ORIGINAL ARTICLE

Volanesorsen and Triglyceride Levels in Familial Chylomicronemia Syndrome

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ABSTRACT

BACKGROUND

Familial chylomicronemia syndrome is a rare genetic disorder that is caused by loss of lipoprotein lipase activity and characterized by chylomicronemia and recurrent episodes of pancreatitis. There are no effective therapies. In an open-label study of three patients with this syndrome, antisense-mediated inhibition of hepatic *APOC3* mRNA with volanesorsen led to decreased plasma apolipoprotein C-III and triglyceride levels.

METHODS

We conducted a phase 3, double-blind, randomized 52-week trial to evaluate the safety and effectiveness of volanesorsen in 66 patients with familial chylomicronemia syndrome. Patients were randomly assigned, in a 1:1 ratio, to receive volanesorsen or placebo. The primary end point was the percentage change in fasting triglyceride levels from baseline to 3 months.

RESULTS

Patients receiving volanesorsen had a decrease in mean plasma apolipoprotein C-III levels from baseline of 25.7 mg per deciliter, corresponding to an 84% decrease at 3 months, whereas patients receiving placebo had an increase in mean plasma apolipoprotein C-III levels from baseline of 1.9 mg per deciliter, corresponding to a 6.1% increase (P<0.001). Patients receiving volanesorsen had a 77% decrease in mean triglyceride levels, corresponding to a mean decrease of 1712 mg per deciliter (19.3 mmol per liter) (95% confidence interval [CI], 1330 to 2094 mg per deciliter [15.0 to 23.6 mmol per liter]), whereas patients receiving placebo had an 18% increase in mean triglyceride levels, corresponding to an increase of 92.0 mg per deciliter (1.0 mmol per liter) (95% CI, –301.0 to 486 mg per deciliter [–3.4 to 5.5 mmol per liter]) (P<0.001). At 3 months, 77% of the patients in the volanesorsen group, as compared with 10% of patients in the placebo group, had triglyceride levels of less than 750 mg per deciliter (8.5 mmol per liter). A total of 20 of 33 patients who received volanesorsen had injection-site reactions, whereas none of the patients who received placebo had such reactions. No patients in the placebo group had platelet counts below 100,000 per microliter, whereas 15 of 33 patients in the volanesorsen group had such levels, including 2 who had levels below 25,000 per microliter. No patient had platelet counts below 50,000 per microliter after enhanced platelet-monitoring began.

CONCLUSIONS

Volanesorsen lowered triglyceride levels to less than 750 mg per deciliter in 77% of patients with familial chylomicronemia syndrome. Thrombocytopenia and injection-site reactions were common adverse events. (Funded by Ionis Pharmaceuticals and Akcea Therapeutics; APPROACH Clinical Trials.gov number, NCT02211209.)

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Familial chylomicronemia syndrome is a rare genetic disorder characterized by reduced or absent lipoprotein lipase (LPL) activity.[1-4] LPL mediates lipolysis of plasma triglycerides in chylomicrons and other triglyceride-rich lipoproteins,[5] and its absence leads to marked fasting and postprandial chylomicronemia, with triglyceride levels 10 to 100 times above the normal level of 150 mg per deciliter (1.7 mmol per liter). Familial chylomicronemia syndrome results from inactivating mutations in both alleles of the *LPL* gene or from mutations in other genes encoding proteins required for LPL activity, such as apolipoproteins C-II and A-5 (*APOC2* and *APOA5*), glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (*GPIHBP1*), and lipase maturation factor 1 (*LMF1*).[6-8] Occasionally, patients with familial chylomicronemia syndrome phenotypes do not present with mutations in these genes.[9,10] Patients with this syndrome may exhibit eruptive xanthoma, lipemia retinalis, and hepatosplenomegaly and may have recurrent episodes of abdominal pain and acute pancreatitis.[11] Although lipoprotein apheresis can lower greatly elevated triglyceride levels in the short term, the only current long-term therapy is restriction of total fat intake to less than 10 to 15% of daily calories (15 to 20 g per day), which is often ineffective in preventing chylomicronemia and acute pancreatitis.[2,3,11,12]

Elevated plasma levels of apolipoprotein C-III are a major risk factor for hypertriglyceridemia.[13-15] Previously, it was thought that apolipoprotein C-III raised plasma triglyceride levels primarily by inhibiting LPL activity. However, in a recent pilot study in three patients with familial chylomicronemia syndrome, plasma apolipoprotein C-III levels were reduced by antisense targeting of hepatic *APOC3* mRNA with volanesorsen, which led to reductions in triglyceride levels of 56 to 86%, findings that support the hypothesis that apolipoprotein C-III also inhibits an LPL-independent pathway of triglyceride-rich lipoprotein clearance.[16] These preliminary results led us to conduct the APPROACH trial, a 52-week placebo-controlled trial of volanesorsen-mediated inhibition of plasma apolipoprotein C-III in 66 patients with familial chylomicronemia syndrome.

Methods

Trial Design

The APPROACH trial was a randomized, double-blind, phase 3 trial that evaluated the efficacy and safety of volanesorsen sodium (at a dose of 300 mg subcutaneously once a week) as compared with placebo in patients older than 18 years of age who had familial chylomicronemia syndrome. Patients were eligible to participate if the syndrome was confirmed either by genetic testing or by documentation of low LPL activity (levels <20% of the normal range) and if fasting triglyceride levels were at or above 750 mg per deciliter (8.5 mmol per liter). Genetic confirmation of the syndrome was based on detection of homozygosity, compound heterozygosity, or double heterozygosity for known loss-of-function mutations in *LPL*, *APOC2*, *APOA5*, *GPIHBP1*, or *LMF1* genes. Alternatively, patients were eligible if their post-heparin LPL activity, measured in a central laboratory, was less than 20% of the normal range. Enrollment of patients without a documented history of previous pancreatitis was capped at 28% of the total cohort.

After a 6-week diet stabilization run-in period during which patients were asked to follow a diet that included less than 20 g of fat per day, patients with fasting baseline triglyceride levels of 750 mg per deciliter or higher were randomly assigned in a 1:1 ratio to volanesorsen at a dose of 300 mg per week or placebo, with stratification according to history or no history of pancreatitis and according to receipt or no receipt of concurrent fibrates, prescription n–3 fatty acids, or both. Volanesorsen or placebo was administered as a single subcutaneous injection once a week for 52 weeks. During the trial, a reduction of dosing to once every other week was allowed if unacceptable side effects or adverse events occurred. The primary end point was the percentage change in fasting triglyceride levels from baseline to the time the primary analysis was performed at the end of month 3; the fasting triglyceride level for month 3 was the average of fasting assessments made at week 12 (day 78) and week 13 (day 85).

Trial Oversight

We conducted the trial at 40 centers in 12 countries from August 2014 through March 2017. The protocol was approved by the institutional review board at each participating center and by an ethics committee (Quorum Review IRB), and the trial was performed in accordance with the principles of the Declaration of Helsinki and current Good Clinical Practice guidelines. All patients provided written informed consent before enrollment.

We assessed safety by determining the incidence, severity, and dose relationship of adverse events and changes in laboratory measurements. Episodes of acute pancreatitis were evaluated for safety and independently adjudicated according to the revised Atlanta criteria.[17] Further details on evaluations of safety are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.

The trial was designed and conducted, and the data analyzed, by the sponsors (Ionis Pharmaceuticals and Akcea Therapeutics). All the authors interpreted the data and collaborated in the preparation of the manuscript. The first draft of the manuscript and all revisions were written by the first author with assistance from two coauthors employed by Ionis Pharmaceuticals and were reviewed by all the authors. All the authors approved the final version, made the decision to submit the manuscript for publication, vouch for the completeness and accuracy of the data, and affirm that the trial was conducted and reported with fidelity to the protocol and statistical analysis plan (available at NEJM.org). Additional details on the methods are provided in the Supplementary Appendix.

STATISTICAL ANALYSIS

The primary end point was the percentage change in fasting triglyceride levels from baseline to month 3 among patients who received volanesorsen as compared with patients who received placebo in the full analysis set. The data were evaluated with the use of an analysis of covariance (ANCOVA) model, with two randomization stratification factors (presence or absence of history of pancreatitis and receipt or no receipt of concurrent n–3 fatty acids, fibrates, or both) along with the treatment group as a factor and log-transformed baseline triglyceride levels as a covariate. For the primary analysis end point at 3 months, there were no missing data on triglyceride levels. All patients had triglyceride levels measured at either week 12 or week 13, and 76% had triglyceride levels measured at both visits. The primary end point at 3 months was defined as the average of levels at week 12 and week 13 for patients who had triglyceride data recorded at both weeks; for patients who had triglyceride levels measured at only one of these two visits, that level was used.

Nine secondary end points were prespecified and prioritized by rank to be analyzed in hierarchical order (details are provided in the Supplementary Appendix). If analysis of the first end point showed significance, the second end point in the hierarchy would be tested for significance, and so on. If a test of an end point was nonsignificant, analysis of all subsequent end points in the hierarchy would be considered to be exploratory.

The percentage changes from baseline to 6 months and to 12 months were compared between treatment groups with the use of an ANCOVA model similar to that of the primary analysis; in these analyses, missing triglyceride data were imputed with the multiple imputation method (details are available in the Supplementary Appendix). We also performed analyses, not prespecified in the protocol, of the reduction in triglyceride levels at 6 months and 12 months using the observed data for various patient subgroups, such as a subgroup defined according to whether patients received full doses or reduced doses of volanesorsen. All statistical tests were conducted with two-sided tests at 5% type 1 error rates.

Exploratory analyses of other lipid and lipoprotein results to investigate the difference between treatment groups in the percentage change from baseline to 3 months were performed with ANCOVA models similar to those used for the primary analysis.

RESULTS

CHARACTERISTICS OF THE PATIENTS

A total of 130 patients were screened, and 67 patients underwent randomization from December 2014 through January 2016. One patient assigned to the placebo group withdrew from the trial before receiving the placebo and is not included in this analysis (Fig. S1 in the Supplementary Appendix). A full description of the population was published previously.[18] Selected baseline characteristics of the 66 remaining patients who underwent randomization are shown in Table 1, and in Table S1 in the Supplementary Appendix. Of the 66 patients, 41 were homozygous or compound heterozygous for at least one of 25 different inactivating mutations in *LPL*, and 11 patients had biallelic mutations in accessory proteins or were double heterozygous for *LPL* and *APOA5* or *LMF1* mutations; 14 patients did not have defined mutations but were enrolled on the basis of their clinical phenotype

Table 1. Characteristics of the Patients at Baseline.*

Characteristic	Placebo (N=33)	Volanesorsen (N=33)	All Patients (N=66)
Mean age (range) — yr	46 (20–68)	47 (22–75)	46 (20–75)
Sex — no.			
Female	19	17	36
Male	14	16	30
Body-mass index†	24.1±4.7	25.9±6.5	25.0±5.7
Triglycerides — mg/dl	2152	2267	2209
History of pancreatitis — no. (%)	26 (79)	24 (73)	50 (76)
Baseline use of n–3 fatty acids, fibrates, or both — no. (%)	16 (48)	19 (58)	35 (53)
Genetic mutations — no. (%)			
LPL	24 (73)	17 (52)	41 (62)
APOA5	1 (3)	1 (3)	2 (3)
GPIHBP1	0	5 (15)	5 (8)
LMF1	0	1 (3)	1 (2)
APOC2	0	1 (3)	1 (2)
LPL/LMF-1	0	1 (3)	1 (2)
LPL/APOA5	1 (3)	0	1 (2)
Not identified‡	7 (21)	7 (21)	14 (21)

* Plus-minus values are means ±SD. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. *APOA5* denotes apoprotein A-5, *APOC2* apoprotein C-II, *GPIHBP1* glycosylphosphatidylinositol-anchored high-density–lipoprotein-binding protein 1, *LMF-1* lipase maturation factor 1, and *LPL* lipoprotein lipase.
† The body-mass index is the weight in kilograms divided by the square of the height in meters.
‡ Among 14 patients with genetic mutations that were not identified, 6 had mutations in LPL or accessory proteins not annotated or predicted to be inactivating, and 5 had no known relevant mutations. Genetic testing was not performed in 3 patients because of lack of consent; these patients were enrolled on the basis of other criteria.

and low LPL activity. A detailed description of the genetic diagnoses and associated phenotypes was published previously.[19]

Patients were 20 to 75 years of age, 80% were white, and 55% (36 patients) were women; the mean (±SD) body-mass index (the weight in kilograms divided by the square of the height in meters) was 25.0±5.7. The investigator-reported age at which familial chylomicronemia syndrome was diagnosed (data available for 46 patients) ranged from 1 year or younger to 75 years of age. The syndrome was diagnosed in 6 of the patients in the first 5 years of life and in 14 in the first 20 years of life. Lipemia retinalis occurred in 14 (21%) and eruptive xanthomata in 15 (23%) of the 66 patients. At the time of screening, 50 patients (76%) had a documented history of acute pancreatitis, and of these, 23 patients had had a total of 53 adjudicated episodes of acute pancreatitis in the previous 5 years. Seven patients had chronic pancreatitis. At baseline, 35 of the 66 patients (53%) were taking fibrates, n–3 fatty acids, or both, and 13 (20%) were receiving statins. Seven patients (11%) had been treated with alipogene tiparvovec more than 2 years before they were enrolled. There were no significant differences in baseline triglyceride levels between patients who were receiving lipid-lowering medication and those who were not. Mean fasting triglyceride levels were elevated — 2209±1199 mg per deciliter (25.0±13.6 mmol per liter; normal level, <150 mg per deciliter [<1.7 mmol per liter]) — as were mean chylomicron triglyceride levels (1849±1176 mg per deciliter [20.9±13.3 mmol per liter]; normally not present) and mean apolipoprotein B-48 levels (10.2±6.6 mg per deciliter [normal level, <0.83 mg per deciliter]). Mean plasma apolipoprotein C-III levels were 30.2±14.2 mg per deciliter (normal level, <20 mg per deciliter).

EFFICACY END POINTS

Effects on Plasma Apolipoprotein C-III and Triglyceride Levels

Volanesorsen reduced mean apolipoprotein C-III levels from baseline by 84% after 3 months of therapy and by 83% after 6 months of therapy (P<0.001 for both comparisons), which corresponded to decreases of 25.7 mg per deciliter and 25.6 mg per deciliter, respectively; in contrast, mean apolipoprotein C-III levels increased by 6.1% (1.9 mg per deciliter) after 3 months of therapy and decreased by 5.2% (1.7 mg per deciliter) at 6 months among patients who received placebo (Table 2 and Fig. 1A). With respect to the primary efficacy end point, the mean percentage change in triglyceride levels between baseline and 3 months was a decrease of 77% in the volanesorsen group, as compared with an 18% increase in the placebo group (P<0.001) (Table 2), which corresponded to a mean decrease of 1712 mg per deciliter (19.3 mmol per liter) (95% confidence interval [CI], 1330 to 2094 mg per deciliter [15.0 to 23.6 mmol per liter]) in the volanesorsen group, as compared with an increase of 92.0 mg per deciliter (1.0 mmol per liter) (95% CI, −301 to 486 mg per deciliter [−3.4 to 5.5 mmol per liter]) in the placebo group (P<0.001) (Fig. 2).

Secondary Efficacy End Points

The results of the analysis of the first-ranked secondary end point — treatment response rate, with response defined as a fasting plasma triglyceride level of less than 750 mg per deciliter at 3 months — were significant. A waterfall plot of results at 3 months (Fig. S2 in the Supplementary Appendix) shows that 77% of patients treated with volanesorsen, as compared with 10% of patients who received placebo, achieved triglyceride levels below 750 mg per deciliter (odds ratio, 186.16; 95% CI, 12.86 to could not be estimated; P<0.001). The result of the second-ranked secondary end point — the percentage change in fasting triglyceride levels from baseline to 6 months — was also significant. Volanesorsen treatment effects were sustained for 6 months, at which time triglyceride levels were lower by a mean of 53% in the volanesorsen group (decrease, 1380 mg per deciliter [15.6 mmol per liter]), as compared with a 25% mean increase in the placebo group (increase, 224 mg per deciliter [2.53 mmol per liter]). The relative

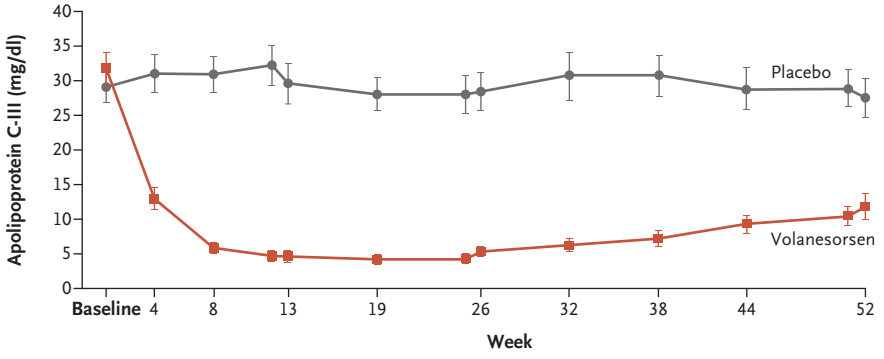
Table 2. Effects on Fasting Lipid Variables at Month 3.*

Lipid	Normal Range	Placebo		Volanesorsen		Change from Baseline		P Value
		Baseline	3 Months	Baseline	3 Months	Placebo	Volanesorsen	
		milligrams per deciliter				*% change (95% CI)†*		
Total triglyceride	50 to 150	2152±1153	2367±1315	2267±1259	590±497	17.6 (−4.0 to 39.2)	−76.5 (−97.4 to −55.5)	<0.001
Chylomicron triglyceride	NA	1785±1149	1991±1279	1913±1216	436±480	29.2 (−1.7 to 60.2)	−82.7 (−112.6 to −52.7)	<0.001
VLDL cholesterol	NA	41±29	42±36	39±32	13±10	15.2 (−10.4 to 40.9)	−58.3 (−88.4 to −28.3)	<0.001
LDL cholesterol	50 to 130	28±13	29±18	28±19	61±39	5.7 (−30.6 to 42.0)	135.6 (100.8 to 170.3)	<0.001
HDL cholesterol	35 to 60	17±4	17±5	17±4	25±11	6.8 (−6.4 to 20.0)	46.1 (33.2 to 59.1)	<0.001
Non–HDL cholesterol	80 to 160	267±125	287±134	276±135	131±51	11.9 (−2.4 to 26.2)	−45.9 (−59.9 to −31.9)	<0.001
Apolipoprotein B	NA	69.38±19.78	70.41±22.74	64.69±19.45	75.85±27.13	3.6 (−7.4 to 14.6)	19.5 (9.0 to 30.0)	0.03
Apolipoprotein B-48	0.15 to 0.83	9.25±5.96	9.92±6.90	11.18±7.14	2.59±2.38	13.6 (−4.2 to 31.4)	−75.9 (−93.0 to −58.8)	<0.001
Apolipoprotein A-1	110 to 205	99±20	100±19	101±21	114±24	2.3 (−2.0 to 6.7)	14.2 (10.0 to 18.5)	<0.001
Apolipoprotein C-III	5 to 20	28.94±13.08	30.70±16.11	31.42±15.29	4.58±2.79	6.1 (−2.1 to 14.3)	−84.2 (−92.2 to −76.1)	<0.001

* Plus-minus values are means ±SD. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. CI denotes confidence interval, HDL high-density lipoprotein, LDL low-density lipoprotein, NA not available, and VLDL very-low-density lipoprotein.

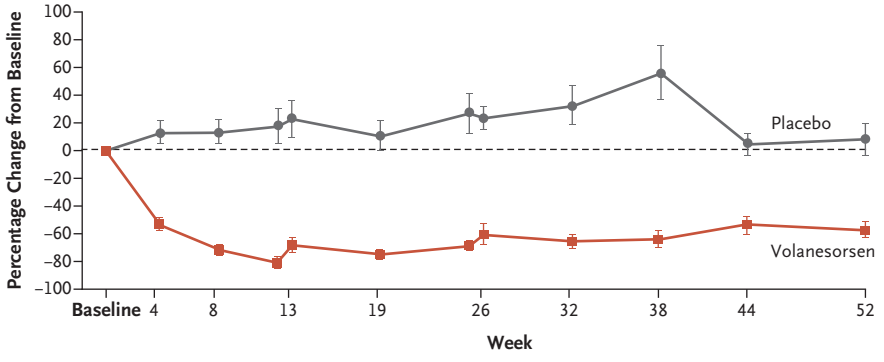
† The values are least-squares means. The least-squares means values, corresponding 95% confidence intervals, and P values are derived from analysis of covariance models, with percentage change from baseline as the dependent variable; treatment group, presence or absence of pancreatitis, and receipt or no receipt of concurrent n−3 fatty acids, fibrates, or both as factors; and log-transformed baseline triglyceride levels as a covariate.

A Change in Fasting Apolipoprotein C-III Levels over Time



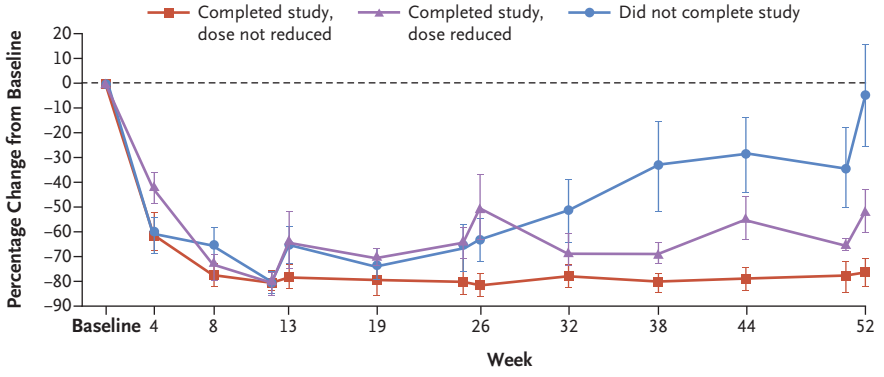
No. at Risk											
Placebo	33	31	33	26	32	32	26	30	31	29	30	26	26
Volanesorsen	33	30	33	28	30	28	22	27	25	24	25	23	24

B Change in Triglyceride Levels over Time



No. at Risk											
Placebo	31	33	26	32	31	26	30	31	29	30	26
Volanesorsen	30	33	28	30	28	22	27	25	24	25	24

C Change in Triglyceride Levels over Time with and without Volanesorsen Dose Adjustment



No. at Risk											
Completed study, dose not reduced	6	5	6	6	6	6	5	5	5	6	6	4	6
Completed study, dose reduced	13	13	13	12	12	13	11	13	13	13	13	12	12
Did not complete study	14	12	14	10	12	9	6	9	7	5	6	7	6

Figure 1 (facing page). Changes in Fasting Apolipoprotein C-III and Triglyceride Levels.

Panel A shows the change in levels of apolipoprotein C-III over the 52-week treatment period among patients who were randomly assigned to receive volanesorsen at a dose of 300 mg per week and those who were assigned to receive placebo. Panel B shows the percentage change in triglyceride levels over the 52-week trial for all patients. Panel C shows the percentage change in triglyceride levels among patients in the volanesorsen group who had a dose reduction during the 52-week trial and those who did not have a dose reduction during the trial, as well as in patients who did not complete the trial. Data presented are means; I bars indicate standard errors.

between-group difference in the percentage change was −77.8% (95% CI, −106.4 to −49.1; P<0.001).

The result of the analysis of the third-ranked secondary end point — the percentage change in fasting triglyceride levels from baseline to 12 months — was significant. At 12 months, triglyceride levels were reduced by 40% in the volanesorsen group (decrease, 986 mg per deciliter [11.1 mmol per liter]), whereas the levels at 12 months were increased by 9% in the placebo group (increase, 39 mg per deciliter [0.44 mmol per liter]). The between-group relative difference in percentage change was −49.1% (95% CI, −94.7 to −3.5; P=0.03). The result of the analysis of the fourth-ranked secondary end point, the average of maximum intensity of patient-reported abdominal pain during the treatment period, did not reach statistical significance. Descriptions and results of exploratory analyses of the other secondary end points, which are also considered to be nonsignificant, are provided in the Supplementary Appendix.

Figure 1A and 1B shows the mean percentage change in apolipoprotein C-III and plasma triglyceride levels over the full 52-week trial among all patients who underwent randomization. Of the patients who received volanesorsen, 19 completed the full 52-week treatment period: 6 received the starting dose (300 mg per week) for the entire treatment period, and among the remaining 13, doses were reduced in frequency (to 300 mg every 2 weeks), treatment was paused, or both. Figure 1C shows the results of an analysis, not prespecified in the protocol, of the mean percentage change in triglyceride levels among patients who had volanesorsen dose adjustments

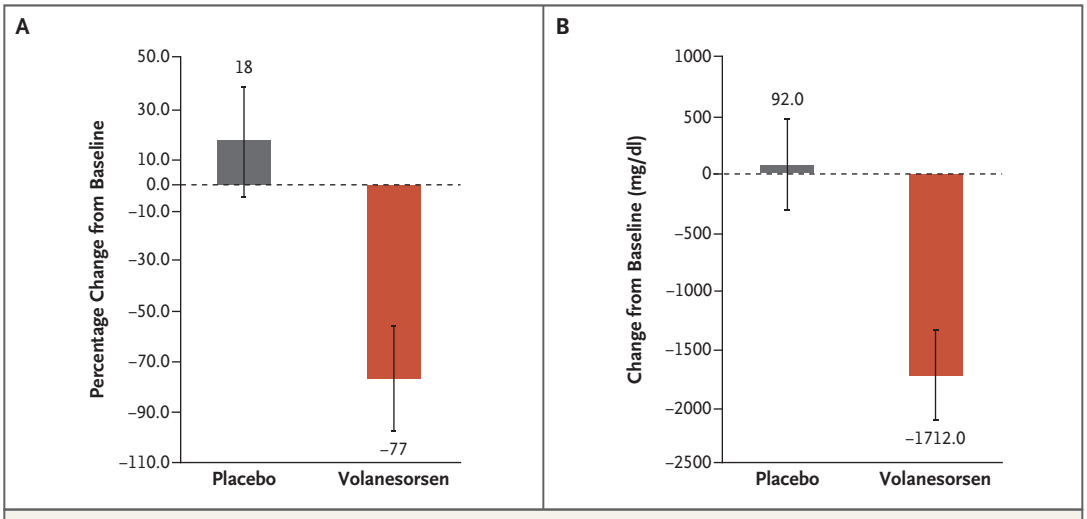


Figure 2. Change in Triglyceride Levels in Patients at 3 Months.

Panel A shows the percentage change in triglyceride levels from baseline to 3 months (the primary end point) among patients receiving placebo and those receiving volanesorsen at a dose of 300 mg per week. Panel B shows the reductions in those levels in milligrams per deciliter. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. Data represent the full analysis set and are shown as least-squares means. I bars indicate 95% confidence intervals.

and those whose doses were not adjusted. Among the 6 patients who did not have a dose reduction, the mean decrease in triglyceride levels from baseline was 79% at 3 months, 80% at 6 months, and 72% at 12 months (mean decreases from baseline of 1670 mg per deciliter [18.9 mmol per liter], 1656 mg per deciliter [18.7 mmol per liter], and 1454 mg per deciliter [16.4 mmol per liter], respectively), whereas corresponding decreases in triglyceride levels among the 13 patients whose doses were reduced was 71% at 3 months, 52% at 6 months, and 54% at 12 months (mean decrease from baseline of 1933 mg per deciliter [22.5 mmol per liter], 1564 mg per deciliter [17.7 mmol per liter], and 1400 mg per deciliter [15.8 mmol per liter], respectively). Among the 6 patients whose doses were not reduced, 5 had triglyceride levels below 750 mg per deciliter at 6 months, and 4 had triglyceride levels below 750 mg per deciliter at 12 months. Of the 13 patients whose doses were reduced, 6 had triglyceride levels below 750 mg per deciliter at 6 months, and 6 had triglyceride levels below 750 mg per deciliter at 12 months; 3 patients had triglyceride levels below 750 mg per deciliter at both 6 and 12 months.

Table 3. Adverse Events.*

Adverse Event	Placebo (N=33)	Volanesorsen (N=33)
	number (percent)	
Injection-site reaction	0	20 (61)
Platelet count decreased	1 (3)	11 (33)
Abdominal pain	7 (21)	9 (27)
Fatigue	3 (9)	7 (21)
Headache	5 (15)	7 (21)
Nausea	2 (6)	6 (18)
Asthenia	3 (9)	5 (15)
Myalgia	1 (3)	5 (15)
Diarrhea	2 (6)	5 (15)
Epistaxis	0	5 (15)
Vomiting	3 (9)	5 (15)
Nasopharyngitis	7 (21)	5 (15)
Petechiae	0	4 (12)
Arthralgia	0	4 (12)
Pain in extremity	1 (3)	4 (12)
Thrombocytopenia	0	4 (12)
Diabetes mellitus	0	4 (12)

* Shown are adverse events that were reported in 10% or more of the patients and were more common in the volanesorsen group than in the placebo group.

Exploratory Analyses

Table 2 shows an analysis of the effects of volanesorsen at 3 months on other lipoprotein and apoprotein variables. Patients who received volanesorsen had decreases in levels of chylomicron triglyceride (by 83%), apolipoprotein B-48 (by 76%), non–high-density lipoprotein (HDL) cholesterol (by 46%), and very-low-density lipoprotein (VLDL) cholesterol (by 58%) and increases in levels of HDL cholesterol (by 46%), apolipoprotein A1 (by 14%), low-density lipoprotein (LDL) cholesterol (by 136%), and total apolipoprotein B (by 20%).

Volanesorsen lowered triglyceride levels irrespective of the patients' genetic diagnoses. At 3 months, mean triglyceride levels were decreased by 65% in the 17 patients with biallelic mutations in *LPL* and by 75% in the 9 patients with non-*LPL* genetic deficiencies. Patients with mutations in *APOC2*, *GPIHBP1*, *APOA5*, and *LMF1* all showed marked triglyceride decreases (by 69 to 88%). Treatment was also effective irrespective of baseline triglyceride levels and was equally effective in patients receiving baseline n–3 fatty acids, fibrate therapy, or both and patients not receiving those therapies (mean decrease from baseline to 3 months, 76% [95% CI, 42 to 110] and 73% [95% CI, 50 to 96], respectively).

Because the size of the trial was limited by the low prevalence of familial chylomicronemia syndrome, a change in the number of episodes of acute pancreatitis during the trial was not a prescribed efficacy end point. However, in exploratory analyses, we assessed adjudicated episodes of acute pancreatitis that occurred during the trial. During the treatment period, three patients in the placebo group had four episodes of acute pancreatitis, whereas one patient in the volanesorsen group had one episode (9 days after receiving the final dose).

SAFETY

The most common adverse events that occurred during treatment among patients who received volanesorsen were those related to injection-site reactions and decreases in platelet counts (Table 3, and Table S3 in the Supplementary Appendix). In the volanesorsen group, 20 patients (61%) had at least one injection-site reaction (categorized as mild to moderate), and, on average, 12% of

volanesorsen injections, as compared with 0% of placebo injections, were associated with these reactions. One patient withdrew from the trial early owing to an injection-site reaction (Table S3 in the Supplementary Appendix).

Confirmed nadir platelet counts below the normal level of 140,000 per microliter were observed in 25 patients (76%) in the volanesorsen group and in 8 patients (24%) in the placebo group; confirmed nadir levels below 100,000 per microliter were observed in 16 patients (48%) who received volanesorsen but in no patients who received placebo (Table 4). Because there was no documented history of marked decreases in platelet counts in humans treated with antisense drugs of this class,[20] the initial protocol required platelet monitoring at 4-to-6-week intervals. However, midway through the trial (May 2016), grade 4 thrombocytopenia (<25,000 platelets per microliter) developed in two patients in the volanesorsen group, and their trial treatment was discontinued. No major bleeding events occurred in either patient, and the two patients reached normal platelet counts 23 and 33 days after discontinuation of the drug. One patient received oral prednisone at a dose of 60 mg for 23 days. The other patient received methylprednisolone at a dose of 125 mg for 11 days, followed by oral prednisone at a dose of 70 mg tapered to 50 mg for 21 days, as well as intravenous immune globulin at a dose of 60 g and 80 g on 2 successive days, followed 4 days later by immune globulin at a dose of 40 g daily for 5 more days. Three other patients with lesser grades (grade 1 or 2) of decreased platelet counts were withdrawn from the trial because of concerns on the part of the site investigators.

After the two cases of severe thrombocytopenia occurred, a more intensive platelet monitoring program was implemented that consisted of assessment of platelet counts every 2 weeks, a threshold (<100,000 per microliter) for reduction in dose frequency to every 2 weeks, and a new threshold (75,000 per microliter, changed from 50,000 per microliter) for an interruption in dosing. After these rules were implemented, no patients had platelet-count declines to less than 50,000 per microliter, and no platelet-related dose discontinuations occurred. Dose reduction of volanesorsen occurred in 13 patients; dose reductions in 9 of those patients occurred because of decreases in platelet counts.

Table 4. Platelet Counts before and after Enhanced Platelet Monitoring.

Confirmed Platelet Count	Placebo (N=33)	Volanesorsen (N=33)*
	no. of patients (before/after)†	
<140,000/μl	8 (4/4)	25 (15/10)
<100,000/μl	0	16 (10/6)
100,000 to <140,000/μl	8 (4/4)	9 (5/4)
75,000 to <100,000/μl	0	6 (2/4)
50,000 to <75,000/μl	0	7 (5/2)
25,000 to <50,000/μl	0	1 (1/0)
0 to <25,000/μl	0	2 (2/0)‡

* No patients had a major bleeding event.
† Shown are numbers of patients who had the indicated platelet level during the trial; "before/after" indicates the numbers of patients who had that level before and after enhanced platelet monitoring was implemented.
‡ Platelet counts in both patients normalized after treatment was discontinued and glucocorticoids (and intravenous immune globulin in one patient) were administered.

Fourteen patients assigned to volanesorsen, as compared with 2 patients assigned to placebo, did not complete the 52-week trial (Fig. S1 in the Supplementary Appendix). Among the patients treated with volanesorsen, 9 discontinued the trial because of adverse events that occurred during treatment — 5 because of platelet decreases and 4 because of other volanesorsen-related effects (one each because of an injection-site reaction and fatigue; fatigue; chills and sweating; and generalized erythema). Four patients in the volanesorsen group voluntarily withdrew from the trial (reasons provided were dehydration, uncertainty of drug efficacy, travel schedule, and duration of trial visits), and one patient was withdrawn by an investigator for nonadherence. Among patients in the placebo group, one patient had been erroneously assigned to that group and did not receive the assigned placebo, and one patient withdrew for reasons not related to adverse events that occurred during the treatment period. There were no deaths during the trial.

DISCUSSION

The APPROACH trial showed that volanesorsen-mediated lowering of plasma apolipoprotein C-III levels in patients with familial chylomicronemia syndrome effectively lowered plasma triglycerides below the threshold of 750 to 880 mg per deciliter

(8.5 to 10 mmol per liter) that has been associated with triglyceride-induced acute pancreatitis.[6,21-26] Although the mean LDL cholesterol level increased from 28 to 61 mg per deciliter (136%), the mean baseline level of LDL cholesterol was very low. Moreover, non–HDL cholesterol, a general measure of atherogenic lipoproteins, decreased by 45%. These data show that volanesorsen-mediated lowering of apolipoprotein C-III effectively enhances clearance of triglyceride-rich lipoproteins in patients without LPL activity.[27] Because traditional triglyceride-lowering therapies such as fibrates, n–3 fatty acids, and statins depend on LPL activity, inhibition of hepatic VLDL output, or both, they are largely ineffective in familial chylomicronemia syndrome, as evidenced by the fact that half the patients in this trial were receiving one or more of these agents at the time they were recruited.[12]

Although patients with familial chylomicronemia syndrome have many physical and cognitive complications from sustained chylomicronemia,[3,11,28,29] potentially recurrent and even fatal acute pancreatitis is the complication that is most feared. The reported prevalence of acute pancreatitis among patients with this syndrome is not known with certainty but may be as high as 67%,[29] with an associated risk of major complications that is two or three times as high as the risk among patients with pancreatitis without hypertriglyceridemia, as well as an associated 6% risk of death from acute pancreatitis.[26,29,30] Maintaining triglyceride levels below 750 mg per deciliter would be expected to decrease the risk for pancreatitis. Although the small size of the APPROACH trial did not allow us to draw firm conclusions about the effect of volanesorsen on pancreatitis, we performed an exploratory analysis of the effect of volanesorsen on patients with multiple previous occurrences of acute pancreatitis, since these patients are known to be at greater risk for subsequent episodes.[24,31-33] There were 11 patients with two or more adjudicated episodes of acute pancreatitis in the previous 5 years, with a total of 41 previous episodes (Table S4 in the Supplementary Appendix). Of those 11 patients, 4 were assigned to placebo; 3 of the 4 in the placebo group had four episodes of acute pancreatitis. No episodes of acute pancreatitis occurred among the 7 patients who received volanesorsen. Observational data suggest that the incidence of acute pancreatitis increases approximately 3% for every increment of 100 mg per deciliter in triglyceride levels over 1000 mg per deciliter[34]; we would therefore speculate that a decrease in triglyceride levels to any extent below this threshold would result in a decrease in the risk of acute pancreatitis.

Volanesorsen therapy was associated with mild-to-moderate injection-site reactions (in 12% of injections). One patient withdrew from the trial owing to these reactions. Another side effect was thrombocytopenia, which was defined as a platelet count below 140,000 per microliter. A recently reported natural history study of 87 patients with familial chylomicronemia syndrome over a 15-year period indicates considerable lability in platelet counts: 55.2% had thrombocytopenia on at least one occasion, and 2.4% had platelet levels of less than 50,000 per microliter, whereas 12.6% presented with thrombocytosis.[35] During the APPROACH trial, 8 patients (24%) in the placebo group and 25 (76%) in the volanesorsen group had platelet-level decreases to below 140,000 per microliter. Before the initiation of the APPROACH trial, more than 2600 patients had been treated with 16 different 2′-O-methoxyethyl (2′MOE) antisense agents (the class of drugs to which volanesorsen belongs) in placebo-controlled and open-label clinical trials, with treatment durations as long as 4.6 years. There was no evidence of a class effect on platelet counts and no incidence of confirmed platelet levels below 50,000 per microliter.[20] Thus, platelet monitoring in APPROACH was initially performed at 4-to-6-week intervals; however, because platelet counts of less than 25,000 per microliter developed in 2 patients, a more intensive platelet-monitoring protocol was instituted. After implementation of these rules, there were no platelet-level declines to less than 50,000 per microliter and no platelet-related discontinuations of volanesorsen in the trial. The underlying mechanisms responsible for the declines in platelet levels are not clear at present. Nearly all the patients received an injection of heparin at the beginning of the trial and at approximately 26 weeks to enable measurement of LPL activity, but a classic heparin-induced mechanism for thrombocytopenia was excluded, as were antisense-mediated effects on platelet production. There was some evidence for associated immunologic

response in the most severe events. It is important to note that decreases in platelet levels were reversible with an interruption in dosing, and for the most part appeared to be dose-dependent. The establishment of more frequent platelet monitoring and either an interruption in dosing or reductions in the dose, or both, appeared to be adequate for preventing severe thrombocytopenia and maintaining stable platelet counts for most patients.

Fourteen patients in the volanesorsen group discontinued the trial: 9 because of adverse events that occurred during treatment (including 5 who had decreases in platelet counts and 4 because of other drug-related side effects), and 5 primarily because of issues related to requirements of the protocol. The combination of an unexpected, potentially serious level of thrombocytopenia occurring during the trial and the lack of a prespecified protocol for responding to it in a timely manner led to patient and investigator uncertainty and resulted in patients withdrawing from the trial.

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APPENDIX

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