safety, as assessed by adverse events, and efficacy, as assessed by factor IX levels at week 26. Secondary end points included changes in annualized bleeding rates and consumption of factor IX concentrate, development of factor IX inhibitors, and clearance of viral genomes. A complete list of end points is provided in the protocol. The objective of long-term follow-up was to assess safety and durability for 15 years.

Factor IX activity was measured by a one-stage clotting assay at a central laboratory. (Details are provided in the Supplementary Methods section.) Baseline annualized data for bleeding and factor IX consumption were collected retrospectively starting at 3 years before treatment. Annualized bleeding and factor IX consumption after the receipt of gene therapy were evaluated starting on day 15 after gene therapy through the last follow-up assessment.

VECTOR DESIGN AND PRODUCTION

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

STATISTICAL ANALYSIS

The trial was terminated early in October 2020 after changes were made in the clinical development plan. The long-term follow-up study is ongoing. Preliminary data from the two studies were pooled as of the data-cutoff date of September 20, 2021, and descriptive statistics were produced for this article. Details regarding changes to the development plan and the statistical analysis plan are provided in the Supplementary Methods section.

RESULTS

PATIENTS

From December 2017 through March 2020, a total of 17 patients underwent screening, and 10 men with severe or moderately severe hemophilia B were treated with FLT180a (Fig. S2). Patients were excluded from treatment because of the following reasons: liver dysfunction (in 1 patient), presence or history of a factor IX inhibitor (in 2 patients), a lack of a negative result on screening for AAVS3 neutralizing antibodies (in 2 patients), withdrawal from participation in the trial (in 1 patient), and early termination of the trial (in 2 patients). One patient had two reasons for exclusion: a history of a factor IX inhibitor and lack of a negative result on screening for AAVS3 neutralizing antibodies.

All 10 patients who received FLT180a completed the 26-week trial and enrolled in the long-term follow-up study. The characteristics of the

Table 1. Characteristics of the 10 Patients at Screening and Factor IX Levels after Gene Transfer.*

Characteristic	Patient Number and Gene-Therapy Dose									
	1 3.84×10^{11}	2 3.84×10^{11}	3 1.28×10^{12}	4 6.40×10^{11}	5 6.40×10^{11}	6 1.28×10^{12}	7 8.32×10^{11}	8 8.32×10^{11}	9 8.32×10^{11}	10 8.32×10^{11}
At screening										
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‡	21.2	21.4	27.0	23.0	26.5	32.1	29.5	31.0	29.3	25.2
After gene therapy										
Follow-up (mo)§	41.2	42.4	36.0	30.2	30.4	24.2	21.9	21.7	20.0	19.1
Factor IX level (%)										
At 26 wk	44	46	71	7	64	280	53	180	190	143
From 12 mo to last follow-up	47.8±4.0	38.2±3.6	80.1±8.7	10.5±6.8¶	61.0±4.2‖	279.0±26.9‖	52.3±2.9	63.8±19.7	40.9±4.2	28.2±5.3
At last follow-up	51 at 42 mo	43 at 36 mo	78 at 30 mo	9 at 30 mo¶	57 at 24 mo	260 at 24 mo	58 at 22 mo	59 at 21 mo	36 at 19 mo	23 at 18 mo

* Plus–minus values are means ±SD. Gene-therapy doses are listed as the number of vector genomes per kilogram. A more detailed list of the patients' characteristics is provided in Table S1.
† Race was reported by the patients.
‡ The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters.
§ Follow-up was measured from the time of the gene-therapy infusion until the last visit as of the data-cutoff date on September 20, 2021.
¶ Patient 4 resumed factor IX prophylaxis at month 13.
‖ Fewer than five values were available for this calculation.

patients are described in Tables 1 and S1; the representativeness of this patient sample for the broader hemophilia B population is described in Table S6.

SAFETY

No patients withdrew from the trial because of toxic effects, and no deaths were reported. No infusion reactions and no discontinuations of infusions occurred. At the time of the data-cutoff date, no inhibitors of factor IX had been detected. Adverse events that were considered by the investigators to be related to FLT180a are listed in Table 2. Additional details, including serious adverse events and adverse events related to immunosuppression, are listed in Tables S2 to S5. Of all adverse events, approximately 10% were considered to be related to FLT180a and 24% to immunosuppression. Of the 12 serious adverse events that were thought to be associated with FLT180a, 9 were increases in liver aminotransferase levels. All the patients had at least 1 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 4 weeks after treatment. Details regarding these analyses are provided in the Supplementary Results section.

TREATMENT RESPONSE, ACCORDING TO DOSE LEVEL

The ascending–descending adaptive dosing design is shown in Figure 1. Factor IX levels at the week 26 visit, from month 12 onward, and at the latest follow-up for all patients are provided in Table 1. Factor IX levels, alanine aminotransferase (ALT) levels, and immunosuppression over time are shown in Figure 2. The annualized bleeding rate and factor IX consumption before and after gene therapy are provided in Figure 3 and Table S1.

Dose 1 — 3.84×10^{11} vg per Kilogram

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 aminotransferase levels or in adverse events related to FLT180a. The mean (±SD) factor IX level was

Table 2. Adverse Events Related to FLT180a, According to Dose.*

Event	3.84×10^{11} vg/kg (N=2)		1.28×10^{12} vg/kg (N=2)		6.40×10^{11} vg/kg (N=2)		8.32×10^{11} vg/kg (N=4)	
	Incidence	No. of Events	Incidence	No. of Events	Incidence	No. of Events	Incidence	No. of Events
	no. (%)		*no. (%)*		*no. (%)*		*no. (%)*	
Any adverse event	0	0	2 (100)	14	2 (100)	4	4 (100)	11
Increased aminotransferase†	0	0	2 (100)	5	2 (100)	3	4 (100)	5
Fatigue or malaise	0	0	1 (50)	2	0	0	1 (25)	2
Increased coagulation factor IX	0	0	2 (100)	2	0	0	0	0
Muscle spasm, musculoskeletal pain, or myalgia	0	0	0	0	0	0	1 (25)	3
Dyspepsia or eructation	0	0	1 (50)	2	0	0	0	0
Arteriovenous fistula thrombosis	0	0	1 (50)	1	0	0	0	0
Decreased coagulation factor IX	0	0	0	0	1 (50)	1	0	0
Headache	0	0	0	0	0	0	1 (25)	1
Pulmonary sepsis	0	0	1 (50)	1	0	0	0	0
Somnolence	0	0	1 (50)	1	0	0	0	0

* Listed are adverse events that were reported during the 26-week B-AMAZE trial and the follow-up long-term study until the data-cutoff date of September 20, 2021, that were considered by the investigators to be related to FLT180a. The timing of follow-up varies according to the dose level. Additional details regarding adverse events are provided in Tables S2 to S5.
† Included in this category are increased levels of either alanine aminotransferase or aspartate aminotransferase.

47.8±4.0% in Patient 1 from month 12 through month 42 and 38.2±3.6% in Patient 2 from month 12 through month 36. Neither patient received exogenous factor IX after day 5 following gene therapy. In Patient 1, two minor bleeding episodes after trauma resolved without treatment.

Dose 2 — 1.28×10^{12} vg per Kilogram

The highest dose was administered nonconsecutively to Patients 3 and 6. Factor IX levels rose steadily in Patient 3 and reached 167% by week 5. Prophylactic prednisolone was initiated at 60 mg daily at week 4. The patient was treated with intravenous methylprednisolone and tacrolimus for vector-related increases in ALT levels at week 7. The mean factor IX level from month 12 through month 30 was 80.1±8.7%. Patient 3 had no bleeding since day 3 after gene therapy and had not received exogenous factor IX.

Factor IX levels in Patient 6 increased into the normal range approximately 1 week after infusion. Prophylactic prednisolone was initiated at a dose of 90 mg daily at week 3. Because the factor IX levels continued to increase above the normal range, a thrombosis risk assessment was performed. Subsequent prophylactic anticoagulation with apixaban was initiated at week 3 in consideration of the patient's older age (67 years), renal impairment, hypertension, body-mass index of 32.1, and use of glucocorticoids. At week 4, the factor IX level was 310% and the ALT level had increased to a peak of 69 U per liter. The ALT level responded to intravenous methylprednisolone and tacrolimus and normalized within 4 days. Apixaban (at doses ranging from 2.5 and 5.0 mg twice daily) was used for anticoagulation for approximately 7 months. After a temporary interruption in apixaban owing to suspected bleeding, the development of an arteriovenous fistula thrombosis in the right arm resulted in hospitalization and anticoagulation with dalteparin.

After discharge, Patient 6 continued to receive apixaban prophylaxis at a dose of 2.5 mg twice a day. His factor IX level was stable above the normal range at a mean of 279.0±26.9% from 12 to 24 months after gene therapy. He also had four serious adverse events (epigastric pain, increased troponin and amylase levels, and

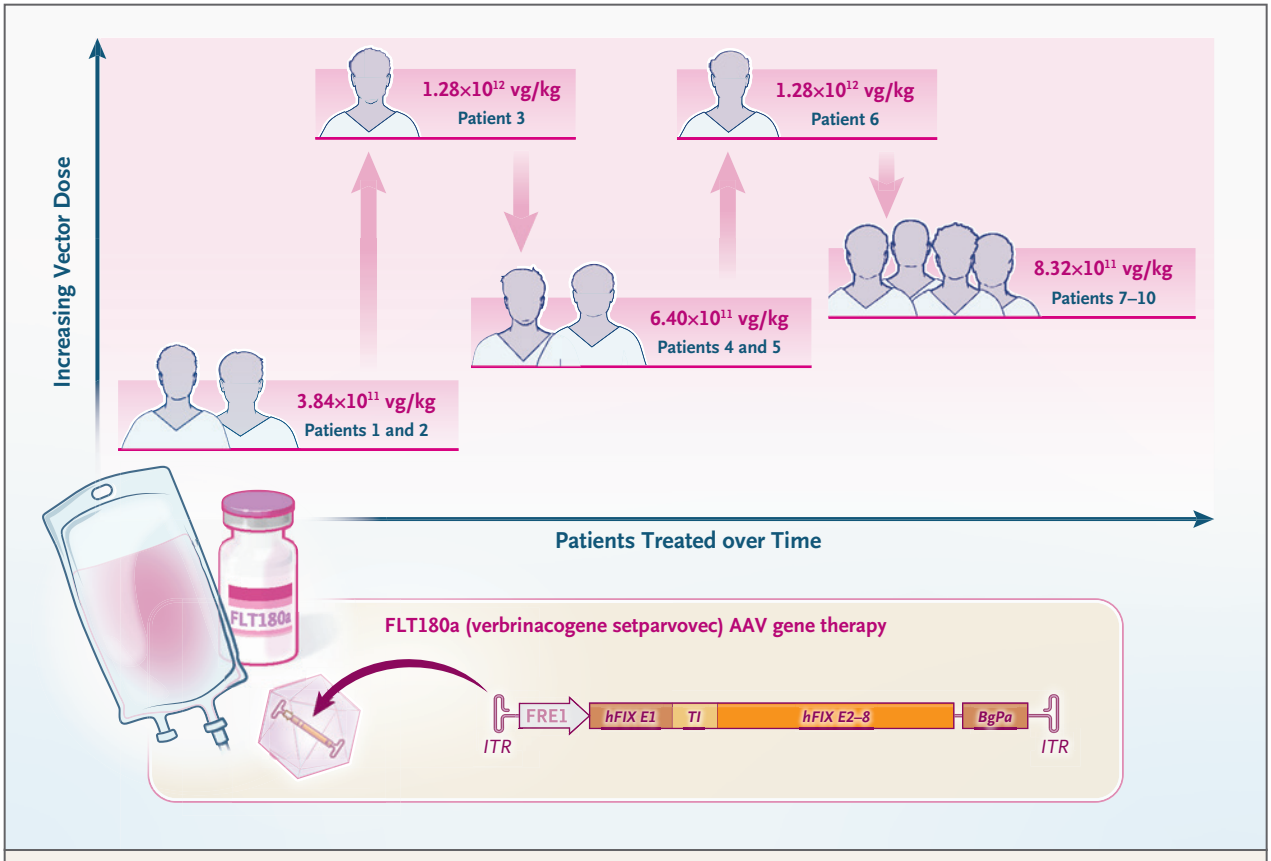

Figure 1. Ascending–Descending Adaptive Dosing of FLT180a in the 10 Trial Patients.

Patients 1 and 2 received the initial, lowest dose of of FLT180a of 3.84×10^{11} vector genomes (vg) per kilogram. Factor IX levels increased to nearly the lower limit of the normal range in these patients, which led to a protocol-driven dose increase to 1.28×10^{12} vg per kilogram. Patient 3 had a rapid increase in the factor IX level to 167% by week 5 but then had an increase in the alanine aminotransferase (ALT) level, which led to a decrease in the factor IX level; that level subsequently stabilized in the normal range after treatment with intravenous methylprednisolone and tacrolimus. The high initial factor IX level and immune response that was observed in Patient 3 led to a decrease in dose to an intermediate level of 6.40×10^{11} vg per kilogram in Patients 4 and 5. In these patients, the factor IX level also increased to approximately the lower limit of the normal range. The factor IX response at the dose of 6.40×10^{11} vg per kilogram prompted a dose increase back to 1.28×10^{12} vg per kilogram in Patient 6. However, after the factor IX level exceeded the normal range, the dose was subsequently reduced to 8.32×10^{11} vg per kilogram in Patients 7 through 10.

The FLT180a recombinant vector genome includes inverted terminal repeats (ITRs) at each end, which are the only adeno-associated virus (AAV) DNA sequences included in the vector and are required to enable replication and packaging of the expression cassette during production. The expression construct contains a liver-specific promoter (FRE1) and a partially codon-optimized coding DNA for factor IX with the Padua mutation (replacement of arginine with leucine at residue 338). The factor IX–Padua coding DNA is interrupted between exons 1 and 2 (E1 and E2) by a truncated version of the corresponding native intron (TI), which enhances factor IX expression. BgPa denotes bovine growth factor polyadenylation signal.

chest sepsis) over an approximate 2-week period that began 10 weeks after FLT180a treatment. A narrative of these events is provided in the Supplementary Results section. The patient reported one minor, spontaneous bleeding episode after FLT180a treatment but did not receive exogenous factor IX.

Dose 3 — 6.40×10^{11} vg per Kilogram

Patients 4 and 5 received an intermediate FLT180a dose. Patient 4 had an initial steady increase in the factor IX level to 47% by week 5. Prophylactic immunosuppression was initiated with 70 mg of prednisolone daily at week 4. After the ALT level increased from approximate-

ly 10 U per liter in weeks 1 to 4 to 57 U per liter in week 5, he was treated with intravenous methylprednisolone and tacrolimus. While he was receiving 2.5 mg of prednisolone daily, an increase in the ALT level occurred approximately 22 weeks after gene therapy. This increase was unanticipated owing to the length of time since the FLT180a infusion, which led to delayed recognition. Prednisolone was increased to 40 mg daily, and tacrolimus was reinitiated. However, factor IX levels decreased to less than 2% at month 11, and factor IX prophylaxis was resumed during month 13. The delayed recognition of the increased ALT level in Patient 4 led to an extension of twice weekly ALT monitoring in subsequent patients.

Patient 5 started prophylactic prednisolone at a dose of 80 mg daily at week 3. At week 4, he had a mild increase in the ALT level (39 U per liter) that was treated with intravenous methylprednisolone and tacrolimus. The mean factor IX level from month 12 through month 24 was 61.0±4.2%. Since receiving FLT180a, he reported having one traumatic bleeding episode and one minor, spontaneous bleeding episode but did not receive treatment with exogenous factor IX.

Dose 4 — 8.32×10^{11} vg per Kilogram

On the basis of changes in factor IX levels observed at dose levels 2 and 3, the next dose was selected to fall between 6.40×10^{11} and 1.28×10^{12} vg per kilogram. The immunosuppression regimen for Patients 7 through 10 was amended to include prophylactic tacrolimus beginning concurrently with glucocorticoids starting at week 3.

Factor IX levels in Patient 7 reached 228% at week 4. He had two episodes of increases in liver aminotransferase levels: an initial breakthrough at week 5 and another at week 16 after tapering of glucocorticoids. His factor IX levels decreased after the second episode of elevation but subsequently reached steady levels with a mean of 52.3±2.9% for months 12 through 22. Tacrolimus troughs in Patient 7 were difficult to get into the desired range (10 to 15 ng per milliliter), potentially caused by a drug interaction with carbamazepine.[18]

Patients 8, 9, and 10 all had factor IX levels of more than 150% by week 4 or 5. Prophylactic immunosuppression with glucocorticoids and tacrolimus suppressed vector-related increases in liver aminotransferase levels, and high factor IX levels were sustained throughout the 26-week trial period. Patients 8, 9, and 10 received tacrolimus for 17 or 18 weeks with tacrolimus continued 6 to 8 weeks after cessation of glucocorticoids. After the completion of tacrolimus, all three patients had increases in aminotransferase levels at or near month 6. Immunosuppression was reinitiated, but factor IX levels ultimately decreased before stabilizing at levels consistent with mild hemophilia B or near the lower limit of the normal range (Tables 1 and S1).

Among Patients 7 through 10, six minor traumatic bleeding episodes were noted after FLT180a treatment. One such bleeding episode in Patient 8 was treated with factor IX replacement on the basis of the physician's choice despite the presence of an endogenous factor IX level within the normal range.

BLEEDING AND EXOGENOUS FACTOR IX CONSUMPTION

Among the 10 patients, the mean annualized bleeding rate at baseline was 2.93 events per year (range, 0 to 7.33), as compared with a mean of 0.71 events per year (range, 0 to 1.70) after gene therapy. (Data for post-treatment bleeding episodes include both treated and untreated episodes.) Annualized factor IX consumption per patient decreased from a baseline mean of 226,026 IU per year (range, 83,263 to 423,333) to a mean of 9723 IU per year (range, 0 to 95,532) after gene therapy.

DISCUSSION

The maintenance of factor IX levels within the normal range is an important therapeutic goal for hemophilia B gene therapy. Normalization of hemostasis would be expected to protect against spontaneous bleeding as well as excessive bleeding associated with trauma or surgery and with damaging microbleeding episodes.[19] Vector-related immune responses are a substantial barrier to predictable and durable expression after liver-directed AAV gene therapy,[20-22] but maintenance of normal factor IX levels after treatment is critical because retreatment with AAV vectors is unlikely to be successful because of the persistence of capsid-specific neutralizing antibodies.

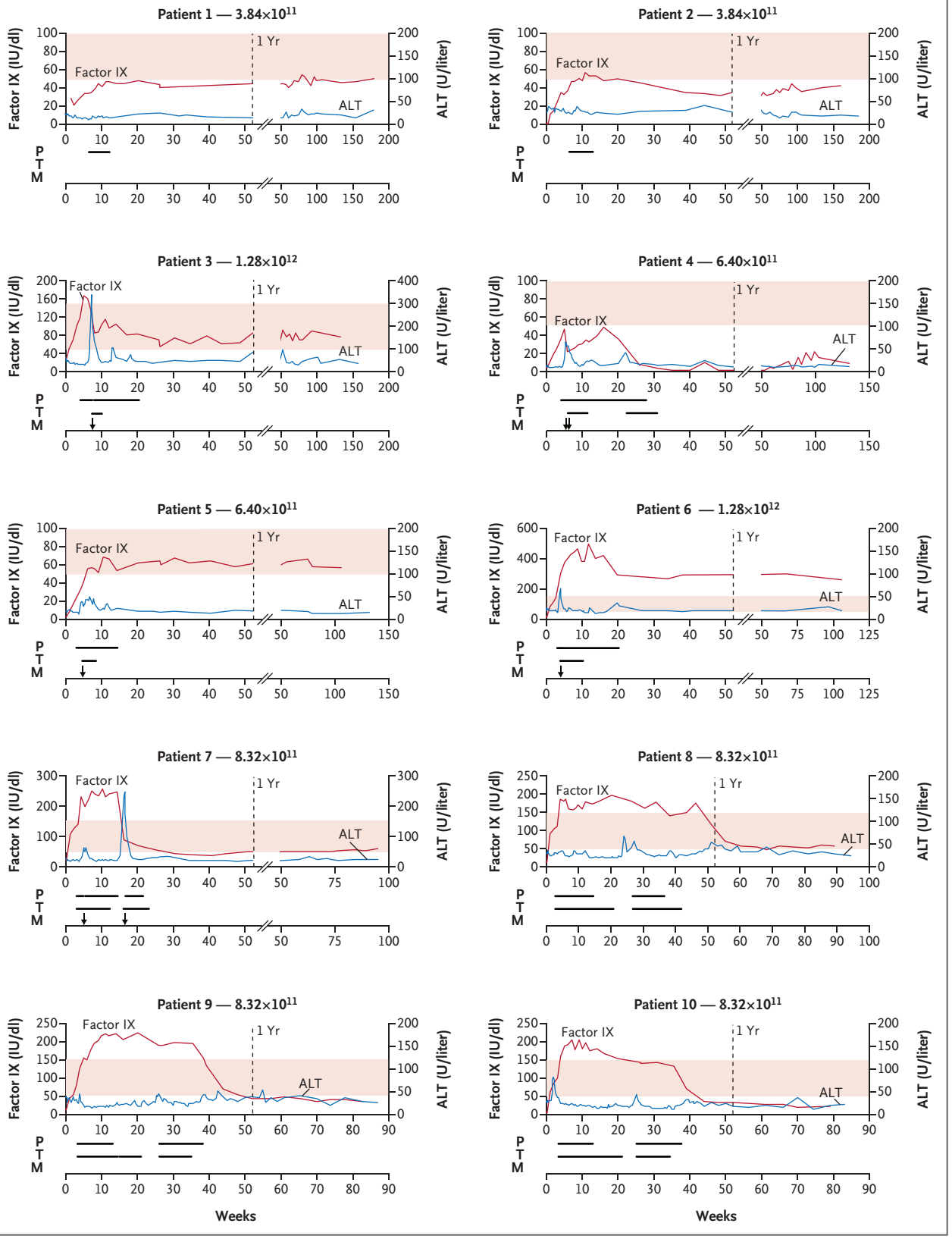

Figure 2 (facing page). Factor IX Levels, Alanine Aminotransferase (ALT) Levels, and Immunosuppression after Gene Therapy.

Shown are factor IX levels (left axes, red curve) and serum ALT levels (right axes, blue curve) over time for each patient throughout the trial and long-term follow-up. The dose in vector genomes per kilogram is indicated for each patient. The normal range for factor IX levels (50 to 150 IU per deciliter) is indicated in light red shading in each plot. The axes that are shown in each plot have been rescaled for each patient for clarity. For some patients, the x axis has been split at 1 year to show more resolution in the early part of treatment, when elevated aminotransferase levels were occurring. In other patients, there is no split at 1 year because the follow-up was shorter and the decreases in factor IX levels were still occurring. The immunosuppression regimen for each patient is shown according to the timing of administration of prednisolone (P) and tacrolimus (T) (black bars) and methylprednisolone (M) (arrows). Decreases in factor IX levels occurred in response to increases in liver aminotransferase levels and the withdrawal of glucocorticoids. Patient 4 resumed factor IX prophylaxis at month 13, so factor IX values beyond that point include exogenous factor IX. Factor IX activity was determined at a central laboratory by a one-stage clotting assay; ALT values were determined at a local laboratory. Data regarding doses and tapering of the immunosuppressive agents in each patient are available in the Supplementary Results section.

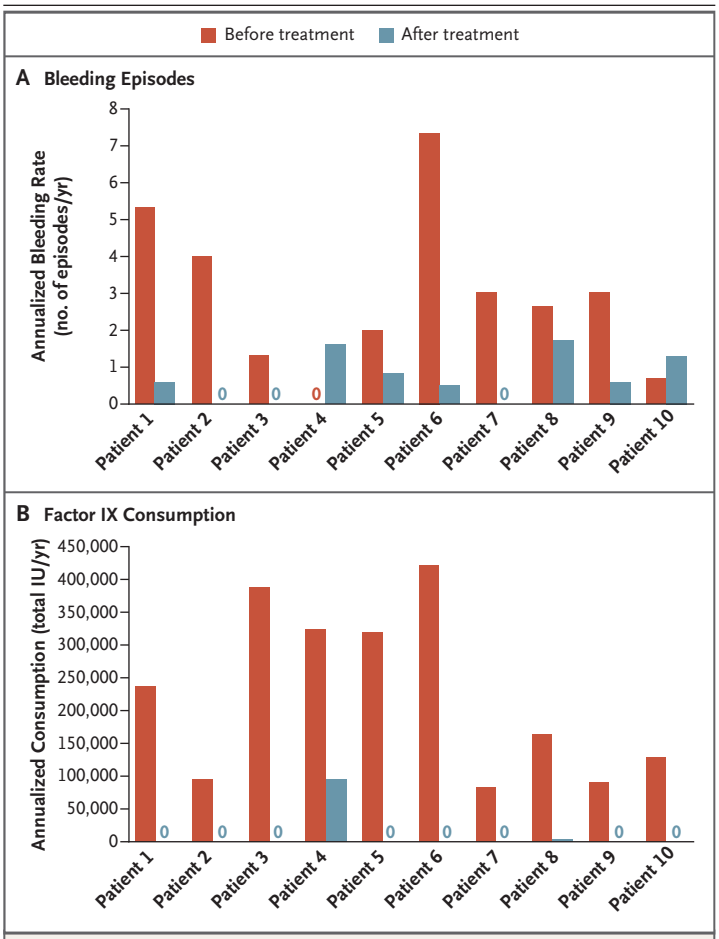

Figure 3. Effect of Gene Therapy on Annualized Bleeding Rate and Total Factor IX Consumption.

Bar charts of the annualized bleeding rate (Panel A) and annualized total factor IX consumption (Panel B) for each patient are shown. Pretreatment data were collected retrospectively for the 3 years before the patients received gene therapy. Post-treatment data include bleeding events and factor IX consumption from day 15 after gene therapy to the latest follow-up as of the data-cutoff date. Patient 4 resumed factor IX prophylaxis at month 13. Two other patients had spontaneous bleeding episodes (one episode each in Patients 5 and 6). All other bleeding episodes were traumatic. Only Patients 4 and 8 received exogenous factor IX after treatment. Patient 8 received a single factor IX infusion to treat a bleeding episode, which contributed to an annualized total factor IX consumption of less than 2000 IU per year.

To date, mean factor IX levels after AAV gene therapy for hemophilia B have generally been below the normal range despite the use of the factor IX Padua variant.[9,12] Although levels of more than 50% have been reported on an occasional basis in some studies, such levels were observed in the context of wide variability across patients. In this trial, we selected patients who did not have neutralizing antibodies against AAVS3. We also adopted a prophylactic immunosuppression regimen to improve the predictability of the dose response and to increase the chances that normal factor IX levels would be reached and maintained in these patients.

Our results confirm that gene therapy with FLT180a can result in factor IX levels in the normal range with relatively low vector doses. Initial factor IX expression in patients who received FLT180a was dose-dependent and had a threshold effect, in which patients who received lower doses had a plateau in factor IX activity that was near the lower limit of the normal range, whereas patients who received higher doses had levels of more than 150% within a few weeks after treatment. Episodes of vector-related increases in liver aminotransferase levels were the most common adverse events and were associated with reductions in factor IX activity in some cases. However, only one patient resumed factor IX prophylaxis, which occurred after the failure of his immunosuppression regimen because of delayed recognition of an immune response occurring approximately 22 weeks after treatment.

Steady factor IX activity (ranging from 28 to 279%) was reached in the remaining nine patients starting at month 12, with correspondingly low frequencies of bleeding and exogenous factor IX consumption.

Despite the high level of sustained factor IX expression, unexpected late episodes of increases in liver aminotransferase levels and decreases in factor IX levels were observed. The factor IX activity patterns in Patients 8, 9, and 10 were particularly consistent. Increases in aminotransferase levels occurred on initial withdrawal of an extended course of prophylactic tacrolimus. Immunosuppression was reinitiated, and factor IX expression was initially steady but subsequently declined when immunosuppression was stopped. In these three patients, the factor IX levels stabilized in the mild or normal range with a similar trajectory.

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 various disease states, routes of administration, and capsid serotypes. The cytosine guanine dinucleotide (CpG) content of the transgene has been identified as one potential trigger for immune response. The transgene of FLT180a has only 5 CpG motifs, as compared with 99 in the factor IX transgene, which was hypothesized to lead to an immune response in a previous clinical trial.[24] Therefore, it seems unlikely that the immune responses to FLT180a were due to CpG motifs.

The decreases in factor IX levels that were reported approximately 9 to 12 months after treatment occurred only in the patients who had received prolonged courses of prophylactic tacrolimus beyond the glucocorticoid taper and who had late increases in aminotransferase levels. Tacrolimus has multiple attributes that make it a good candidate for investigation in AAV gene therapy, since 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 on the basis of 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, in a recent study of AAV gene therapy involving patients with hemophilia A, the potential utility of tacrolimus as a steroid-sparing agent was shown.[29] Work is ongoing to refine the immunosuppression regimen with the goal of reducing vector-related immune responses in the early period after treatment and enabling durable factor IX expression without late decreases in values.

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

Thus, we found that normal factor IX levels can be achieved in patients with severe or moderately severe hemophilia B with the use of relatively low vector doses of FLT180a. In all but one patient, gene therapy led to durable factor IX expression, eliminated the need for factor IX prophylaxis, and eliminated spontaneous bleeding leading to factor IX replacement. Our trial results support further evaluation of FLT180a in clinical trials to confirm the dose and immunosuppressive regimen that are necessary for the maintenance of adequate hemostasis in patients with hemophilia B.

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