ORIGINAL ARTICLE

Targeting of Antithrombin in Hemophilia A or B with RNAi Therapy

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ABSTRACT

BACKGROUND

Current hemophilia treatment involves frequent intravenous infusions of clotting factors, which is associated with variable hemostatic protection, a high treatment burden, and a risk of the development of inhibitory alloantibodies. Fitusiran, an investigational RNA interference (RNAi) therapy that targets antithrombin (encoded by *SERPINC1*), is in development to address these and other limitations.

METHODS

In this phase 1 dose-escalation study, we enrolled 4 healthy volunteers and 25 participants with moderate or severe hemophilia A or B who did not have inhibitory alloantibodies. Healthy volunteers received a single subcutaneous injection of fitusiran (at a dose of 0.03 mg per kilogram of body weight) or placebo. The participants with hemophilia received three injections of fitusiran administered either once weekly (at a dose of 0.015, 0.045, or 0.075 mg per kilogram) or once monthly (at a dose of 0.225, 0.45, 0.9, or 1.8 mg per kilogram or a fixed dose of 80 mg). The study objectives were to assess the pharmacokinetic and pharmacodynamic characteristics and safety of fitusiran.

RESULTS

No thromboembolic events were observed during the study. The most common adverse events were mild injection-site reactions. Plasma levels of fitusiran increased in a dose-dependent manner and showed no accumulation with repeated administration. The monthly regimen induced a dose-dependent mean maximum antithrombin reduction of 70 to 89% from baseline. A reduction in the antithrombin level of more than 75% from baseline resulted in median peak thrombin values at the lower end of the range observed in healthy participants.

CONCLUSIONS

Once-monthly subcutaneous administration of fitusiran resulted in dose-dependent lowering of the antithrombin level and increased thrombin generation in participants with hemophilia A or B who did not have inhibitory alloantibodies. (Funded by Alnylam Pharmaceuticals; ClinicalTrials.gov number, NCT02035605.)

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Hemophilia A and B are inherited bleeding disorders arising from insufficient thrombin generation to prevent bleeding that is caused by deficiencies in coagulation factors VIII and IX, respectively. Without effective treatment, patients have recurrent bleeding, which can be life-threatening and lead to disability from chronic hemarthropathy.[1]

Current management is based on replacement therapy with concentrates of factor VIII or IX, administered either on demand or prophylactically.[2] The benefits of prophylaxis therapy have been established,[3-6] but such treatment poses a substantial treatment burden.[7,8] Standard regimens require two or three intravenous infusions per week to maintain factor trough levels of more than 1 IU per deciliter, yet maintenance of higher levels may provide greater hemostatic protection.[9-12] The development of a new generation of factor concentrates with an extended half-life[13-15] has reduced the frequency of administration, most notably for factor IX products.[16-18] However, these products still require intravenous infusion. Finally, treatment with factor-replacement products can result in the development of inhibitory alloantibodies in up to 30% of patients with severe hemophilia A[19] and in 5% of those with hemophilia B,[20] which renders factor treatment ineffective.[21] Thus, there remains a major unmet need for new therapeutic agents that can provide safe, effective, and consistent prevention of bleeding, including in patients with inhibitory alloantibodies, while avoiding the development of such antibodies and reducing the treatment burden.

Genetic studies have identified potential alternative strategies to address insufficient thrombin generation in hemophilia. Evidence suggests that the coinheritance of deficiency in natural anticoagulants may ameliorate the hemophilia phenotype.[22-26] Reduced antithrombin levels are hypothesized to improve thrombin generation and promote hemostasis in hemophilia.[27] Thus, we are developing fitusiran (ALN-AT3SC), an investigational RNA interference (RNAi) therapy that specifically targets antithrombin messenger RNA (encoded by *SERPINC1*) to suppress the production of antithrombin in the liver, for the treatment of hemophilia. Nonclinical studies of fitusiran have shown dose-dependent lowering of antithrombin levels in multiple species. In murine hemophilia models, antithrombin reduction was associated with increased thrombin generation and enhanced hemostasis, which suggests a potential therapeutic benefit.[27]

In this study, we evaluated the pharmacokinetic and pharmacodynamic characteristics and safety of fitusiran in healthy volunteers and in participants with hemophilia A or B. In addition, we conducted a post hoc exploratory analysis to evaluate the effect of monthly fitusiran on bleeding frequency.

Methods

Study Oversight

This was a multicenter, international, open-label, dose-escalation study involving healthy volunteers and participants with hemophilia A or B. The study was initiated in January 2014 and conducted according to the principles of the International Conference on Harmonization for Good Clinical Practice, the World Health Organization Declaration of Helsinki, and the 1996 Health Insurance Portability and Accountability Act. All the participants provided written informed consent. The study protocol (available with the full text of this article at NEJM.org) was developed by the sponsor, Alnylam Pharmaceuticals, and was approved by the institutional review board or ethics committee at each participating center.

Two of the academic authors and authors employed by the sponsor were involved in the study design, and several authors who were not employed by the sponsor gathered the data. The first author and those employed by the sponsor analyzed the data. The first author and one author employed by the sponsor prepared the first draft of the manuscript with editorial assistance provided by Adelphi Communications, under contract with the sponsor. All the authors who were not employed by Alnylam, as well as their institutions, were required to maintain the confidentiality of the data until the completion of the study at all sites and the first publication of the results. All the authors made the decision to submit the manuscript for publication and assume responsibility for the completeness and integrity of the data and for the fidelity of the study to the protocol.

Study Design and Participants

The study was conducted in three sequentially enrolled phases. In the first phase (Part A), healthy volunteers received a single dose of fitu-

siran or placebo in a randomized, single-blind study. In the next two open-label phases, participants with hemophilia A or B were assigned to receive one of several ascending doses of fitusiran on a once-weekly basis (in Part B) or once-monthly basis (Part C).

In Part A, healthy men between the ages of 18 and 40 years with no known thrombophilic disorder or history of venous thromboembolism were randomly assigned in a 3:1 ratio to receive a single subcutaneous injection of fitusiran (at a dose of 0.03 mg per kilogram of body weight) or placebo. Higher doses were not administered because per-protocol antithrombin lowering was limited to 40% (60% of the residual level, relative to baseline) in healthy volunteers. In Parts B and C, men between the ages of 18 and 65 years who had moderate or severe hemophilia A or B and who had received previous prophylaxis were eligible to participate in the study if the prophylactic factor had been discontinued at least 5 days before the initiation of the study drug. Participants with a history of venous thromboembolism, a known coexisting thrombophilic disorder, liver dysfunction, or inhibitory alloantibodies were excluded. Full details regarding the inclusion and exclusion criteria are provided in the protocol.

In Part B, three cohorts of participants received three once-weekly subcutaneous injections of fitusiran at doses of 0.015, 0.045, or 0.075 mg per kilogram. In Part C, four cohorts of participants received three once-monthly subcutaneous injections of fitusiran at doses of 0.225, 0.45, 0.9, or 1.8 mg per kilogram, and a fifth cohort received three once-monthly subcutaneous injections of a fixed dose of 80 mg (which corresponded to a weight-based range of 0.92 to 1.3 mg per kilogram). The participants were followed for 56 days in Part A, 70 days in Part B, and 112 days in Part C until they transitioned to the open-label extension study or until antithrombin levels returned to more than 80% of the baseline value, whichever period was longest. Bleeding episodes were managed with factor VIII or IX replacement therapy.

OUTCOME MEASURES

Measurements of plasma antithrombin levels were determined by an activity-based chromogenic assay, and thrombin generation was determined by calibrated automated thrombogram assay.[28] Noncompartmental pharmacokinetic measurements were calculated from plasma samples with the use of Phoenix WinNonlin software, version 6.4 or higher. The safety evaluation included the monitoring of adverse events, clinical laboratory assessments (e.g., hematologic, biochemical, and coagulation measurements), vital signs, and 12-lead electrocardiography. All adverse events were categorized with the use of the *Medical Dictionary for Regulatory Activities*, version 16.0. The effect of fitusiran on the rate of bleeding was an exploratory end point.

STATISTICAL ANALYSIS

The sample size was not based on power calculations. The safety-analysis population included all the participants who had received at least a partial dose of fitusiran. The pharmacokinetic and pharmacodynamic populations included all the participants who had received at least one dose of fitusiran and had at least one plasma sample that could be evaluated. Statistical analyses were primarily descriptive and performed with the use of SAS software, version 9.2 or higher. Descriptive statistics were presented for continuous variables, and frequencies and percentages for categorical and ordinal variables. Percentages were based on the number of nonmissing values.

RESULTS

STUDY POPULATION

A total of 4 healthy volunteers were enrolled in Part A, 12 participants with hemophilia in Part B, and 18 participants with hemophilia in Part C (Fig. S1 in the Supplementary Appendix, available at NEJM.org). Five participants who completed Part B while enrollment was ongoing in Part C underwent additional screening and also participated in Part C (Table S1 in the Supplementary Appendix). In Parts B and C, 18 participants had hemophilia A and 7 had hemophilia B; the majority of the participants had severe disease. The baseline demographic and clinical characteristics are provided in Table 1; more detailed information according to dose cohort is provided in Table S2 in the Supplementary Appendix. The study population consisted of participants who had been receiving both on-demand and prophylaxis treatment regimens, so there was a diverse range in the reported annualized rates of bleeding before enrollment.

Table 1. Demographic and Clinical Characteristics of the Participants at Baseline.*

Characteristic	Part A (N=4)	Part B (N=12)	Part C (N=18)
Age (yr)			
Mean	31.5	37.5	36.6
Range	21–38	19–61	20–61
Weight (kg)			
Mean	86.5	79.3	75.8
Range	58.8–114.5	57.8–116.4	57.0–93.8
Type of hemophilia (no.)			
A	NA	10	13
B	NA	2	5
Disease severity (no.)			
Severe (<1% factor activity)	NA	12	15
Moderate (1–5% factor activity)	NA	0	3
Prestudy therapy (no.)			
On demand	NA	8	9
Prophylaxis	NA	4	9
No. of bleeding episodes per year†			
Median	NA	13	2
Range	NA	0–38	0–32
Previous hepatitis C virus infection (no.)	0	9	12

* Of the 25 participants who were enrolled in Parts B and C, 5 were enrolled in both cohorts, so these participants are listed twice in this table (i.e., data are for 30 participants). NA denotes not applicable because the volunteers in Part A did not have hemophilia.

† Each participant's historical annualized rate of bleeding and occurrence of clinically significant bleeding was based on a 6-month history.

PHARMACOKINETIC AND PHARMACODYNAMIC OUTCOMES

After the administration of fitusiran at doses ranging from 0.015 to 1.8 mg per kilogram, the mean peak plasma levels of the drug were generally observed after 2 to 6 hours. Fitusiran levels decreased rapidly in plasma, with a mean elimination half-life that ranged from 2.6 to 5.3 hours. Plasma levels of fitusiran increased in an approximately dose-proportional manner (Fig. 1). Plasma exposures were similar after the first and last doses, which indicated that there was no accumulation of fitusiran after repeated administration. Further pharmacokinetic details are provided in Table S3 in the Supplementary Appendix.

At baseline, the antithrombin levels were similar among the healthy volunteers (mean, 102.7%; range, 97.8 to 109.7%) and among the participants with hemophilia (mean, 103.3%; range, 84.9 to 121.0%). In the healthy volunteers, a single subcutaneous dose of fitusiran at 0.03 mg per kilogram elicited a mean (±SE) maximum antithrombin lowering of 19±4.4% on day 21, with recovery toward baseline values by day 56 (Fig. 2A). No further dose escalation was done in the healthy volunteers, and the study advanced to Part B.

In participants who received three once-weekly subcutaneous injections of fitusiran, a dose-dependent lowering of the antithrombin level from baseline was observed, with a mean maximum lowering of 61±8% at the highest dose of 0.075 mg per kilogram (Fig. 2B). Similarly, three once-monthly subcutaneous injections of fitusiran led to a dose-dependent mean maximum lowering of the antithrombin level, ranging from 70±9% at 0.225 mg per kilogram to 89±1% at 1.8 mg per kilogram (Fig. 2C). In the cohort of 6 participants who received a fixed

dose of 80 mg of fitusiran, the mean maximum lowering of the antithrombin level was 87±1% (Fig. 2C). A summary of dose-dependent antithrombin lowering according to dose cohort in Parts A, B, and C is shown in Figure S2 in the Supplementary Appendix. The minimum post-dose antithrombin level that was observed during the study was 9.8% (in the 80-mg cohort). After the discontinuation of fitusiran, the rate of recovery in the antithrombin level had a mean slope of 10 to 15% per month (Fig. 2C).

The association between the antithrombin level and thrombin generation showed that a lowering of the antithrombin level led to increased thrombin generation in the participants with hemophilia (Fig. 3). This relationship was similar in participants with hemophilia A and in those with hemophilia B. A reduction in the antithrombin level by more than 75% from baseline resulted in median peak thrombin values at the lower end of the range observed in healthy volunteers (Fig. S3 in the Supplementary Appendix).

In a post hoc exploratory analysis to determine the effect of monthly fitusiran administration on bleeding rates, there were fewer bleeding episodes per month after treatment with fitusiran than before treatment, a finding that was consistent with the reduction in antithrombin levels (Table S4 in the Supplementary Appendix). All the bleeding episodes were successfully managed with factor replacement (Table S5 in the Supplementary Appendix).

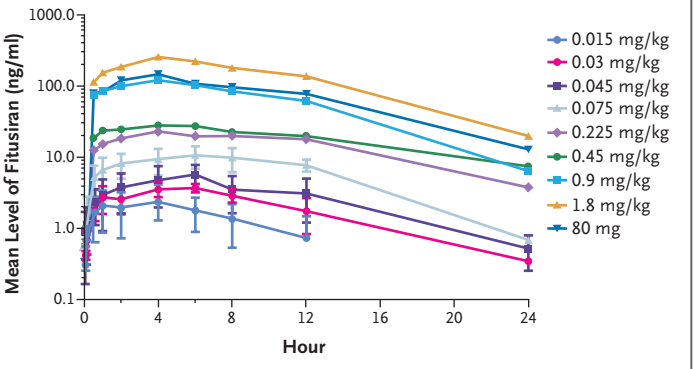

Figure 1. Pharmacokinetic Characteristics of Fitusiran in Plasma after Subcutaneous Injection.

Shown are mean plasma levels of fitusiran over time after a single subcutaneous injection, according to dose. The I bars represent standard errors, which were calculated only for cohorts with at least two participants.

SAFETY OUTCOMES

Adverse events were graded as mild, moderate, or severe. An adverse event was further classified as a serious adverse event if it met prespecified criteria.

Three healthy volunteers received a single dose of fitusiran at 0.03 mg per kilogram (one received placebo), and a total of five mild adverse events were reported in two of the volunteers who received fitusiran. Four of these events were considered by the investigators to be either not related or unlikely to be related to fitusiran, and one event (headache) was considered to be possibly related. All the adverse events resolved spontaneously. The adverse events that were reported in Part A are summarized in Table S6 in the Supplementary Appendix. There were no serious adverse events or discontinuations among the healthy volunteers.

In Parts B and C, 25 participants with hemophilia received fitusiran, and 19 participants (76%) reported having an adverse event during the study. Most of these events were mild to moderate in severity. (All the adverse events that occurred in at least 2 participants who received fitusiran are shown in Table 2, and in Table S7 in the Supplementary Appendix.) Nine of 25 participants (36%) in Parts B and C had adverse events that were considered to be related to fitusiran. Of these events, the most common were injection-site pain (in 6 participants [24%]), injection-site erythema (in 4 participants [16%]), and an increased alanine aminotransferase level (in 2 participants [8%]). More adverse events were reported in the cohort that received the 80-mg fixed dose than in the other cohorts, but this increased rate appears to have been driven mainly by a higher reporting of injection-site reactions and potentially by a larger group size (6 participants), which led to a higher number of adverse events in a single participant. Participants who received the higher weight-based dose of 1.8 mg per kilogram had a lower frequency of adverse events, so no clear conclusion with respect to the relationship between dose and adverse events can be made.

Three participants in Part C had adverse events that were graded as severe during the study. One participant who was receiving 0.45 mg of fitusiran per kilogram had severe, nonserious hypertriglyceridemia, tooth fracture, and toothache.

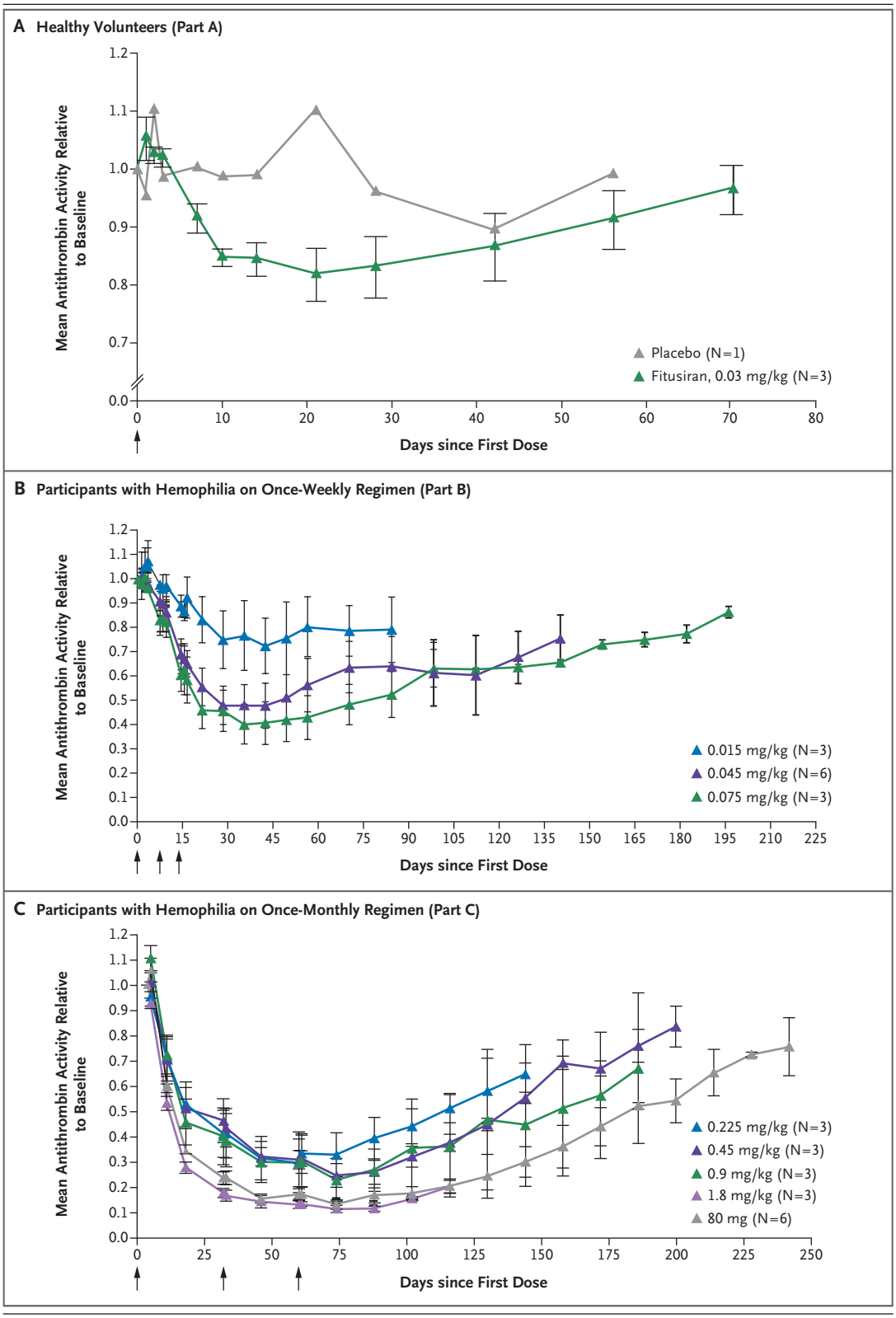

Figure 2 (facing page). Pharmacodynamic Characteristics of Fitusiran.

Shown are the mean plasma antithrombin levels among the study participants, normalized to the activity level at baseline, after a single injection of fitusiran in healthy volunteers (Panel A) and in participants with hemophilia after three once-weekly injections (Panel B) and after three once-monthly injections (Panel C). The arrows below the graph indicate the timing of the injections. The I bars represent standard errors, which were calculated only for cohorts with at least two participants.

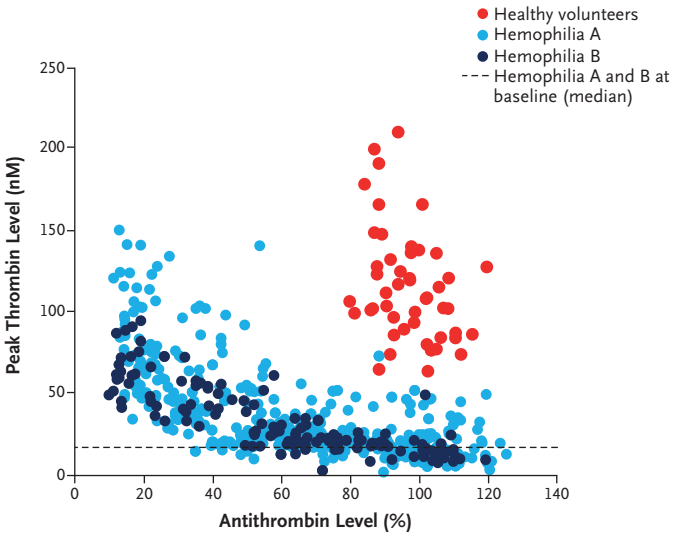

Figure 3. Relationship between Antithrombin Level and Thrombin Generation.

Shown are paired antithrombin levels and peak values for thrombin generation in all the participants with hemophilia A or B and in healthy volunteers for whom data were available. The dashed line shows the median baseline values for the participants with hemophilia A or B. Antithrombin levels were determined relative to a standard human plasma reagent with a defined antithrombin activity level calibrated against a World Health Organization reference.

A second participant, who was receiving 1.8 mg of fitusiran per kilogram, had a severe, serious event of viral pneumonia. A third participant who was receiving the fixed dose of 80 mg of fitusiran had a severe, nonserious event of noncardiac chest pain that was considered to be possibly drug-related on day 44 and which led to study discontinuation on day 45 (after the last dose of fitusiran had been received on day 28). The chest pain was accompanied by elevations in levels of C-reactive protein, alanine aminotransferase, aspartate aminotransferase, and D-dimer. Extensive evaluation did not reveal any substantial medical abnormalities; pulmonary embolism, portal-vein thrombosis, and deep-vein thrombosis were ruled out on repeated computed tomographic angiography and Doppler ultrasonography of the liver and lower limbs. Serologic results for viral hepatitis were negative. Ultrasonography of the abdomen showed the presence of gallbladder sludge, but a definitive diagnosis was not made. The event resolved with the use of analgesics and antacids, and laboratory values normalized.

Of the 25 participants in Parts B and C, 2 (8%) reported one serious adverse event each. One of these events was a reactivation of hepatitis C viral infection in a participant who had received 0.45 mg of fitusiran per kilogram (see the Supplementary Appendix for details), and the other was the above-mentioned viral pneumonia. Neither serious adverse event led to a discontinuation of fitusiran.

There were no clinically significant changes on physical examination, in vital signs, or in electrocardiographic measurements in any participant. Asymptomatic, transient elevations in the alanine aminotransferase level (>1.5 times the upper limit of the normal range or >1.5 times the baseline value [in participants with a baseline value that was above the upper limit of the normal range]) were observed in 9 of 25 participants (36%), but no events were associated with an increased bilirubin level or led to treatment discontinuation (Figs. S4 and S5 in the Supplementary Appendix). Of these 9 participants, 8 had peak elevations that were less than 3 times the upper limit of the normal range; 1 participant had an increase of more than 3 times the upper limit of the normal range in association with the above-mentioned chest pain. The 9 participants with elevated alanine aminotransferase levels were distributed across the dose cohorts, and no clear dose–response relationship could be determined (Table S8 in the Supplementary Appendix). Eight of the participants with an increased alanine aminotransferase level had a medical history of hepatitis C infection and had not received curative treatment. Elevations in the D-dimer level were observed in some participants; however, we were

Table 2. Adverse Events among the Participants Who Received Fitusiran.*

Event	All Participants (N=28)
	no. (%)
Any adverse event	27 (96)
Serious adverse event	2 (7)
Reactivation of hepatitis C virus infection	1 (4)
Viral pneumonia	1 (4)
Adverse event leading to study-drug discontinuation	1 (4)
Most common adverse events†	
Injection-site pain	6 (21)
Injection-site erythema	4 (14)
Arthralgia	4 (14)
Upper respiratory tract infection	
Any	3 (11)
Viral	2 (7)
Increase in alanine aminotransferase	2 (7)
Headache	2 (7)
Decrease in joint range of motion	2 (7)
Osteoarthritis	2 (7)
Pain in limb	2 (7)

* Listed are the data for all the participants who received fitusiran in Parts A, B, and C. Of these participants, data for the 5 who were enrolled in both Part B and Part C are listed only once in this table.

† All the listed events were reported during the study period in at least 2 participants.

unable to identify any dose–response relationships or consistent trends related to fitusiran treatment (Fig. S6 in the Supplementary Appendix). Furthermore, the changes in D-dimer levels were not accompanied by clinically significant alterations in coagulation markers, such as fibrinogen, prothrombin time, activated partial thromboplastin time, and platelets (Figs. S7 through S10 in the Supplementary Appendix).

Antibodies to fitusiran did not develop in any of the participants during the study. One participant had positive antidrug-antibody results before the administration of fitusiran and throughout the study.

DISCUSSION

Single-dose and weekly administration of fitusiran showed that the antithrombin-lowering effect could support a regimen with an interval beyond once-weekly doses. This observation led us to explore repeated administration of fitusiran in a once-monthly regimen. Monthly doses of 0.225 to 1.8 mg of fitusiran per kilogram resulted in a clear, nonlinear, dose-dependent lowering of antithrombin levels from baseline of 70±9% and 89±1%, respectively. Higher monthly weight-based doses of fitusiran led to incrementally lower levels of antithrombin, which prompted the exploration of a fixed 80-mg dose. The 80-mg fixed dose resulted in robust and predictable antithrombin lowering of 87±1%. After the onset of the effect, we observed minimal variation in antithrombin lowering during the monthly interval, which potentially allowed for more constant hemostatic protection between doses.

A relationship between reduced levels of antithrombin and increased thrombin generation was observed in the participants regardless of the type of hemophilia. Thrombin-generation values that were associated with the greatest lowering in antithrombin levels (>75% from baseline) were consistent with those reported for mild hemophilia,[29] which suggests that long-term fitusiran treatment may allow for the functional conversion of severe hemophilia to a milder clinical phenotype. An exploratory assessment of the effect of fitusiran on bleeding frequency revealed an apparent lower rate of bleeding episodes than before study enrollment. However, our study was not designed to assess efficacy, and larger studies of a longer duration of treatment are required to draw conclusions about the efficacy of fitusiran.

We observed no thromboembolic events during the study. One participant reported severe chest pain, and although thrombosis was ruled out, this event led to treatment discontinuation. No antidrug antibodies developed in healthy volunteers or in the participants with hemophilia during the study. The largest class of adverse events was injection-site reactions, which were mild in intensity; none required treatment or led to the discontinuation of fitusiran. We observed transient increases in liver aminotransferase values in 9 of 25 participants (36%). Because fitusiran is a liver-targeted agent, these increases may be drug-related; however, the small sample size and presence of other potential confounders precluded a clear determination of causality or dose relationship. Of note, a medical history of hepatitis C viral infection was reported in 16 of 25

participants (64%) in the study and in 8 of 9 participants (89%) with increased aminotransferase values. Therefore, it is possible that in some participants, the observed aminotransferase changes may have been due to chronic hepatitis C infection or to liver fibrosis caused by viral hepatitis.[30-32]

The study limitations include its open-label design, short duration, small number of participants who may not represent the overall population of those with hemophilia, and (with the exception of Part A) its lack of randomization and concurrent control group. Therefore, these results must be regarded as preliminary and require confirmation in larger clinical studies of longer duration. An extension study (ClinicalTrials.gov number, NCT02554773) is currently ongoing to allow longer-term assessment of the safety and efficacy of fitusiran. Although the mechanism of action of fitusiran is expected to be equally applicable to all patients with hemophilia regardless of the presence or absence of inhibitory alloantibodies, the presence of such antibodies was an exclusion criterion for Parts B and C of our study. To address this limitation, we have amended the study to include a Part D to enroll patients with such antibodies. Finally, the study does not address the use of fitusiran in high-risk settings such as surgery or trauma. Additional clinical data will need to be generated with fitusiran in such settings.

In conclusion, we found that the subcutaneous injection of fitusiran lowered antithrombin levels and increased thrombin generation in participants with hemophilia A or hemophilia B. In addition, the study provided information on the side-effect profile of this investigative agent.

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

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APPENDIX

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