ORIGINAL ARTICLE

Multiyear Factor VIII Expression after AAV Gene Transfer for Hemophilia A

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ABSTRACT

BACKGROUND

The goal of gene therapy for patients with hemophilia A is to safely impart long-term stable factor VIII expression that predictably ameliorates bleeding with the use of the lowest possible vector dose.

METHODS

In this phase 1–2 trial, we infused an investigational adeno-associated viral (AAV) vector (SPK-8011) for hepatocyte expression of factor VIII in 18 men with hemophilia A. Four dose cohorts were enrolled; the lowest-dose cohort received a dose of 5×10^{11} vector genomes (vg) per kilogram of body weight, and the highest-dose cohort received 2×10^{12} vg per kilogram. Some participants received glucocorticoids within 52 weeks after vector administration either to prevent or to treat a presumed AAV capsid immune response. Trial objectives included evaluation of the safety and preliminary efficacy of SPK-8011 and of the expression and durability of factor VIII.

RESULTS

The median safety observation period was 36.6 months (range, 5.5 to 50.3). A total of 33 treatment-related adverse events occurred in 8 participants; 17 events were vector-related, including 1 serious adverse event, and 16 were glucocorticoid-related. Two participants lost all factor VIII expression because of an anti–AAV capsid cellular immune response that was not sensitive to immune suppression. In the remaining 16 participants, factor VIII expression was maintained; 12 of these participants were followed for more than 2 years, and a one-stage factor VIII assay showed no apparent decrease in factor VIII activity over time (mean [±SD] factor VIII activity, 12.9±6.9% of the normal value at 26 to 52 weeks when the participants were not receiving glucocorticoids vs. 12.0±7.1% of the normal value at >52 weeks after vector administration; 95% confidence interval [CI], –2.4 to 0.6 for the difference between matched pairs). The participants had a 91.5% reduction (95% CI, 88.8 to 94.1) in the annualized bleeding rate (median rate, 8.5 events per year [range, 0 to 43.0] before vector administration vs. 0.3 events per year [range, 0 to 6.5] after vector administration).

CONCLUSIONS

Sustained factor VIII expression in 16 of 18 participants who received SPK-8011 permitted discontinuation of prophylaxis and a reduction in bleeding episodes. No major safety concerns were reported. (Funded by Spark Therapeutics and the National Heart, Lung, and Blood Institute; ClinicalTrials.gov numbers, NCT03003533 and NCT03432520.)

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N Engl J Med 2021;385:1961-73.
DOI: 10.1056/NEJMoa2104205

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Hemophilia A is an X-linked bleeding disorder caused by the deficiency or dysfunction of coagulation factor VIII. Factor VIII, which is endogenously produced primarily in liver sinusoidal endothelial cells, imparts a procoagulant cofactor effect within the intrinsic tenase enzyme complex.[1,2]

The hemophilia phenotype consists of recurrent joint hemorrhage that, over repeated incidences, results in arthropathy. The hemophilia phenotype correlates with measured plasma factor VIII activity. Spontaneous hemorrhage classically occurs frequently in severe hemophilia (factor VIII activity, <1% of the normal value), infrequently in moderate hemophilia (1 to <5% of the normal value), and rarely in mild hemophilia (5 to <40% of the normal value). Endogenous factor VIII activity greater than 10% of the normal value is associated with a low risk of spontaneous joint bleeding.[3,4] Either recurrent intravenous infusion of exogenous factor or subcutaneous administration of a factor VIII–mimetic bispecific monoclonal antibody, emicizumab, is currently used to confer a moderate or mild hemophilia phenotype in patients with hemophilia A.[3,5,6]

Multiple ongoing clinical trials of gene therapy for hemophilia A involve the use of recombinant adeno-associated viral (AAV) vectors to target hepatocyte factor VIII expression with the goal of a one-time disease-altering therapy. Data from an ongoing trial of gene therapy for hemophilia A showed an average decrease of approximately half of transgene expression from year 1 to year 2 after vector administration; the most recently presented 4-year follow-up data showed that this decrease continued.[7-9] The results of this trial suggested that long-term, stable factor VIII activity may not be achieved with hepatocyte-directed AAV gene transfer in humans. These results were unexpected given observations from clinical trials involving patients with hemophilia B that showed durable factor IX transgene-derived expression for 8 years after hepatocyte-directed AAV-mediated gene transfer and from studies in large-animal models of hemophilia A that showed durable factor VIII transgene-derived expression for 10 years.[10-12]

One obstacle to efficacious AAV gene transfer is a cellular immune response against the AAV capsid, which was identified in trials of gene therapy for hemophilia B.[13,14] This response is vector-dose dependent and classically manifested by an increase in the level of the liver enzyme alanine aminotransferase, a decrease in transgene expression, and identification of circulating capsid-specific T cells. Left untreated, this capsid immune response results in complete loss of transgene expression. However, clinical trial results have suggested that this cellular immune response can be controlled by immune suppression in most, but not all, recipients of AAV gene therapy.[15-17]

We hypothesized that safe and durable hepatocyte expression of factor VIII could provide sufficient endogenous prophylaxis in persons with hemophilia A. Here, we report data from our phase 1–2 trial on the safety and preliminary efficacy of an AAV vector (SPK-8011).

METHODS

TRIAL DESIGN AND OVERSIGHT

This open-label, multicenter, nonrandomized, dose-escalation, phase 1–2 trial evaluated SPK-8011 for hemophilia A. The protocol, available with the full text of this article at NEJM.org, was approved by the institutional review board at each investigative site. All the trial participants provided written informed consent for enrollment into one of four dose cohorts, which ranged from a low dose of vector to a high dose (i.e., from 5×10^{11} vector genomes [vg] per kilogram of body weight to 2×10^{12} vg per kilogram).

Immediately before vector administration, the participants received a factor VIII product to attain factor VIII activity that was 100% of the normal value. Thereafter, the participants received a single, intravenous infusion of SPK-8011 on an outpatient basis. Some participants received glucocorticoids within 52 weeks after vector administration either to prevent or to treat a presumed AAV capsid immune response. The participants were followed for 52 weeks after vector administration, and all the participants subsequently were enrolled in a 4-year long-term follow-up trial. The first participant was enrolled on January 26, 2017, and the data cutoff date for the analysis was May 3, 2021.

The authors designed the trial. Data were collected by clinical site investigators (listed as authors). All the authors analyzed the data and vouch for the accuracy and completeness of the data presented and for the adherence of the trial to the protocol. The first author wrote the first

draft of the manuscript with subsequent input from the other authors and without editorial assistance. Previous versions of the figures were developed with editorial assistance funded by Spark Therapeutics, a study sponsor and the vector manufacturer.

VECTOR DESIGN AND PRODUCTION

SPK-8011 is a recombinant-AAV vector consisting of SPK200 (a bioengineered capsid derived from AAV3 [specifically, subtype LK03])[18] with a liver-specific, truncated transthyretin enhancer and promoter, a synthetic intron sequence, and codon-optimized *F8* complementary DNA encoding FVIII-SQ, a B-domain–deleted form of factor VIII (Fig. S1 in the Supplementary Appendix, available at NEJM.org).[19] SPK-8011 was manufactured with transient triple transfection of human embryonic kidney cells (HEK293 cells), and titers were determined by means of quantitative polymerase chain reaction (Supplementary Methods section in the Supplementary Appendix).

PARTICIPANTS

We enrolled men (≥18 years of age) who had congenital hemophilia A and baseline factor VIII activity that was 2% or less of the normal value, no history of factor VIII inhibitory antibodies, and SPK200 neutralizing antibody titers of 1:5 or less. The enrolled participants met all screening criteria outlined in the Supplementary Appendix. Annualized bleeding rates and factor VIII infusions were retrospectively determined by means of review of the participants' medical records for the year before enrollment and were compared with prospectively collected phenotypic information after the infusion of vector. Serologic and imaging studies were performed as outlined in the trial protocol.

OBJECTIVES

The primary objective of the trial was to evaluate the safety and preliminary efficacy of SPK-8011. Safety end points included adverse events and the changes from baseline in findings on physical examination and laboratory values. Efficacy end points included factor VIII activity determined by a central laboratory on the basis of a steady-state one-stage factor VIII assay and the number of factor VIII infusions and bleeding events after vector administration.

Additional objectives were to characterize expression pharmacokinetics and the immune response to SPK200 and expressed factor VIII-SQ protein. The cellular immune response was assessed by monitoring factor VIII activity, the alanine aminotransferase level, and the participants' response to SPK200-derived peptides as determined by an interferon-γ enzyme-linked immune absorbent spot (ELISpot) assay of peripheral-blood mononuclear cells (PBMCs).[16] Vector shedding was also assessed.

STATISTICAL ANALYSIS

Descriptive statistics were used to analyze data from all the enrolled participants. For each participant, we used the differences between the mean factor VIII activity in weeks 26 to 52 (when they were not receiving glucocorticoids) and the mean factor VIII activity more than 52 weeks after vector administration to construct a confidence interval for the paired difference. This confidence interval was used to describe the multiyear durability of factor VIII expression. All interval estimates were descriptive in nature to show precision of the estimate; thus, no multiplicity adjustment was performed.

Prospective bleeding events and factor VIII infusions after the administration of vector were compared with retrospectively collected data and were summarized with means and confidence intervals for the differences. We used Bayesian negative binomial regression with noninformative priors on model parameters to analyze the relationship between the annualized rate of bleeding events after vector administration and the proportion of time the factor VIII activity was greater than 10% of the normal value (as determined in a one-stage factor VIII assay).

RESULTS

TRIAL POPULATION

Of the 34 men with hemophilia A who underwent screening, 18 men (18 to 52 years of age) were enrolled, received vector, and were followed for safety for a median of 36.6 months (range, 5.5 to 50.3) after administration of SPK-8011. Participant 5 was lost to follow-up 32.6 months after administration of vector. All participant data were analyzed (Fig. S2 in the Supplementary Appendix). Thirteen of the 18 participants were receiving factor prophylaxis before vector administration; the remainder received factor VIII

Table 1. Characteristics of the Participants at Baseline and after Gene Transfer.*

Variable	SPK-8011 Dose																	
	5×10^{11} vg/kg		1×10^{12} vg/kg			2×10^{12} vg/kg									1.5×10^{12} vg/kg			
	participant number																	
	1	*2*	*3*	*4*	*6*	*5*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*
At baseline																		
Age — yr	52	45	34	34	48	27	28	18	34	39	21	21	18	34	39	40	20	39
Weight — kg	68	89	89	82	89	60	79	93	72	78	78	83	69	121	60	115	60	95
BMI†	22	29	25	26	28	19	24	29	22	26	24	24	28	30	21	32	22	30
Factor VIII activity — % normal value	<1	<1	<1	<1	<1	<1	1–2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Genotype	Ser1070x	Arg218 2His	Arg81 4x	Intr 22 inv	Arg301 Leu	Intr 1 inv	Intr 22 inv	Intr 22 inv	Arg21 66X	His855 Leufs*2	Intr 22 inv	Ex 20–22 del	Ph2294 Serfs*90	Val402 Tyrfs*	Arg218 2His	Glu98 Lys	Intr 22 inv	Tyr1834 Asp
Cross-reacting material status‡	–	–	–	–	+	–	–	–	–	–	–	+	–	+	–	+	–	+
Use of prophylaxis	No	Yes	Yes	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Treatment type	rFVIII	EHL-rFVIII	EHL-rFVIII	EHL-rFVIII	pdFVIII	rFVIII	rFVIII	rFVIII	rFVIII	rFVIII	EHL-rFVIII	EHL-rFVIII	EHL-rFVIII	rFVIII	rFVIII	EHL-rFVIII	EHL-rFVIII	EHL-rFVIII
Factor VIII dose — IU/kg	NA	65 twice weekly	30 twice weekly	50 twice weekly and 25 weekly	NA	50 every other day	NA	25 every other day	NA	20 twice weekly	45 every 4 days	60 twice weekly	60 weekly	40 twice weekly	NA	50 weekly	55 twice weekly	50 every 5 days
Arthropathic joints — no.	6	2	4	5	5	5	1	2	4	3	2	0	2	3	2	3	1	2
Infection history§																		
HCV	+	+	+	+	+	–	–	–	+	+	–	–	–	–	+	–	–	+
HBV	–	+	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–	–
HIV	+	–	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–
Liver fibrosis (test)¶	F1 (TE)	F2 (SMF)	F0 (SMF)	F1–F2 (SMF)	F1 (SMF)	F0 (SMF)	F0 (TE)	F0 (SMF)	F0–F1 (SMF)	F0 (APRI)	F0 (SMF)	F0 (SMF)	F0 (SMF)	F0 (APRI)	F1 (TE)	F0 (SMF)	F0–F1 (SMF)	F0 (SMF)

After gene transfer																		
Immune suppression																		
Use	No	No	Yes	Yes	No	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Wk	NA	NA	8	6	NA	13	5	18	NA	NA	8	14	16	44‖	32‖	66‖	52‖	3
Factor VIII activity — IU/dl**																		
Peak																		
OSA	19	12	6	20	25	16	63	39	14	23	70	22	194	209	43	48	55	94
CSA	10	8	5	15	14	16	37	20	8	15	46	16	117	117	29	29	38	70
Mean, after 52 wk																		
OSA	11	8	5	8	22	NA	25	3	8	16	16	NA	6	17	12	4	5	NA
CSA	6	5	3	5	12	NA	14	3	5	11	10	NA	3	11	8	3	3	NA
Maximum AFP level — ng/ml††	9.0	3.5	2.2	1.4	6.6	1.8	4.1	2.5	1.1	2.7	1.6	2.3	1.8	3.7	3.2	1.7	2.6	8.7
Duration of safety follow-up — mo	50.3	47.8	45.5	44.2	41.1	32.6	40.9	36.8	36.7	36.5	36.1	35.9	31.7	25.4	23.7	20.7	19.8	5.5

* AFP denotes alpha-fetoprotein, APRI aspartate aminotransferase–to-platelet ratio index, del deletion, EHL extended half-life, ex exon, HBV hepatitis B virus, HCV hepatitis C virus, HIV human immunodeficiency virus, int intron, inv inversion, NA not applicable, pdFVIII, plasma-derived factor VIII, rFVIII, recombinant factor VIII, SMF serum markers of fibrosis, and TE transient elastography.

† Body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters.

‡ Positive results (plus sign) for cross-reacting material indicate that FVIII protein is detectable but dysfunctional at baseline, whereas negative results (minus sign) indicate no detectable FVIII protein at baseline.

§ A positive HCV, HBV, or HIV status (plus sign) indicates that the participant had serologic findings consistent with a history of infection but a negative viral polymerase-chain-reaction test at enrollment. A minus sign indicates that the participant did not have serologic findings consistent with a history of infection.

¶ Liver fibrosis was assessed on a scale of F0 to F4, with higher stages indicating a greater degree of fibrosis.

‖ Participants 14 through 17 received immune suppression for an extended time because of the use of glucocorticoid-sparing agents.

** On the one-stage factor VIII assay (OSA) and chromogenic factor VIII assay (CSA), the normal range for factor VIII activity is 50 to 150 IU per deciliter.

†† The upper limit of the normal range for AFP is 8.9 ng per milliliter.

Table 2. Drug-Related Adverse Events and Drug-Related Serious Adverse Events.*

Event	2×10^{12} vg/kg Cohort (N=9)		1.5×10^{12} vg/kg Cohort (N=4)		All Participants (N=18)	
	No. of Participants (%)	No. of Events	No. of Participants (%)	No. of Events	No. of Participants (%)	No. of Events
Adverse event related to SPK-8011						
Any event	4 (44)	12	4 (100)	5	8 (44)	17
Elevated alanine aminotransferase level	3 (33)	8	4 (100)	5	7 (39)	13
Pyrexia	1 (11)†	1	0	0	1 (6)	1
Myalgia	1 (11)†	1	0	0	1 (6)	1
Back pain	1 (11)†	1	0	0	1 (6)	1
Vomiting	1 (11)†	1	0	0	1 (6)	1
Serious adverse event related to SPK-8011						
Any serious adverse event	1 (11)	1	0	0	1 (6)	1
Elevated liver aminotransferase level	1 (11)	1	0	0	1 (6)	1
Adverse event related to glucocorticoid						
Any event	2 (22)	7	2 (50)	9	4 (22)	16
Adrenal insufficiency	1 (11)	1	0	0	1 (6)	1
Gastroesophageal reflux	1 (11)	1	0	0	1 (6)	1
Osteoporosis	0	0	1 (25)	1	1 (6)	1
Weight gain	1 (11)	2	1 (25)	1	2 (11)	3
Generalized edema	0	0	1 (25)	1	1 (6)	1
Hypomagnesemia	0	0	1 (25)	1	1 (6)	1
Muscle spasms	1 (11)	1	0	0	1 (6)	1
Acne	0	0	1 (25)	1	1 (6)	1
Irritability	0	0	1 (25)	1	1 (6)	1
Psychomotor hyperactivity	0	0	1 (25)	1	1 (6)	1
Tremor	0	0	1 (25)	1	1 (6)	1
Insomnia	1 (11)	1	0	0	1 (6)	1
Hypertension	1 (11)	1	0	0	1 (6)	1
Hot flash	0	0	1 (25)	1	1 (6)	1

* The relationship between the events and SPK-8011 or glucocorticoid was determined by the clinical investigators. Data are from the safety observation period. There were no drug-related adverse events or serious adverse events in two vector-dose cohorts (5×10^{11} vg per kilogram and 1×10^{12} vg per kilogram).
† One participant had an infusion reaction consisting of pyrexia, myalgia, back pain, and vomiting.

on demand. All but 1 participant had baseline hemophilic arthropathy (mean [±SD] number of arthropathic joints, 2.8±1.7; range, 0 to 6.0). Nine participants had a history of hepatitis C virus infection with baseline stage 0–2 liver fibrosis, and 3 participants had a coexisting human immunodeficiency virus infection (Table 1).

SAFETY OF SPK-8011 INFUSION AND IMMUNE-MODULATING AGENTS

Participant 5 had an acute infusion reaction involving vomiting, myalgia, back pain, and pyrexia 12 hours after vector administration. This reaction resolved within 72 hours with outpatient antipyretic therapy. Seven participants had elevated alanine aminotransferase levels. All these

elevations were mild except in Participant 12, who was hospitalized on an elective basis to receive intravenous methylprednisolone for a grade 2 elevation in the alanine aminotransferase level (3.0 to 4.9 times the upper limit of the normal range); this elevated level constituted the only serious adverse event in the trial (Table 2). Among four participants, there were seven nonsustained elevations in alanine aminotransferase levels that met protocol-specified toxicity criteria: six elevations were grade 1 (>2.5 to 2.9 times the upper limit of the normal range), and one elevation was grade 2 (Fig. S3). All these nonsustained elevations in alanine aminotransferase levels occurred in participants in two vector-dose cohorts (1.5×10^{12} vg per kilogram and 2×10^{12} vg per kilogram), and these elevations occurred during a cellular immune response against SPK200 that resolved with the use of glucocorticoids.

Four participants had adverse events related to glucocorticoids (Table 2). Concomitant use of glucocorticoid-sparing immune-modulating agents (e.g., azathioprine, tacrolimus, and mycophenolate mofetil) in some participants did not result in adverse events (Fig. S8). All the participants had vector shedding that was below the quantification limit in saliva, serum, urine, and semen by 3 weeks after vector infusion and in PBMCs by 12 weeks after vector infusion (Fig. S4). Factor VIII inhibitory antibodies did not develop in any of the participants. One participant had an isolated alpha-fetoprotein value of 9.0 ng per milliliter, which is above the upper limit of the normal range (8.9 ng per milliliter) (Table 1); this value was within the normal range on repeat analysis. All the other participants had alpha-fetoprotein values that remained within the normal range. No new findings were noted on ultrasonographic assessment of the liver; these assessments were performed to screen for hepatocellular carcinoma, which was identified in preclinical studies as a concern with respect to the long-term safety of systemic administration of AAV.[20,21]

FACTOR VIII EXPRESSION AND DURABILITY

All the participants had factor VIII expression after vector administration. Two of the 18 participants, both of whom were in the cohort that received 2×10^{12} vg per kilogram, lost expression after a cellular immune response against SPK200 detailed below and in the Supplementary Results section in the Supplementary Appendix. Among the 15 participants in whom factor VIII expression was maintained and who were followed for more than 52 weeks, the mean factor VIII activity more than 52 weeks after vector administration was 11.0±6.8% of the normal value (range, 3.2 to 24.8) in a one-stage factor VIII assay and 6.9±3.8% of the normal value (range, 3.0 to 14.3) in a chromogenic factor VIII assay. Consistent with findings in other trials of therapy for hemophilia A,[7,22] factor VIII activity that was determined with the use of a one-stage factor VIII assay was 1.5 times as high as that determined with the use of a chromogenic factor VIII assay (Fig. S5).

Across all the dose cohorts, the initial peak factor VIII expression occurred 6 to 12 weeks after vector administration (Fig. 1 and Figs. S6 though S8). Participants in the cohorts that received 5×10^{11} vg per kilogram or 1×10^{12} vg per kilogram reached plateau factor VIII activity by 12 weeks; these kinetics of expression are consistent with previous observations in trials of AAV gene therapy for hemophilia B.[16] In contrast, participants in the cohorts that received 1.5×10^{12} vg per kilogram or 2×10^{12} vg per kilogram (except Participant 6) had decreases in peak expression from week 6 to 12 to plateau expression by 26 to 52 weeks after vector administration. Although peak expression in the cohorts that received 5×10^{11} vg per kilogram or 1×10^{12} vg per kilogram was lower than that observed in the cohorts that received 1.5×10^{12} vg per kilogram or 2×10^{12} vg per kilogram, a clear dose response in steady-state factor VIII activity was not observed more than 52 weeks after vector administration. This finding may be related to a narrow dose escalation, interparticipant variability, or both that have been observed in previous trials of gene therapy for hemophilia.[8,16]

Among the 16 participants in whom factor VIII expression was maintained outside the capsid immune response window, 12 were followed for more than 2 years and had apparent multiyear stable expression. Specifically, for the difference between matched pairs (i.e., an individual participant's mean factor VIII activity when not receiving glucocorticoids during the period from 26 to 52 weeks vs. that participant's mean

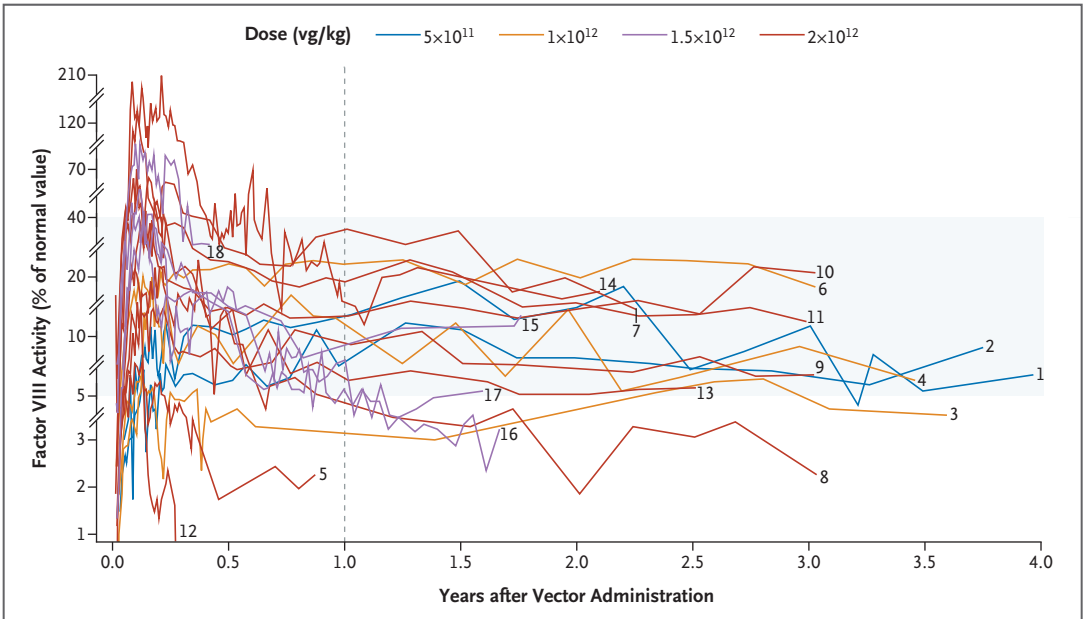

Figure 1. Factor VIII Activity after Infusion of SPK-8011 in 18 Participants.

The SPK-8011 vector was administered in four doses: 5×10^{11} vector genomes (vg) per kilogram of body weight, 1×10^{12} vg per kilogram, 1.5×10^{12} vg per kilogram, and 2×10^{12} vg per kilogram. On the y axis, factor VIII activity is shown on a log scale. The number to the right of each line is the participant number. Factor VIII activity was determined with the use of a one-stage factor VIII assay. The gray shaded area indicates the range of factor VIII activity in mild hemophilia A. Participants 5 and 12 lost transgene expression.

factor VIII activity after 52 weeks), the confidence interval was –2.4 to 0.6 (Fig. 1 and Table S1). In addition, among these 12 men, the combined mean factor VIII activity when the participants were not receiving glucocorticoids for 26 to 52 weeks (12.9±6.9% of the normal value) was analogous to the combined mean factor VIII activity after more than 52 weeks (12.0±7.1%).

CELLULAR IMMUNE RESPONSE AGAINST THE AAV CAPSID AND IMMUNE MODULATION COURSE

The initial dosing protocol called for the first 12 participants to receive glucocorticoids when at least one of the following trial assessments indicated a capsid immune response: a decrease in factor VIII activity, an increase in the alanine aminotransferase level to at least 1.5 times the level at baseline, or a positive PBMC interferon-γ ELISpot to SPK200 peptides. Five participants did not receive glucocorticoids. Eight participants received glucocorticoids for 3 to 18 weeks (within the first 52 weeks after vector infusion), and vector expression was maintained in 6 of these participants. Although they received glucocorticoids, Participants 5 and 12 lost all factor VIII expression within 52 weeks after vector infusion because of a capsid immune response; emicizumab was initiated in Participant 5, and the use of factor VIII prophylaxis was resumed in Participant 12.

In response to the loss of factor VIII expression in two participants, the protocol was amended, and the subsequent five participants (Participants 13 through 17) received prophylactic glucocorticoids that were initiated early (2 to 4 weeks after vector administration); transgene expression was maintained in all these participants. However, attempts to wean four of these participants (Participants 14 through 17) off oral glucocorticoids resulted in an increase in the alanine aminotransferase level, positive SPK200 ELISpot assays, or both outside the 3-month window that is typical of a capsid cellular immune response. Thus, in Participants 14 through 17, treatment was transitioned to glucocorticoid-sparing immune-modulating agents (azathioprine or mycophenolate mofetil, tacrolimus, or both) for a total of 32 to 66 weeks, and factor VIII expression was maintained when the participants were weaned off all immune-modulating

agents (Table 1). Because of the prolonged use of immune modulation in the participants who received prophylactic glucocorticoids, Participant 18 received glucocorticoids when evidence of an immune response to the vector was noted and expression was maintained. Consistent with the presence of a glucocorticoid response element in the promoter, factor VIII expression increased in all the participants while they were receiving glucocorticoids.

PRELIMINARY EFFICACY OF SPK-8011

The analysis involving all 18 participants (median efficacy follow-up, 33.4 months; range, 3.7 to 47.6) showed a 91.5% reduction (95% confidence interval [CI], 88.8 to 94.1) in the annualized rate of bleeding events (median, 8.5 events per year [range, 0 to 43] before vector administration vs. 0.3 events per year [range, 0 to 6.5] after vector administration) (Fig. 2A) and a 96.4% reduction (95% CI, 95.7 to 97.1) in the annualized number of factor VIII infusions (median, 57.5 infusions [range, 24 to 245] per year before vector administration vs. 0.6 [range, 0 to 28.6] after administration) (Fig. 2B). For Participants 5 and 12, the reported efficacy data were based on observations before loss of factor VIII expression. The percentage of participants with no bleeding events ranged from 60 to 100% at years 1 through 4 of follow-up (Fig. 2C), and the cumulative percentage was 39% over the entire follow-up period (Fig. 2A). Prophylaxis was discontinued in the 16 participants who had sustained factor VIII expression.

A Bayesian negative binomial regression analysis showed that the mean spontaneous bleeding rates were less than 1 event per year, with a posterior probability of 0.99 when transgene-derived factor VIII activity was more than 10% of the normal value at least 50% of the time in a one-stage factor VIII assay (Fig. 3). These data indicate that among participants who had factor VIII activity that was greater than 10% of the normal value in a one-stage factor VIII assay at least half the time, the probability of an annualized rate of bleeding events of less than 1 event was 99%.

DISCUSSION

Our phase 1–2 trial data showed that SPK-8011 imparted multiyear durable factor VIII expression that significantly reduced bleeding. These data provide support for our hypothesis that hepatocyte expression of factor VIII after AAV gene transfer is a viable approach for long-term stable phenotypic amelioration of hemophilia A.

No major safety concerns were identified after the administration of SPK-8011. In our trial, nonsustained, drug-related elevations in alanine aminotransferase levels that met toxicity criteria (>2.5 to 3.6 times the upper limit of the normal range) occurred in 22% of the participants who had a cellular immune response against SPK200. These findings are consistent with the results of previous trials of AAV-mediated gene therapy for hemophilia B[15,16] and contrast with the elevated alanine aminotransferase levels of unclear cause lasting for multiple months that were observed in a previous trial of AAV5-hFVIII-SQ gene therapy for hemophilia A.[7,8] Although the suspected immune response against SPK200 did not arouse major safety concerns, it limited the efficacy of SPK-8011 in some participants.

The complete loss of factor VIII expression in two participants in the highest-dose cohort showed that the AAV capsid immune response was not universally sensitive to glucocorticoids; this observation was noted in another trial of AAV gene transfer.[17] Although the use of prophylactic glucocorticoids prevented loss of expression, attempts to wean participants from these agents were complicated by the recurrence of laboratory findings that were consistent with a cellular immune response. However, in a small cohort, the use of azathioprine or tacrolimus, with or without mycophenolate mofetil, enabled participants to discontinue glucocorticoids and allowed for maintenance of transgene expression; further evaluation in a larger cohort is necessary before conclusions can be drawn.

The effect of immune suppression on the clearance of the AAV capsid antigen and the consequent duration of the presence of antigen after vector administration are unknown. Similarly, aside from the CpG (cytosine–guanine dinucleotide) content in the vector cassette,[17] whether the capsid serotype, the manufacturing method, or both play a role in the incidence and severity of a capsid immune response has not been established.

As expected, a small amount of durable factor VIII expression conferred a marked clinical benefit in the 15 participants with sustained factor

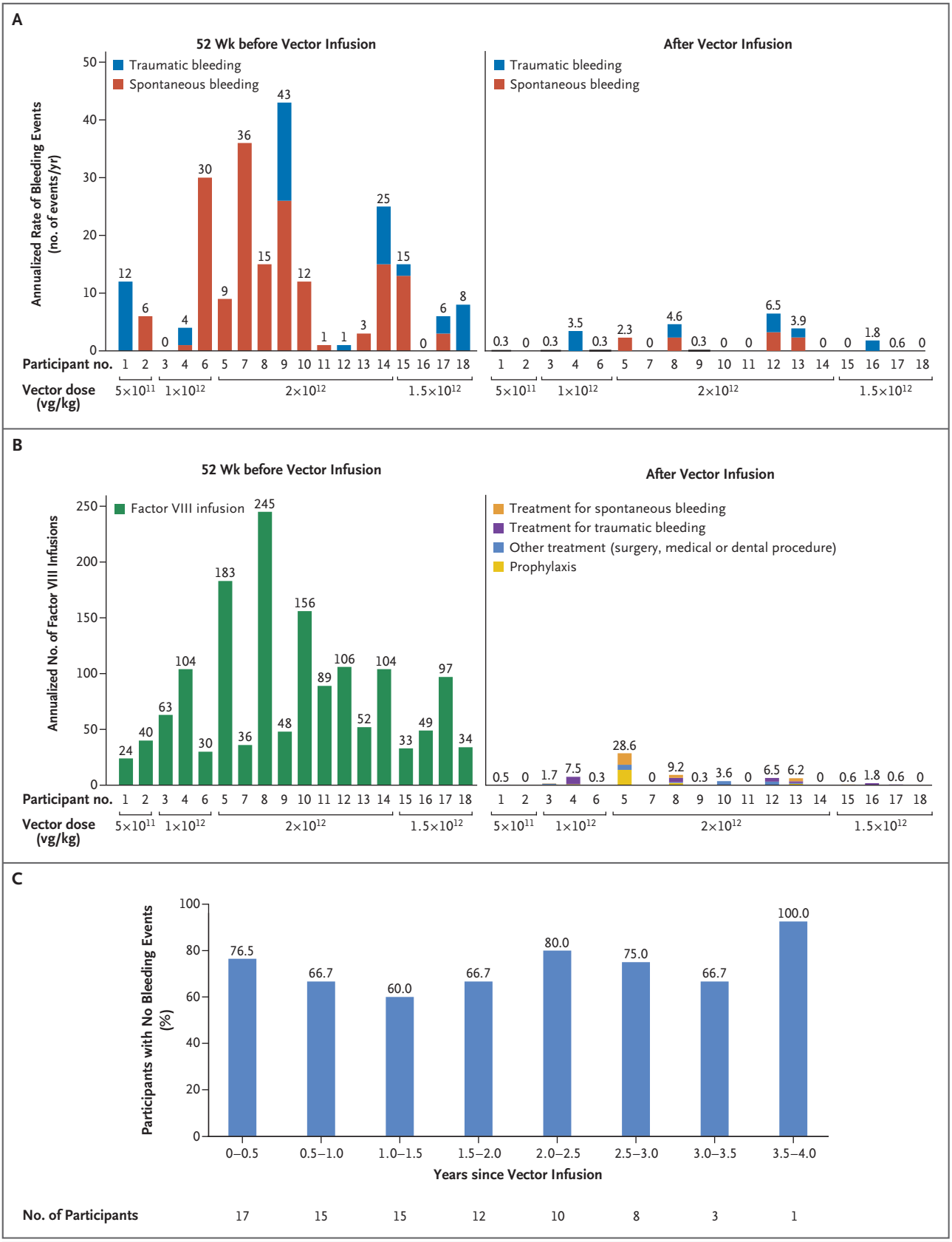

Figure 2 (facing page). Preliminary Efficacy of SPK-8011.
Panel A shows the annualized rate of bleeding events before and after vector infusion. Panel B shows the annualized number of exogenous factor VIII infusions before and after vector infusion. Participants 5 and 12 lost all transgene expression within 1 year after vector administration, and the data shown are for the period of transgene expression only. Panel C shows the percentage of participants with no bleeding events after SPK-8011 administration. The number of participants who completed each follow-up interval is indicated below the x-axis.

VIII expression who were followed for more than 52 weeks. Specifically, transgene-derived factor VIII activity that was greater than 10% of the normal value in a one-stage factor VIII assay eliminated spontaneous joint bleeding; this finding is consistent with natural history data indicating a relationship between joint bleeding and the activity of endogenous factor VIII determined by a one-stage factor VIII assay in persons with hemophilia.[4] Thus, factor VIII activity that was determined by a one-stage factor VIII assay may correlate with the in vivo hemostatic effect of hepatocyte-derived factor VIII. Furthermore, the observed annualized bleeding rate of 0 events in 60 to 100% of 16 participants with sustained factor VIII expression is similar to the results with emicizumab prophylaxis in severe hemophilia A.[6] Collectively, these data suggest that multiyear, durable factor VIII expression of greater than 10% as determined by a one-stage factor VIII assay may be a threshold for minimally targeted therapeutic efficacy after gene transfer and may confer a phenotype analogous to emicizumab prophylaxis.

The multiyear, generally stable hepatocyte factor VIII expression observed in our trial contrasts with expression in a hemophilia A gene-transfer trial evaluating AAV5-hFVIII-SQ in which approximately half of transgene expression was lost in nearly all participants from year 1 to year 2 after vector infusion and in which expression continued to decrease throughout the 4 years of available follow-up.[7-9] Both vectors encode the same B-domain–deleted factor VIII variant (FVIII-SQ). AAV5-hFVIII-SQ differs from SPK-8011 in that it was manufactured with baculovirus transduction of *Spodoptera frugiperda* (Sf9) cells. In addition, AAV5-hFVIII-SQ differs from SPK-8011 with respect to codon optimization of the expression cassette, AAV capsid serotype, vector dose

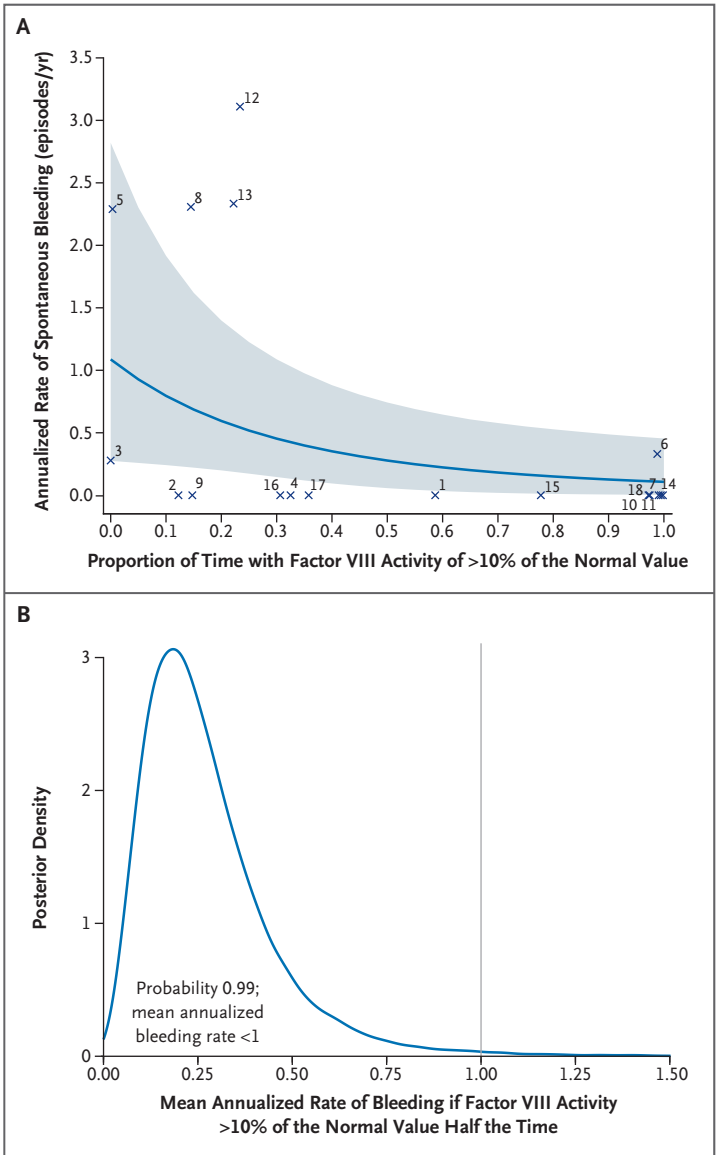

Figure 3. Correlation between Factor VIII Activity and the Rate of Spontaneous Bleeding Events.
Panel A shows the factor VIII activity relative to the annualized rate of spontaneous bleeding among Participants 1 through 18. Factor VIII activity was determined with the use of a one-stage factor VIII assay. A Bayesian negative binomial regression analysis with noninformative priors was used to analyze the relationship between the annualized rate of spontaneous bleeding after vector administration and the proportion of time participants had factor VIII activity that was greater than 10% of the normal value. The blue curve indicates the regression line, and the shaded area the 95% credible interval. Each X denotes one participant, and the participant numbers are shown. Data shown for Participants 5 and 12 are based on observations before the loss of transgene expression. Panel B shows the posterior density of the mean annualized rate of spontaneous bleeding among participants with factor VIII activity of more than 10% of the normal value at least half the time; among these participants, there was a 99% probability of an annualized rate of spontaneous bleeding of less than 1 event. The gray vertical line indicates an annualized rate of spontaneous bleeding of 1 event.

(30 to 120 times as high with AAV5-hFVIII-SQ), and the promoter.[7,8]

Previous AAV gene-therapy studies of hemophilia B that used an AAV serotype 5 vector manufactured with the baculovirus vector-production system and administered at doses that were similar to those of AAV5-hFVIII-SQ[7,8] showed stable factor IX protein expression in participants for more than 2 years[23] after vector administration. These findings suggest that the capsid, manufacturing platform, or vector dose alone is not responsible for differences in the durability of factor VIII expression across trials of gene therapy for hemophilia A. Furthermore, both SPK-8011 and AAV5-hFVIII-SQ have cassette sizes of approximately 5 kb, so the length of DNA packaged is not different; whether other factors (e.g., charge differences in the capsid sequence) affect DNA packaging, inverted terminal repeat–dependent formation of stable concatemers, or both is unclear.[24] Although both vectors used promoters targeting hepatocyte expression of factor VIII, cell-specific expression was not confirmed, which leaves open the possibility that expression from heterogeneous cell sources may account for differences in durability.

Finally, on average, AAV5-hFVIII-SQ was associated with factor VIII activity that was 10 times as high at 1 year after vector administration as that of SPK-8011.[8,9] This outcome was not unexpected given that vector doses of AAV5-hFVIII-SQ were orders of magnitude higher than those of SPK-8011. This finding raises the possibility that the level of factor VIII expression itself may contribute to durability. One proposed explanation is that factor VIII expression induces an unfolded protein response that, unmitigated, can produce apoptosis and loss of expression[25]; this has been observed in factor VIII mammalian cell culture and with supratherapeutic, but not low, levels of factor VIII expression after liver-directed gene transfer with AAV vectors in mice.[26-31]

Although SPK-8011 led to stable expression of factor VIII after gene transfer in most participants, a presumed dose-dependent capsid immune response prevented sustained expression in 2 of 18 participants. The safe achievement of sustained, stable, and predictable factor VIII levels in all participants, even in the presence of immune responses, remains an unrealized goal of gene therapy for hemophilia A. Potential solutions are under investigation and may include the use of less immunogenic AAV capsids,[32] gain-of-function factor VIII transgenes,[33-35] improved immune modulation regimens, or all of these.

Our trial provided data on multiyear, stable factor VIII expression after hepatocyte-directed AAV gene transfer. This trial began to answer questions that have emerged from previous trials of AAV-mediated gene transfer for hemophilia A. Close monitoring of participants to determine safety and efficacy and to confirm initial multiyear observations of durable expression are ongoing.

Supported by Spark Therapeutics and grants from the National Heart, Lung, and Blood Institute (NIH/NHLBI K08 HL 146991, to Dr. George, and NIH/NHLBI K08 HL 140078, to Dr. Samelson-Jones).

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

APPENDIX

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