ORIGINAL ARTICLE

Phase 1 Trial of an RNA Interference Therapy for Acute Intermittent Porphyria

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ABSTRACT

BACKGROUND

Induction of delta aminolevulinic acid synthase 1 (*ALAS1*) gene expression and accumulation of neurotoxic intermediates result in neurovisceral attacks and disease manifestations in patients with acute intermittent porphyria, a rare inherited disease of heme biosynthesis. Givosiran is an investigational RNA interference therapeutic agent that inhibits hepatic *ALAS1* synthesis.

METHODS

We conducted a phase 1 trial of givosiran in patients with acute intermittent porphyria. In part A of the trial, patients without recent porphyria attacks (i.e., no attacks in the 6 months before baseline) were randomly assigned to receive a single subcutaneous injection of one of five ascending doses of givosiran (0.035, 0.10, 0.35, 1.0, or 2.5 mg per kilogram of body weight) or placebo. In part B, patients without recent attacks were randomly assigned to receive once-monthly injections of one of two doses of givosiran (0.35 or 1.0 mg per kilogram) or placebo (total of two injections 28 days apart). In part C, patients who had recurrent attacks were randomly assigned to receive injections of one of two doses of givosiran (2.5 or 5.0 mg per kilogram) or placebo once monthly (total of four injections) or once quarterly (total of two injections) during a 12-week period, starting on day 0. Safety, pharmacokinetic, pharmacodynamic, and exploratory efficacy outcomes were evaluated.

RESULTS

A total of 23 patients in parts A and B and 17 patients in part C underwent randomization. Common adverse events included nasopharyngitis, abdominal pain, and diarrhea. Serious adverse events occurred in 6 patients who received givosiran in parts A through C combined. In part C, all 6 patients who were assigned to receive once-monthly injections of givosiran had sustained reductions in *ALAS1* messenger RNA (mRNA), delta aminolevulinic acid, and porphobilinogen levels to near normal. These reductions were associated with a 79% lower mean annualized attack rate than that observed with placebo (exploratory efficacy end point).

CONCLUSIONS

Once-monthly injections of givosiran in patients who had recurrent porphyria attacks resulted in mainly low-grade adverse events, reductions in induced *ALAS1* mRNA levels, nearly normalized levels of the neurotoxic intermediates delta aminolevulinic acid and porphobilinogen, and a lower attack rate than that observed with placebo. (Funded by Alnylam Pharmaceuticals; ClinicalTrials.gov number, NCT02452372.)

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Acute hepatic porphyrias are rare, serious, and life-threatening genetic diseases caused by mutations in hepatic heme biosynthesis enzymes.[1-5] The neurotoxic heme intermediates delta aminolevulinic acid (ALA) and porphobilinogen (PBG) accumulate in patients with acute hepatic porphyrias, leading to acute debilitating neurovisceral attacks and, in some patients, disabling chronic symptoms.[2,3,6-8]

The prevalence of mutations among patients with acute intermittent porphyria (the most common subtype of acute hepatic porphyria) is approximately 1 in 1700 in Western countries,[9,10] although disease penetrance is low, with less than 10% of patients ever having disease symptoms develop.[1] Most symptomatic patients have only a few attacks in their lifetime; however, approximately 5 to 8% of symptomatic patients with acute intermittent porphyria have recurrent attacks (≥4 attacks per year), which manifest primarily as severe diffuse abdominal pain typically requiring urgent medical attention or hospitalization.[6,9-11] No pharmacotherapies for the prevention of attacks are approved, although nontherapeutic measures, such as trigger avoidance (e.g., porphyrinogenic drugs and fasting), have proved successful in preventing attacks in some patients.[11-13] Intravenous hemin is approved for the treatment of acute attacks and is sometimes used prophylactically for the prevention of attacks.[14-16] However, hemin has a short duration of action, requires venous access (often through an indwelling venous catheter), and can have associated side effects (e.g., infusion-site reactions and headache); long-term administration may lead to iron overload, venous scarring, and catheter-related infection.[14,16] In refractory cases, liver transplantation has been performed; gene therapy is currently in early stages of research.[17-19]

Givosiran is an investigational RNA interference therapeutic agent intended to specifically lower hepatic delta aminolevulinic acid synthase 1 (*ALAS1*) messenger RNA (mRNA) levels, thereby decreasing neurotoxic ALA and PBG accumulation. Nonclinical studies of givosiran in rodent models of acute hepatic porphyria have shown dose-dependent reductions in *ALAS1* mRNA, ALA, and PBG levels, suggesting a potential therapeutic effect.[20]

In the current trial, we evaluated the safety, pharmacokinetic, and pharmacodynamic profiles of givosiran in patients with mutation-confirmed acute intermittent porphyria who had elevated urinary ALA and PBG levels but did not have recent attacks and in those who had recurrent attacks. Exploratory analyses were performed to evaluate the effect of givosiran on rates of porphyria attacks and hemin use among the patients who had recurrent attacks.

Methods

Trial Design and Oversight

This multicenter, randomized, placebo-controlled, phase 1 trial involving patients with acute intermittent porphyria was conducted in three parts. Part A (single injection of an ascending dose) and part B (multiple injections of an ascending dose) were single-blind trials involving patients who had elevated urinary ALA and PBG levels but did not have recent attacks (defined as having no attacks in the 6 months before baseline); these patients were designated as having chronic high excretion. Part C (multiple injections) was a double-blind trial involving patients who had recurrent attacks. Patients were recruited from specialist porphyria centers.

The trial was sponsored by Alnylam Pharmaceuticals and was designed through a collaboration among the sponsor, the principal investigators, and porphyria disease specialists. The trial was approved by central and local institutional review boards or ethics committees and was conducted in accordance with the principles of the International Conference on Harmonisation Good Clinical Practice guidelines, the World Health Organization Declaration of Helsinki, and the 1996 Health Insurance Portability and Accountability Act. All patients provided written informed consent. A safety review committee conducted periodic assessments. Data were collected by trial investigators and staff and were analyzed by the sponsor. Sponsor-employed authors prepared the first draft of the manuscript with editorial assistance provided by Adelphi Communications, under contract with Alnylam Pharmaceuticals. All the authors interpreted the data, collaborated in preparing the manuscript, and vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol, available with the full text of this article at NEJM.org. All the authors, their institutions, and the sponsor were required to maintain data confidentiality during the trial.

Trial Patients

Eligible patients were men and women 18 to 65 years of age who had received a diagnosis of acute

intermittent porphyria and had a confirmed pathogenic mutation in the hydroxymethylbilane synthase (also known as PBG deaminase) gene (*HMBS*). Patients were eligible for parts A and B if they had a urinary PBG level greater than 4 mmol per mole of creatinine (roughly two times the upper limit of the normal range) at screening and no attacks in the 6 months before baseline.[21] Patients with recurrent attacks (defined as ≥2 attacks within 6 months before the run-in period or receiving scheduled hemin prophylaxis at the start of the run-in period) were eligible for part C. Patients who had been receiving scheduled hemin prophylaxis were eligible to participate if they were willing to discontinue the treatment during the run-in and intervention periods (full eligibility criteria are provided in the protocol).

RANDOMIZATION AND TRIAL REGIMEN

In part A, patients with chronic high excretion were randomly assigned, in a 3:1 ratio, to receive a single subcutaneous injection of one of five ascending doses of givosiran (0.035, 0.10, 0.35, 1.0, or 2.5 mg per kilogram of body weight) or placebo. In part B, patients with chronic high excretion were randomly assigned, in a 3:1 ratio, to receive once-monthly injections of one of two doses of givosiran (0.35 or 1.0 mg per kilogram) or placebo (total of two injections 28 days apart). Patients were followed for 42 days after the single injection (part A) or 70 days after the first injection (part B) or until urinary ALA or PBG levels returned to within 80% of the baseline value, whichever occurred first; if the levels had not returned to at least 80% of the baseline value by days 42 or 70, the patients were followed for 1.5 years or until the ALA or PBG levels returned to within 80% of the baseline value. If the levels had not returned to within 80% of the baseline value by 1.5 years, patients were allowed to complete their end-of-trial visit at the discretion of the investigator.

In part C, patients were followed during a run-in period (4 to 24 weeks) and were required to have had at least one attack before randomization. The patients were randomly assigned, in a 3:1 ratio, to receive injections of one of two doses of givosiran (2.5 or 5.0 mg per kilogram) or placebo once monthly (total of four injections) or once quarterly (total of two injections) during a 12-week period, starting on day 0. The patients were followed for an additional 12 weeks after the last injection. Investigators managed attacks according to the local standard of care, which could include use of intravenous hemin.

SAFETY ASSESSMENTS AND OUTCOME MEASURES

Safety assessments included monitoring of adverse events, clinical laboratory assessments, vital signs, 12-lead electrocardiography, and physical examination. Attacks were identified with the use of adverse-event data from case-report forms and were excluded from the summary tables of adverse events and serious adverse events. All adverse events were categorized according to the *Medical Dictionary for Regulatory Activities*, version 17.1, and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

Pharmacodynamic monitoring included measurements of urinary ALA and PBG levels and the circulating hepatic *ALAS1* mRNA level. Urinary ALA and PBG levels (measured as millimoles per mole of urinary creatinine) were determined with the use of the ALA/PBG test (Bio-Rad). *ALAS1* mRNA levels were measured with the use of a circulating extracellular RNA detection assay of exosomes isolated from both serum and urine as previously described.[22,23] Noncompartmental pharmacokinetic measurements were calculated from plasma and urine samples with the use of Phoenix WinNonlin software, version 7.0 or higher (Certara USA).

In part C, the effect of givosiran on rates of porphyria attacks and hemin use were exploratory end points. Attacks were defined as those leading to hospitalization, urgent health care visits, or the use of intravenous hemin at home.

STATISTICAL ANALYSIS

Sample size was not determined on the basis of power calculations. The safety analysis population included all patients in parts A, B, and C who had received at least one injection of givosiran or placebo. The pharmacokinetic-analysis and pharmacodynamic-analysis populations included all patients who had received at least one injection of givosiran or placebo and, for the pharmacokinetic-analysis population, had at least one blood sample that could be evaluated or, for pharmacodynamic-analysis population, had at least one blood or urine sample that could be evaluated. In each trial part, data from the patients who received placebo were combined across cohorts for analysis. Statistical analyses were performed with the use of SAS software, version 9.4 or

higher (SAS Institute). Descriptive statistics are reported for continuous variables, and frequencies and percentages for categorical and ordinal variables. Event count data (numbers of porphyria attacks and doses of hemin administered) are summarized as annualized rates with the standard errors of the mean. A negative binomial regression was fitted to generate statistical inferences on the ratio of annualized attack rate and the annualized number of hemin doses administered in each cohort in part C.

RESULTS

TRIAL POPULATION

The randomization and follow-up of patients are summarized in Figure S1 in the Supplementary Appendix, available at NEJM.org. Enrollment of patients began on May 6, 2015, and 4 patients were enrolled before the trial was registered at ClinicalTrials.gov (May 22, 2015) owing to a misunderstanding of the required timing for trial registration. A total of 23 patients with chronic high excretion underwent randomization in parts A and B (20 were assigned to receive givosiran and 5 to receive placebo; 3 patients participated in both parts A and B, and 2 patients in part A received two injections [either one placebo and one givosiran injection or two givosiran injections at different doses]). A total of 17 patients with acute intermittent porphyria who had recurrent attacks underwent randomization in part C (13 were assigned to receive givosiran and 4 to receive placebo). The baseline demographic and clinical characteristics of the patients are shown in Table 1, and specific *HMBS* mutations in the patients are summarized in Table S1 in the Supplementary Appendix. Part C comprised patients who had received as-needed or prophylactic hemin treatment before the start of the run-in period; consequently, there was a range in the reported annualized attack rate before randomization. The baseline demographic and clinical characteristics of the patients with porphyria disease were generally balanced between the placebo group and the combined givosiran groups in part C (Table 1).

SAFETY OUTCOMES

The most common adverse events across trial parts A through C were abdominal pain, nausea, diarrhea, and nasopharyngitis (Table 2). In parts A and B, all adverse events were mild to moderate in severity, and the proportions of patients (those who received givosiran and those who received placebo) who reported adverse events and serious adverse events in each cohort were similar; no patient discontinued the trial regimen.

In part A, a serious adverse event of abdominal pain occurred in two patients who received givosiran (one in the 0.035-mg-per-kilogram cohort and one in the 1.0-mg-per-kilogram cohort) (Table S2 in the Supplementary Appendix). In part B, there was a serious adverse event of spontaneous abortion in a patient at 7 weeks after conception (90 days after the last givosiran dose of 1.0 mg per kilogram) (see the Supplementary Appendix).

In part C, the 4 patients (100%) who received placebo and the 13 patients (100%) who received at least two injections of givosiran had an adverse event. Most were mild to moderate in severity, and the proportion of patients who had a severe adverse event was similar in the placebo group and the combined givosiran groups (Table S3 in the Supplementary Appendix). Injection-site reactions were the only adverse events reported in more than 2 patients (3 patients [23%]); all were mild to moderate in severity and resolved spontaneously. No clinically significant changes in laboratory measures or in findings from physical examinations were noted.

A total of five serious adverse events were reported in three patients who received givosiran in part C (Table S2 in the Supplementary Appendix). In two patients, the adverse events (influenza A infection and opioid-induced bowel dysfunction) resolved with treatment. The other three serious adverse events (*Staphylococcus epidermidis* bacteremia, auditory hallucination, and hemorrhagic pancreatitis, which was subsequently fatal) occurred in a single patient who received once-monthly injections of givosiran (5.0 mg per kilogram). This patient had a complex medical history (obesity, hypertension, bacteremia, and quadriparesis from acute intermittent porphyria) and a clinical course that had been complicated by a delay in treatment and hospital admission and complications from an acute pulmonary thromboembolism that resulted in right heart failure (see the Supplementary Appendix). The patient was found to have gallbladder sludge on ultrasonographic imaging at the time of presentation. Subsequent monitoring of other patients did not show any clinically meaningful elevations in lipase levels during this trial (see the Supplementary Appendix).

Table 1. Demographic and Clinical Characteristics of the Patients with Acute Intermittent Porphyria at Baseline.*

Characteristic	Parts A and B Combined (N=23)	Part C	
		Patients Who Received Givosiran (N=13)	Patients Who Received Placebo (N=4)
Median age (range) — yr	47 (30–64)	36 (21–59)	42 (27–60)
Female sex — no. (%)	18 (78)	13 (100)	2 (50)
Body weight — kg	75.9±15.9	70.9±14.5	91.4±20.8
Race — no. (%)†			
White	22 (96)	10 (77)	4 (100)
Asian	1 (4)	1 (8)	0
Black	0	2 (15)	0
Previous therapy for porphyria — no. (%)	NA		
Hemin prophylaxis		6 (46)	2 (50)
GnRH analogue		4 (31)	0
Long-term opioid use		7 (54)	2 (50)
Median no. of porphyria attacks in the past 12 mo (range)	NA	9 (0–36)	10 (5–50)
Urinary ALA level — mmol/mole of creatinine‡	23.1±3.1	37.8±6.5	43.1±9.8
Urinary PBG level — mmol/mole of creatinine‡	24.8±3.6	38.9±5.8	39.2±4.6
Urinary *ALAS1* mRNA level — factor change relative to normal	1.9±0.2	4.0±0.5	2.9±0.4

* Plus–minus values are means ±SD (for body weight) or ±SE (for urinary delta aminolevulinic acid [ALA], porphobilinogen [PBG], and ALA synthase 1 [*ALAS1*] mRNA levels). Hypothesis testing was not performed for the baseline comparisons owing to the small sample size involved. Givosiran and placebo group data are not displayed separately for parts A and B because they represent the same patient population and the efficacy evaluations were only performed among the patients who had recurrent attacks. GnRH denotes gonadotropin-releasing hormone analogue, mRNA messenger RNA, and NA not applicable.

† Race was reported by the patients.

‡ The upper limit of the normal range was 3.8 mmol per mole of creatinine for urinary ALA level and 1.5 mmol per mole of creatinine for urinary PBG level.[24]

Antibodies to givosiran did not develop in any of the patients during the trial. Two patients had positive antidrug antibody results before the administration of givosiran; the antidrug antibody levels did not increase during the trial.

PHARMACOKINETIC AND PHARMACODYNAMIC MEASUREMENTS

Pharmacokinetic profiles showed dose-proportional characteristics for givosiran (Fig. S2 and Table S4 in the Supplementary Appendix). The maximum serum concentration and area-under-the-curve values were similar after the first and second injections of givosiran (either 0.35 or 1.0 mg per kilogram [part B]), indicating no drug accumulation with repeated injections (additional pharmacokinetic data are provided in the Supplementary Appendix).

Baseline *ALAS1* mRNA levels were higher than normal by a factor of 2 in patients with chronic high excretion and by a factor of 3 to 4 in those with recurrent attacks (Table 1, and Fig. S3 in the Supplementary Appendix). In part A, a single 2.5-mg-per-kilogram dose of givosiran led to a rapid, dose-dependent reduction from baseline in the urinary *ALAS1* mRNA level (mean [±SE] maximum reduction, 86±8%) (Fig. 1A). The reduction in the *ALAS1* mRNA level with a single 2.5-mg-per-kilogram dose led to rapid, dose-dependent reduction from baseline in the urinary ALA level (Fig. 1B) and PBG level (Fig. 1C) (maximum reductions, 91±3% and 96±1%, respectively). In part A, the reduction in the *ALAS1* mRNA level was highly correlated with the urinary ALA level (Pearson correlation coefficient, 0.79; P<0.001) and PBG level (Pearson correlation coefficient, 0.80; P<0.001). Urinary ALA level was also highly correlated with PBG level (Pearson correlation coefficient, 0.84; P<0.001).

In part B, reductions in the *ALAS1* mRNA,

Table 2. Adverse Events That Occurred during the Intervention Period among Patients Who Received Givosiran or Placebo (Parts A, B, and C Combined).

Adverse Event*	Patients Who Received Givosiran (N=33)†	Patients Who Received Placebo (N=10)
	no. of patients (%)	
Any adverse event	30 (91)	10 (100)
Any serious adverse event‡	6 (18)	0
Any severe adverse event§	4 (12)	2 (20)
Most common adverse events¶		
Nasopharyngitis	9 (27)	2 (20)
Abdominal pain	8 (24)	1 (10)
Nausea	6 (18)	3 (30)
Diarrhea	4 (12)	1 (10)
Back pain	3 (9)	2 (20)
Fatigue	3 (9)	0
Headache	3 (9)	2 (20)
Injection-site reaction	3 (9)	0
Oropharyngeal pain	3 (9)	0
Rash	3 (9)	0
Vomiting	3 (9)	3 (30)

* If a given adverse event occurred more than once in a patient, that patient was counted only once for that adverse event. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. Severe adverse events were those of grade 3 or higher, and serious adverse events were those that resulted in death, were life-threatening, required hospitalization or prolongation of an existing hospitalization, resulted in persistent or significant disability or incapacity, or required an intervention to prevent one of the previous items.

† Two patients in part A received two injections (either one placebo and one givosiran injection or two givosiran injections at different doses). Three patients were treated in both part A and part B.

‡ Serious adverse events included abdominal pain (2 patients) and spontaneous abortion, influenza A infection, functional gastrointestinal disorder (opioid bowel dysfunction), staphylococcal bacteremia, auditory hallucination, and hemorrhagic pancreatitis (1 patient each). Further details are provided in Table S2 in the Supplementary Appendix.

§ In part B, a severe adverse event occurred in a givosiran-treated patient (elbow bursitis). In part C, severe adverse events occurred in three givosiran-treated patients (influenza A infection and pain in an extremity [after a fall], functional gastrointestinal disorder [opioid bowel dysfunction], staphylococcal bacteremia, auditory hallucination, and hemorrhagic pancreatitis) and in two placebo-treated patients (viral gastroenteritis and diarrhea).

¶ The adverse events listed are those that occurred in more than two patients.

ALA, and PBG levels similar to those in part A occurred after the patients received two once-monthly injections of givosiran (0.35 or 1.0 mg per kilogram) (Fig. S4 in the Supplementary Appendix). The *ALAS1* mRNA, ALA, and PBG levels in the patients in both dose cohorts were still below baseline at day 70.

In part C, among the patients who had recurrent attacks, two once-quarterly injections of givosiran resulted in maximum reductions in *ALAS1* mRNA level of 49±3% in the 2.5-mg-per-kilogram cohort and 53±7% in the 5.0-mg-per-kilogram cohort (Fig. 2A). Among the patients who received four once-monthly injections of givosiran, the maximum reductions in *ALAS1* mRNA level from baseline were 67±3% in the 2.5-mg-per-kilogram cohort and 74±6% in the 5.0-mg-per-kilogram cohort (Fig. 2A). Residual *ALAS1* mRNA levels after once-monthly injections of givosiran were at or above the levels seen in healthy persons.[22] The reductions in *ALAS1* mRNA level with givosiran were sustained until the end of the trial (day 168); there was no evidence of *ALAS1* mRNA returning to baseline levels at day 168 in the two dose cohorts that received once-monthly injections.

The reduction in *ALAS1* mRNA level was accompanied by rapid and sustained reductions in ALA and PBG levels, with a mean maximum reduction from baseline of greater than 90% at both once-monthly doses (Fig. 2B and 2C). Once-monthly injections of givosiran led to greater, sustained reductions in ALA and PBG levels and lower peak-to-trough fluctuations than once-quarterly injections. Normalization of ALA and PBG levels occurred in both once-monthly dose cohorts (the upper limit of the normal range was 3.8 mmol per mole of creatinine for ALA level and 1.5 mmol per mole of creatinine for PBG level).

EXPLORATORY END POINTS

During the intervention period of part C (days 0 to 168), the mean annualized attack rate among the patients who received givosiran was 7.2, as compared with 16.7 among the patients who received placebo (a 57% difference) (Fig. 3A). The mean annualized attack rate was 79% lower among the patients who received two once-monthly injections of givosiran (83% lower among those who received the 2.5-mg-per-kilogram dose and 75% lower among those who received the 5.0-mg-per-kilogram dose) than among those who received placebo. The mean annualized attack rate during the intervention period was also reduced by 56% from the run-in period among the patients who received givosiran, as compared with an 18% reduction among the patients who received placebo (Table S5 in the Supplementary Appendix). When the percentage reduction in ALA level from baseline was divided into five categories (0% [increase or no reduction], >0 to 25%, >25 to 50%, >50 to 75%, and >75%) among all patients in part C (those who received placebo or

givosiran), an association was seen between lowering of ALA level and annualized attack rate — the patients who had the greatest reduction in ALA level had the lowest annualized attack rate (Fig. S5 in the Supplementary Appendix).

The annualized number of hemin doses administered among the patients who received givosiran during the intervention period was 12.1, as compared with 23.4 among the patients who received placebo (a 48% difference) (Fig. 3B). The annualized number of hemin doses administered during the intervention period was reduced by 64% from the run-in period among the patients who received givosiran, as compared with a 34% reduction among the patients who received placebo (Table S6 in the Supplementary Appendix; for patient-level data, see Table S7 in the Supplementary Appendix). The patients who completed part C were enrolled in an ongoing open-label extension study (ClinicalTrials.gov number, NCT02949830).

DISCUSSION

In the current trial, treatment with givosiran, an RNA interference therapeutic agent that targets *ALAS1* to reduce ALA and PBG levels, was associated with mainly mild-to-moderate reversible adverse events in patients with acute intermittent porphyria who had recurrent attacks. Once-monthly treatment with givosiran resulted in sustained reductions in *ALAS1* mRNA, ALA, and PBG levels to near normal. These reductions were accompanied by lower annualized rates of porphyria attacks and hemin use than those observed with placebo and those observed during the run-in period (exploratory end points).

There was no clear difference in the proportion of patients who reported adverse events and severe adverse events between the placebo group and the givosiran group, and there was no clear relationship between givosiran dose and the incidence of adverse events. The most frequently reported adverse events were characteristic symptoms of acute intermittent porphyria[1] and were observed both among the patients who received givosiran and among those who received placebo. Injection-site reactions were reported in six patients who received givosiran (18%) and in no patients who received placebo; all reactions were mild to moderate in severity.

One patient had a serious adverse event of spontaneous abortion at 7 weeks after concep-

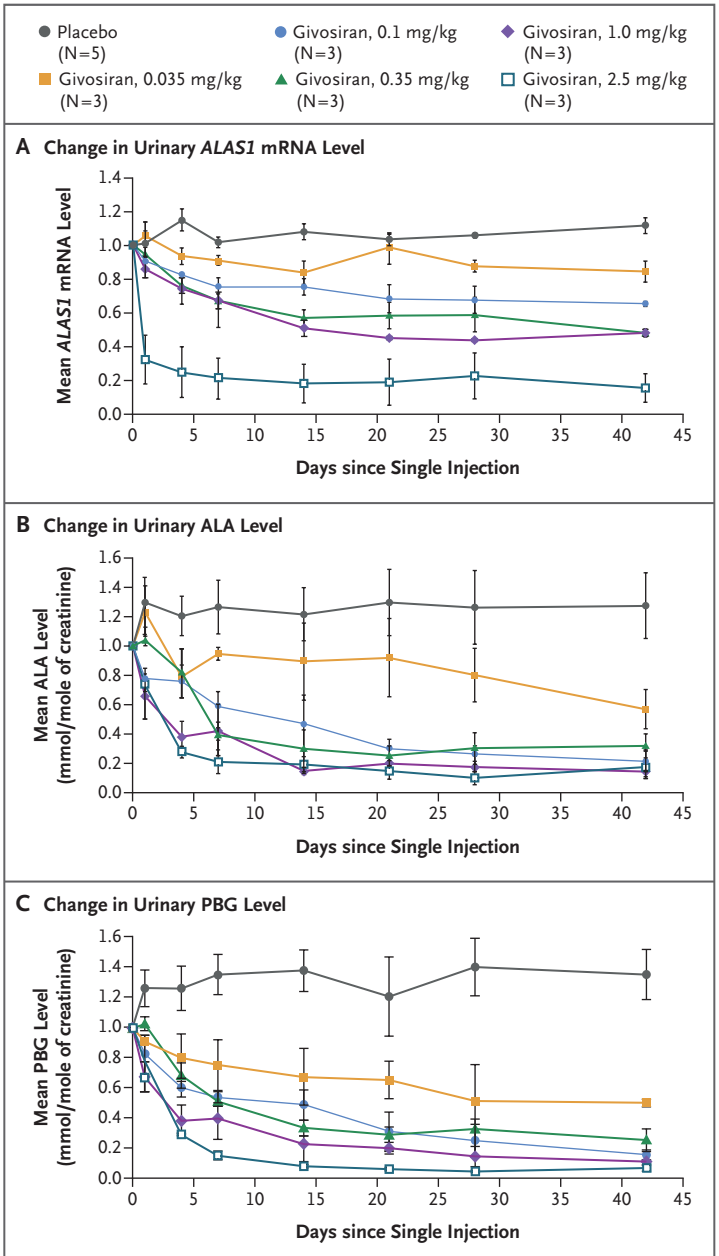

Figure 1. Pharmacodynamic Characteristics of a Single Ascending Dose of Givosiran in Patients with Chronic High Excretion (Part A).

Changes are relative to baseline. In part A of the trial, patients with acute intermittent porphyria who had elevated urinary levels of delta aminolevulinic acid (ALA) and porphobilinogen (PBG) but did not have current attacks (who were designated as having chronic high excretion) were randomly assigned to receive a single subcutaneous injection of one of five ascending doses of givosiran (0.035, 0.10, 0.35, 1.0, or 2.5 mg per kilogram of body weight) or placebo (day 0). ALA synthase 1 (*ALAS1*) messenger RNA (mRNA) levels were measured with the use of a circulating extracellular RNA detection assay of exosomes isolated from urine; the results were normalized to levels found in normal healthy persons (Panel A).[22,23] Urinary ALA (Panel B) and PBG (Panel C) levels were normalized to creatinine concentration. I bars indicate standard errors.

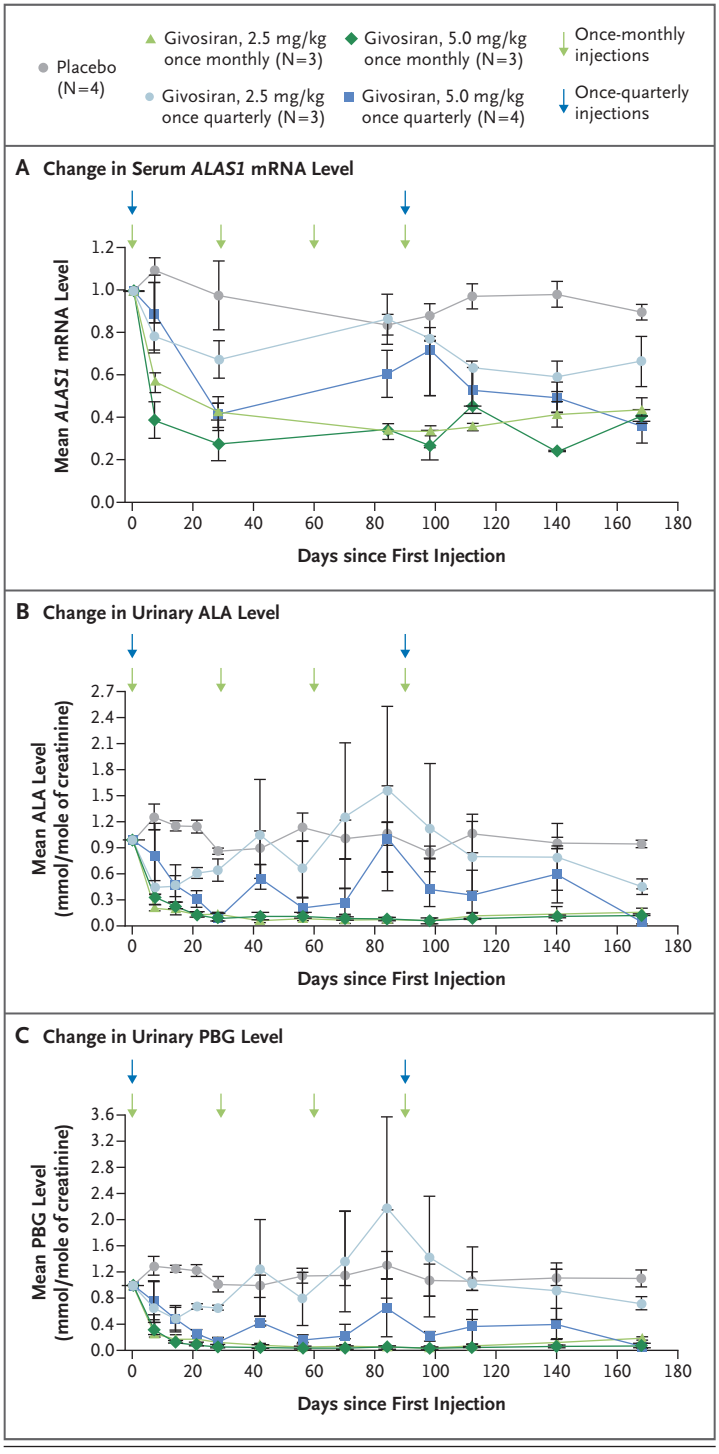

Figure 2. Pharmacodynamic Characteristics of Multiple Ascending Doses of Givosiran in Patients with Recurrent Attacks (Part C).

Changes are relative to baseline. In part C of the trial, patients with acute intermittent porphyria who had recurrent attacks were randomly assigned to receive injections of one of two doses of givosiran (2.5 or 5.0 mg per kilogram) or placebo once monthly (total of four injections) or once quarterly (total of two injections) during a 12-week period, starting on day 0. *ALAS1* mRNA levels were measured with the use of a circulating extracellular RNA detection assay of exosomes isolated from serum; the results were normalized to levels found in normal healthy persons (Panel A).[22,23] Urinary levels of ALA (Panel B) and PBG (Panel C) levels were normalized to creatinine concentration. I bars indicate standard errors.

tion (90 days after the last givosiran dose). We were unable to determine whether this event was a spontaneous abortion independent of treatment because the rate of such events is approximately 20% of all pregnancies,[25] and some studies suggest that the frequency of spontaneous abortion may be higher among patients with acute intermittent porphyria than among healthy women.[26,27] Preclinical biodistribution studies have shown that givosiran is predominantly distributed to the liver, with exposure levels in extrahepatic tissues so low that they would not be expected to result in pharmacodynamic activity. To minimize risks in pregnancy, the use of a highly effective method of birth control while women are taking givosiran is required in all protocols.

One patient with multiple coexisting medical complications and a delay in treatment had a fatal serious adverse event of hemorrhagic pancreatitis. Although the investigator considered that this event was unlikely to be related to givosiran because of the identification of a plausible alternative cause (gallbladder sludge) and the lack of a clear temporal relationship to trial-drug injections, we cannot rule out a causal relationship. In the interests of safety going forward, monthly lipase monitoring was initiated, and no other cases of pancreatitis or lipase elevations greater than three times the upper limit of the normal range in the current trial and the ongoing open-label extension study have been identified to date.

Circulating *ALAS1* mRNA levels confirmed the central importance of liver *ALAS1* induction in the pathophysiology of acute intermittent porphyria, and the higher levels observed in patients with recurrent attacks than in those without attacks showed an association between basal *ALAS1* mRNA levels and disease activity. Greater *ALAS1* induction in patients with recurrent attacks required once-monthly injections of givosiran to attain sustained reductions in the levels of *ALAS1* mRNA (approximately 60% reduction from baseline) and ALA and PBG (approximately 80% reduction from baseline for both) to near normal,

whereas less frequent injections were required in patients with chronic high excretion.[21,22]

It is hypothesized that blocking the heme synthesis pathway could lead to heme deficiency and dysfunction associated with defects in other enzymes containing heme. However, there is little evidence of heme deficiency in the liver of patients with acute intermittent porphyria, as exemplified by normal microsomal heme content and cytochrome P450 isozyme activity in a severely affected patient who underwent liver transplantation.[28] Preclinical research in givosiran-treated mice showed that a 75% reduction in the *Alas1* mRNA level (with a residual level of 25%) did not decrease cytochrome P450 2E1 activity or tryptophan pyrrolase saturation.[20] In the current trial, patients treated with givosiran in part C had residual *ALAS1* mRNA levels at or above the levels found in normal healthy persons, making heme deficiency in the liver unlikely.

Nonclinical and clinical data support the role of ALA as the key mediator of disease manifestations in patients with acute hepatic porphyrias, although a contribution from PBG has not been fully ruled out.[29] In vitro studies have shown that ALA induces oxidative stress, inflammation, and cell death in multiple cell types and potentially binds to γ-aminobutyric acid receptors in the central nervous system.[30-32] In addition, acute symptoms similar to those seen in patients with acute intermittent porphyria occur in patients with ALA dehydratase deficiency porphyria, hereditary tyrosinemia, and lead poisoning, conditions that are associated with increased urinary ALA levels but not PBG levels.[1,3,29] Data from observational studies show that higher ALA levels are associated with greater disease activity, whereas interventions that lower ALA levels (e.g., hemin therapy or liver transplantation) result in the resolution of acute attacks.[19,33,34] In the current trial, a graded relationship was observed between lowering of the ALA level and reduction in annualized attack rate (an exploratory end point). The reduction in attack rate was enhanced with once-monthly givosiran injections, as compared with once-quarterly injections, owing to more sustained reductions in *ALAS1* mRNA and ALA levels. Whether lowering ALA and PBG levels in a sustained manner with givosiran has the potential to affect chronic porphyria symptoms requires further investigation.

In addition to the reduced attack rate, treatment with givosiran reduced the rate of as-needed hemin use (an exploratory end point). Decreasing the number of hemin doses needed to treat acute attacks can potentially reduce the burden of frequent (often weekly) intravenous infusions through a large vein or a chronic indwelling catheter and the side effects associated with long-term administration.[16,34]

Study limitations included a short intervention period and small numbers of patients. These phase 1 results require confirmation in a larger study with the other subtypes of acute hepatic porphyria and a longer treatment duration.

In this small and short phase 1 trial involving patients with acute intermittent porphyria, once-monthly treatment with givosiran was associated with mainly reversible mild-to-moderate adverse

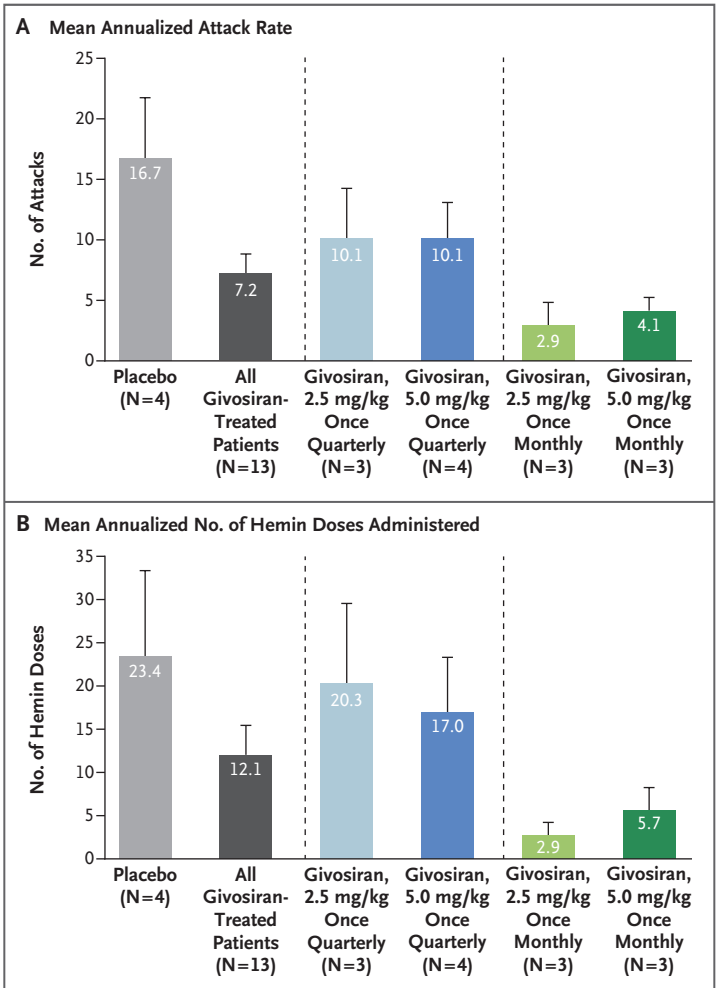

Figure 3. Effect of Givosiran on Annualized Rates of Porphyria Attacks and Hemin Use (Part C).

T bars indicate standard errors.

events and led to sustained reductions in induced *ALAS1* mRNA, ALA, and PBG levels. Exploratory analyses suggested that these reductions in patients with acute intermittent porphyria who have recurrent attacks were associated with lower rates of porphyria attacks and hemin use than those observed with placebo.

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