ORIGINAL ARTICLE

Antisense Inhibition of Apolipoprotein C-III in Patients with Hypertriglyceridemia

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ABSTRACT

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BACKGROUND

Apolipoprotein C-III (APOC3) is a key regulator of plasma triglyceride levels. Elevated triglyceride levels are associated with a risk of adverse cardiovascular events and pancreatitis. ISIS 304801 is a second-generation antisense inhibitor of APOC3 synthesis.

METHODS

We conducted a randomized, double-blind, placebo-controlled, dose-ranging, phase 2 study to evaluate ISIS 304801 in untreated patients with fasting triglyceride levels between 350 mg per deciliter (4.0 mmol per liter) and 2000 mg per deciliter (22.6 mmol per liter) (ISIS 304801 monotherapy cohort), as well as in patients receiving stable fibrate therapy who had fasting triglyceride levels between 225 mg per deciliter (2.5 mmol per liter) and 2000 mg per deciliter (ISIS 304801–fibrate cohort). Eligible patients were randomly assigned to receive either ISIS 304801, at doses ranging from 100 to 300 mg, or placebo, once weekly for 13 weeks. The primary outcome was the percentage change in APOC3 level from baseline.

RESULTS

A total of 57 patients were treated in the ISIS 304801 monotherapy cohort (41 received active agent, and 16 received placebo), and 28 patients were treated in the ISIS 304801–fibrate cohort (20 received active agent, and 8 received placebo). The mean (±SD) baseline triglyceride levels in the two cohorts were 581±291 mg per deciliter (6.6±3.3 mmol per liter) and 376±188 mg per deciliter (4.2±2.1 mmol per liter), respectively. Treatment with ISIS 304801 resulted in dose-dependent and prolonged decreases in plasma APOC3 levels when the drug was administered as a single agent (decreases of 40.0±32.0% in the 100-mg group, 63.8±22.3% in the 200-mg group, and 79.6±9.3% in the 300-mg group, vs. an increase of 4.2±41.7% in the placebo group) and when it was administered as an add-on to fibrates (decreases of 60.2±12.5% in the 200-mg group and 70.9±13.0% in the 300-mg group, vs. a decrease of 2.2±25.2% in the placebo group). Concordant reductions of 31.3 to 70.9% were observed in triglyceride levels. No safety concerns were identified in this short-term study.

CONCLUSIONS

We found that treatment with ISIS 304801 was associated with significant lowering of triglyceride levels, among patients with a broad range of baseline levels, through selective antisense inhibition of APOC3 synthesis. (Funded by Isis Pharmaceuticals; ClinicalTrials.gov number, NCT01529424.)

ELEVATED TRIGLYCERIDE LEVELS ARE ASsociated with several pathologic conditions, including insulin resistance, the metabolic syndrome, diabetes, cardiovascular disease, and hereditary disorders, such as the familial chylomicronemia syndrome, familial combined hyperlipidemia, and familial hypertriglyceridemia.[1,2] Patients with triglyceride levels above 2000 mg per deciliter (22.6 mmol per liter), measured at the peak of abdominal pain, are at high risk for pancreatitis.[3,4] Current guidelines from the Endocrine Society and the European Atherosclerosis Society recommend that fasting triglyceride levels should be maintained at values below 1000 mg per deciliter (11.3 mmol per liter) or 10 mmol per liter, respectively, to prevent intermittent increases in triglyceride levels at which pancreatitis can occur.[1,2] At moderate-to-high elevations in triglyceride levels, patients may also be at risk for new events of coronary heart disease or for recurrence of events in established coronary disease.[5-10]

Apolipoprotein C-III (APOC3) is a key regulator of lipoprotein metabolism and plays a pivotal role in regulating plasma triglyceride levels.[11,12] It is synthesized principally in the liver and is a component of triglyceride-rich lipoproteins.[13] It is known to inhibit lipoprotein lipase–mediated hydrolysis of triglyceride-rich lipoproteins[14] and to adversely affect receptor-mediated hepatic uptake of remnants of triglyceride-rich lipoproteins.[15-20] At higher concentrations, APOC3 also inhibits the activity of hepatic lipase,[21] an enzyme that plays an important role in the conversion of very-low-density lipoprotein (VLDL) to intermediate-density lipoprotein and low-density lipoprotein (LDL),[22] as well as in the remodeling of high-density lipoprotein (HDL).[23] Thus, elevated levels of APOC3 in plasma have been associated with both impaired lipolysis and impaired clearance of triglyceride-rich lipoproteins from the circulation. This impairment results in the accumulation of atherogenic VLDL and chylomicron remnants.[6,13,24,25]

Elevated APOC3 levels are an independent risk factor for cardiovascular disease, especially when APOC3 is present on apolipoprotein B–containing lipoproteins.[13,26,27] Conversely, genetic variants that result in a loss of function and attenuated levels of APOC3 in plasma are associated with a reduced risk of coronary heart disease.[28-30] However, whether targeted reduction of APOC3 will confer such a benefit in patients at high risk for cardiovascular disease, including patients with type 2 diabetes, remains to be determined.[31-33]

ISIS 304801 is a second-generation antisense oligonucleotide that is designed specifically to reduce levels of APOC3 messenger RNA (mRNA). Hybridization of ISIS 304801 to its cognate mRNA, through Watson–Crick base-pair interactions, causes ribonuclease H1–mediated degradation of the target mRNA to prevent production of the APOC3 protein. Selective dose-dependent reduction of APOC3 and concomitant lowering of triglyceride levels have been observed with the use of species-specific antisense oligonucleotides in multiple preclinical animal models, as well as with the use of ISIS 304801 in a phase 1 study in healthy volunteers.[34] Most recently, reductions in APOC3 and triglyceride levels have been reported in a small number of patients with the familial chylomicronemia syndrome who were treated with ISIS 304801.[35] The marked lowering of plasma triglyceride levels in these patients, who had catalytically defective lipoprotein lipase, was presumably a result of enhanced removal of triglyceride-rich lipoproteins. In the current randomized, placebo-controlled, dose-ranging phase 2 study, we evaluated the pharmacodynamic profile of ISIS 304801 as monotherapy and as an add-on to stable doses of fibrate therapy in patients with severe or uncontrolled hypertriglyceridemia.

METHODS

STUDY DESIGN

This phase 2, randomized, double-blind, placebo-controlled, dose-ranging study was designed to evaluate the pharmacodynamic effects of ISIS 304801 on fasting APOC3 levels in adult patients with severe or uncontrolled hypertriglyceridemia. Patients who were not receiving triglyceride-lowering therapy were considered eligible if they had fasting triglyceride levels between 350 mg per deciliter (4.0 mmol per liter) and 2000 mg per deciliter; patients who were receiving a stable dose of fibrate were eligible if they had fasting triglyceride levels between 225 mg per deciliter (2.5 mmol per liter) and 2000 mg per deciliter. Patients assigned to the ISIS 304801 monotherapy cohort were randomly assigned in a 1:1:1 ratio to receive a dose of 100, 200, or 300 mg; the patients in these dose groups were then randomly assigned in a 3:1 ratio to receive active agent or placebo. Patients assigned to the ISIS 304801–fibrate

cohort were randomly assigned in a 1:1 ratio to receive a dose of 200 or 300 mg; patients in these dose groups were then randomly assigned in a 2:1 ratio to receive active agent or placebo. The study drug was administered as a single subcutaneous injection once a week for 13 weeks as monotherapy or as an add-on to fibrate. The primary outcome was the percentage change in fasting total APOC3 levels from baseline (level at day −8) to the end of treatment (mean of the levels at day 85 and day 92).

STUDY OVERSIGHT

The study was conducted at seven sites — one in Chicoutimi, Quebec, and six in North Carolina — from February 2012 through January 2014. The protocol was approved by the institutional review board at Chicoutimi Hospital and by an independent ethics committee (Quorum Review IRB, Seattle), and the study was performed in compliance with the provisions of the Declaration of Helsinki (Washington 2002) and the current Good Clinical Practice guidelines (International Conference on Harmonisation guideline E6[R1]). All study participants gave written informed consent before enrollment.

Safety was assessed by determining the incidence, severity, and dose relationship of adverse events and changes in laboratory measurements. Further details on evaluations of safety are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.

The study was designed by representatives of the sponsor (Isis Pharmaceuticals) and by the first author. Data were collected by study investigators and staff and were analyzed by the sponsor. All the authors interpreted the data and collaborated in the preparation of the manuscript. The first draft of the manuscript was written by the last author and a representative of the sponsor, with review and revision by all authors. All the authors made the decision to submit the manuscript for publication and vouch for the completeness and accuracy of this report and its fidelity to the study protocol, which is available at NEJM.org. Additional details on the methods, including laboratory measurements and secondary outcomes, are provided in the Supplementary Appendix.

STATISTICAL ANALYSIS

In the primary analysis, we compared the percentage change in fasting total APOC3 levels from baseline to the end of treatment (primary outcome) in the 300-mg dose group of the monotherapy cohort with that in the placebo group. Secondary analyses included comparisons of the primary outcome between other dose groups and the placebo group in each cohort. P values were determined with the Wilcoxon rank-sum test or Student's t-test, depending on the normality of the data. All statistical tests were unpaired and two-sided. A P value of less than 0.05 was considered to indicate statistical significance in the primary analysis. Additional details on the statistical analysis are provided in the Supplementary Appendix.

RESULTS

BASELINE CHARACTERISTICS OF THE PATIENTS

A total of 57 patients who underwent randomization were treated in the ISIS 304801 monotherapy cohort (41 received active agent, and 16 received placebo), and 28 were treated in the ISIS 304801–fibrate cohort (20 received active agent, and 8 received placebo) (Fig. S1 in the Supplementary Appendix). Patients ranged in age from 28 to 81 years; 97% were white and 71% were men. The mean body-mass index indicated borderline obesity (Table S1 in the Supplementary Appendix). Among the 85 patients, 31 (36%) received a stable statin regimen from at least 3 months before administration of the first dose of study drug to the end of study. The mean baseline APOC3 levels were elevated in the ISIS 304801 monotherapy and ISIS 304801–fibrate cohorts (22.8±7.1 and 17.6±5.0 mg per deciliter, respectively), relative to normal values (10 to 15 mg per deciliter).[36,37] The corresponding mean baseline triglyceride levels were 581±291 mg per deciliter (6.6±3.3 mmol per liter) and 376±188 mg per deciliter (4.2±2.1 mmol per liter). The mean baseline HDL cholesterol level was 32.4±6.4 mg per deciliter (0.8±0.2 mmol per liter) in the ISIS 304801 monotherapy cohort and 35.5±9.9 mg per deciliter (0.9±0.3 mmol per liter) in the ISIS 304801–fibrate cohort; the corresponding mean baseline LDL cholesterol levels were 88.1±40.0 mg per deciliter (2.3±1.0 mmol per liter) and 102.2±44.5 mg per deciliter (2.6±1.2 mmol per liter). Twenty patients (24%) carried heterozygous loss-of-function mutations in *LPL*, the gene encoding lipoprotein lipase (Table S1B in the Supplementary Appendix). All *APOE* genotypes were represented in this study population (Table S1C in the Supplementary Appendix).

LIPID AND LIPOPROTEIN RESPONSE

ISIS 304801 Monotherapy Cohort

Weekly doses of 100, 200, or 300 mg of ISIS 304801 administered as a single agent produced dose-dependent, prolonged decreases in plasma APOC3 levels (Fig. 1) from baseline to end of treatment; these decreases were substantial as compared with responses in the placebo group (Table 1). In the analysis of the primary outcome, the response was greatest in the 300-mg dose group, and the difference between this group and the placebo group in the percentage change from baseline was significant (a decrease of 79.6% vs. an increase of 4.2%, P<0.001). Concomitant with the reductions in APOC3 levels, a prolonged and dose-dependent decrease in triglyceride levels was also observed. HDL cholesterol levels increased in a dose-dependent manner. The mean change from baseline in triglyceride levels was a decrease of 70.9% at the highest dose of ISIS 304801 (300 mg), as compared with an increase of 20.1% in the placebo group (P<0.001), and HDL cholesterol levels increased by 45.7% from baseline in the 300-mg group, as compared with an increase of 0.7% in the placebo group (P<0.001). Among the patients with end-of-treatment results (Fig. 2), 8 of 12 (75%) in the 200-mg group and 10 of 11 (91%) in the 300-mg group had triglyceride levels below 200 mg per deciliter (2.3 mmol per liter). APOC3 and triglyceride levels were strongly associated with one another (Fig. S2 and Table S2 in the Supplementary Appendix).

We also observed dose-dependent reductions in VLDL cholesterol in the 100-mg, 200-mg, and 300-mg dose groups but not in the placebo group (mean reduction from baseline, 40.0%, 56.1%, and 69.2%, respectively, vs. an increase of 6.3%), as well as dose-dependent reductions in VLDL APOC3 in the three dose groups but not in the placebo group (reductions of 27.3%, 66.7%, and 87.6%, vs. an increase of 21.0%) (Table S3 in the Supplementary Appendix). VLDL apoB, a marker of VLDL particle number, was decreased by 62.7% from baseline to the end of treatment in the 300-mg dose group (Table S4 in the Supplementary Appendix); apoB-48, a marker of chylomicron particle number, was also decreased, by 61.1% (Table S3 in the Supplementary Appendix). The chylomicron–VLDL particle size also had a more substantial reduction in the ISIS 304801–treated groups than in the placebo group (Table S5 in the Supplementary Appendix).

The large decreases in triglyceride levels led to dose-dependent increases in LDL cholesterol levels in ISIS 304801–treated groups, from an overall mean baseline level of 79.5±29.9 mg per deciliter (2.1±0.8 mmol per liter) to a mean level of 127.8±44.9 mg per deciliter (3.3±1.2 mmol per liter) at the end of treatment. This increase in LDL cholesterol level was accompanied by an increase in the LDL apoB concentration (Table S4 in the Supplementary Appendix) and an increase in LDL particle size (Table S5 in the Supplementary Appendix). Non-HDL cholesterol and total apoB levels, however, remained relatively unchanged and similar to those in the placebo group at all doses of ISIS 304801 monotherapy.

Results of a repeated-measurement analysis in the modified intention-to-treat population, which included all patients who received at least one dose of study drug and had at least one postbaseline APOC3 measurement, were similar to those of the per-protocol analysis of the primary and secondary outcomes.

ISIS 304801–Fibrate Cohort

When administered as an add-on to stable fibrate therapy, 200 mg and 300 mg of ISIS 304801 also produced dose-dependent and prolonged mean decreases from baseline in APOC3 levels (Fig. S3 in the Supplementary Appendix). The mean percentage change in APOC3 levels from baseline to the end of treatment was a reduction of 70.9% in the 300-mg dose group (Table 1), as compared with a reduction of 2.2% in the placebo group (P<0.001). In the ISIS 304801–treated groups, the mean percentage decreases in triglyceride levels and mean percentage increases in HDL cholesterol levels from baseline were prolonged and dose-dependent (Fig. S3 in the Supplementary Appendix) and were similar in magnitude to those achieved with monotherapy. Among the patients with end-point results (Fig. S4 in the Supplementary Appendix), 6 of 7 (86%) in the 200-mg group and 8 of 10 (80%) in the 300-mg group had triglyceride levels at the end of treatment that were below 200 mg per deciliter.

Levels of VLDL cholesterol and VLDL APOC3 were also more substantially reduced in the ISIS 304801–treated groups than in the placebo group (Table S3 in the Supplementary Appendix). The VLDL apoB concentration was decreased from baseline to the end of treatment by 64.4% in the 300-mg dose group (Table S4 in the Supplementary Appendix), but there was no substantial change from baseline in apoB-48 concentration
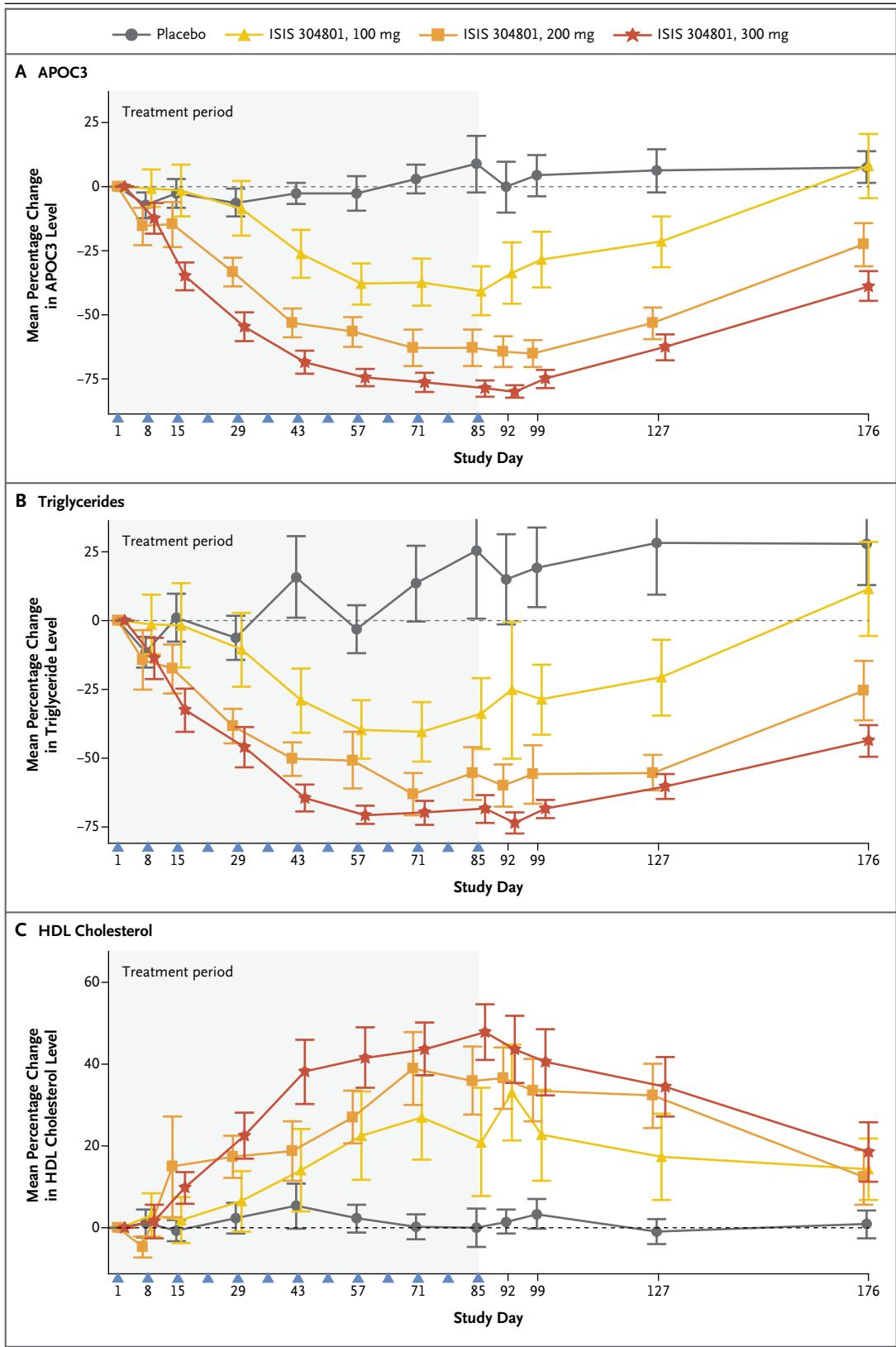
(Table S3 in the Supplementary Appendix) or chylomicron–VLDL particle size (Table S5 in the Supplementary Appendix) among the ISIS 304801–treated groups or the placebo groups. There were no significant differences between the ISIS 304801–treated groups and the placebo group in the changes from baseline in concentrations of total cholesterol, non-HDL cholesterol, LDL cholesterol, and total apoB (Table S3 in the Supplementary Appendix) or in LDL apoB concentrations (Table S4 in the Supplementary Appendix).

SAFETY

Table S6 in the Supplementary Appendix shows the incidence of drug-related adverse events across treatment groups. Local cutaneous reactions at the injection site occurred in association with a mean of 13% of injections of ISIS 304801 in the monotherapy cohort and 15% of injections in the ISIS 304801–fibrate cohort (Table S7 in the Supplementary Appendix). These reactions were typically characterized as mild erythema or pain, were not progressive or associated with systemic sequelae, and resolved spontaneously.

Six of 61 patients (10%) treated with ISIS 304801 discontinued treatment with the study drug because of adverse events; there was no apparent relationship between discontinuation and dosage (Fig. S1 in the Supplementary Appendix). One serious adverse event — a serum sickness–like reaction — occurred 3 days after the 11th weekly dose of 200 mg of ISIS 304801 in the ISIS 304801–fibrate cohort. However, the results of extensive tests were unremarkable, including those involving skin biopsy, an assay of autoantibodies, a vasculitis panel, and C-reactive protein. The patient also tested negative for antibodies to ISIS 304801. One other patient in the ISIS 304801–fibrate cohort had four serious adverse events associated with arterial graft stenosis after the final 300-mg dose on day 85.

Laboratory test results indicated there was no significant effect of ISIS 304801 treatment on renal or hepatic function. Other safety assessments, including vital signs, electrocardiographic findings, and urinalysis results, were clinically unremarkable. In addition, there was no clinical or laboratory evidence of drug–drug interactions in patients receiving concomitant medications, including statins, fibrates, and glucose-lowering agents.

DISCUSSION

In the current dose-ranging phase 2 study, inhibition of APOC3 synthesis with the use of ISIS 304801, a second-generation antisense drug, produced dose-dependent mean reductions of 80% in APOC3 levels and of 71% in triglyceride levels when it was used as a single agent in the treatment of patients with hypertriglyceridemia. A similar incremental reduction in APOC3 and triglyceride levels was also shown in patients receiving ISIS 304801 added to stable fibrate therapy. In both cohorts, reductions in plasma APOC3 levels may have been due to antisense oligonucleotide–induced decreases in hepatic APOC3 production, as well as increased catabolism of triglyceride-rich lipoproteins. In addition to these effects, HDL cholesterol levels increased and VLDL cholesterol levels decreased in a dose-dependent manner in both cohorts. Changes from baseline in other lipid measures were similar between the ISIS 304801–treated groups and the corresponding placebo group, although two exceptions were observed in the ISIS 304801–treated groups in the monotherapy cohort, in which there was a dose-dependent increase in LDL cholesterol and dose-dependent decrease in apoB-48. However, total apoB and non-HDL cholesterol levels remained unchanged in these dose groups.

The reduction in APOC3 levels resulting from short-term treatment with ISIS 304801 is a finding that could have clinical relevance. Increased APOC3 levels are an independent risk factor for coronary heart disease and are implicated in atherogenesis.[13] Conversely, a genetic loss of function or attenuated levels of APOC3 are cardioprotective.[28-30] Thus, a reduction in APOC3 itself might be of benefit. Second, the relationship between APOC3 and circulating triglyceride levels has been known for several decades.[36] The current study further supports this direct relationship, as shown by the strong positive correlations

Table 1. Effect of ISIS 304801 Treatment on Apolipoprotein C-III (APOC3), Triglycerides, and High-Density Lipoprotein (HDL) Cholesterol.*

Measure	ISIS 304801 Monotherapy				ISIS 304801 Added to Fibrate		
	Placebo (N=16)	100 mg (N=11)	200 mg (N=13)	300 mg (N=11)	Placebo (N=8)	200 mg (N=8)	300 mg (N=10)
APOC3 level — mg/dl							
Baseline	22.2±7.7	22.4±7.7	23.1±5.3	22.6±6.3	19.0±5.5	15.5±3.6	18.3±5.9
End point	21.9±10.6	12.0±5.2	7.6±4.1	4.4±2.0	17.7±3.8	6.1±2.9	5.1±2.1
Percentage change	4.2±41.7	−40.0±32.0†	−63.8±22.3‡	−79.6±9.3‡	−2.2±25.2	−60.2±12.5‡	−70.9±13.0‡
Least-squares mean percentage change ±SE	4.2±7.5	−39.2±9.0‡	−64.2±8.3‡	−79.9±8.9‡	−2.2±6.1	−60.5±6.1‡	−71.7±5.3‡
Triglyceride level — mg/dl							
Baseline							
Mean	522.7±369.7	590.6±260.3	641.5±291.7	559.1±224.6	457.0±213.5	281.8±74.5	393.7±227.8
Median (IQR)	459 (356 to 582)	558 (351 to 825)	588 (464 to 717)	566 (355 to 702)	475 (253 to 602)	272 (230 to 295)	271 (241 to 459)
End point							
Mean	547.1±355.4	311.6±142.6	234.7±163.0	139.5±36.4	371.7±125.0	141.1±69.8	134.0±60.9
Median (IQR)	456 (343 to 640)	277 (226 to 394)	180 (157 to 262)	120 (115 to 185)	338 (290 to 394)	120 (103 to 172)	116 (91 to 191)
Percentage change	20.1±72.0	−31.3±56.8†	−57.7±28.3§	−70.9±14.1‡	−7.7±33.8	−51.0±13.5§	−64.0±8.9§
Median percentage change (IQR)	12.1 (−24.6 to 28.9)	−37.8 (−71.5 to −7.0)	−70.3 (−79.5 to −43.4)	−72.2 (−82.8 to −67.0)	−10.4 (−29.8 to 9.7)	−43.8 (−63.5 to −39.8)	−65.4 (−70.7 to −54.6)
Least-squares mean percentage change ±SE	20.1±12.6	−31.2±15.1†	−58.6±13.9§	−70.9±15.0‡	−7.7±7.3	−52.3±7.4‡	−64.9±6.4‡
HDL cholesterol level — mg/dl							
Baseline	33.0±7.5	31.1±4.2	32.3±4.1	33.6±9.6	34.9±8.4	36.9±9.8	33.9±12.3
End point	33.2±8.8	38.5±8.9	42.9±8.3	48.3±15.1	36.3±8.6	52.4±19.3	51.3±19.9
Percentage change	0.7±14.6	26.6±38.0	36.2±26.0‡	45.7±24.0‡	5.9±22.2	50.6±32.0†	51.80±23.7§
Least-squares mean percentage change ±SE	0.7±6.3	28.1±7.3§	32.6±6.8§	46.0±7.3‡	5.9±9.3	47.5±9.5§	49.4±8.2§

* Plus–minus values are means ±SD, except where indicated otherwise. Results are presented for the per-protocol population, except for the results for least-squares mean percentage changes from baseline, which are presented for the modified intention-to-treat population (see the Supplementary Appendix). Baseline is defined as the day −8 value, and the end-point value represents the mean of the values at day 85 and day 92. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. IQR denotes interquartile range, and SE standard error.

† P<0.05 for the comparison with placebo.

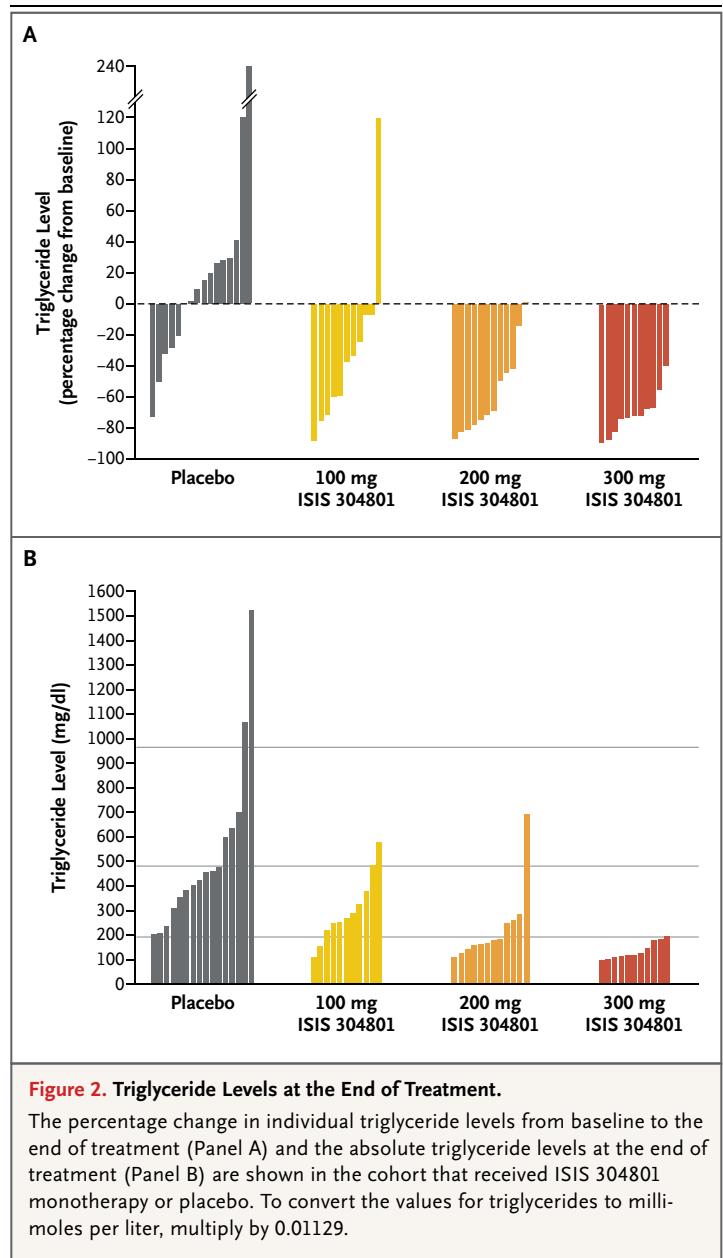‡ P<0.001 for the comparison with placebo.

§ P<0.01 for the comparison with placebo.

between APOC3 and triglyceride levels over time in patients treated with ISIS 304801. Third, in line with the role of APOC3 in regulating plasma triglyceride levels, treatment with ISIS 304801 consistently resulted in reductions in total plasma triglyceride levels, even in patients with baseline levels as high as 1390 mg per deciliter (15.7 mmol per liter).

Prospective case studies have indicated that interventions that decrease fasting triglyceride levels can reduce or eliminate relapse of pancreatitis in patients with severe hypertriglyceridemia and a history of recurrence.[38,39] In a finding consistent with these reports, a recent retrospective analysis showed that lowering triglyceride levels in patients with baseline levels above 500 mg per deciliter (5.7 mmol per liter) was associated with a concentration-dependent reduction in episodes of pancreatitis and cardiovascular events.[40] Patients whose levels were reduced to below 200 mg per deciliter had the greatest benefit. In this respect, the current study showed that after 13 weeks of treatment, approximately 80% of patients who received the higher doses of ISIS 304801 had triglyceride levels below 200 mg per deciliter.

In addition to elevated triglyceride levels, high levels of VLDL and dietary remnant cholesterol levels (gradient density, <1.006 g per milliliter) also characterized patients in the current study, with baseline mean levels that were up to 4 times as high as the normal level of 30 mg per deciliter (0.8 mmol per liter) in the ISIS 304801 monotherapy cohort.[41] The clinical consequences of these increases are unknown without direct study. Remnant cholesterol has been implicated in ischemic heart disease[42] and in an increase in all-cause mortality.[43] After 13 weeks of treatment with ISIS 304801, elevated VLDL cholesterol levels fell to within the normal range in the 300-mg dose group in both cohorts. The dose-dependent decrease in apoB-48 concentrations in patients treated with ISIS 304801 as a single agent indicated an enhanced removal of chylomicron remnants.

High triglyceride levels and low HDL cholesterol levels are characteristic of an atherogenic mixed dyslipidemia, particularly in the context of obesity and insulin resistance.[9] This lipid profile was evident in the current study population at baseline. In concordance with triglyceride lowering, treatment with ISIS 304801 raised

Figure 2. Triglyceride Levels at the End of Treatment.

The percentage change in individual triglyceride levels from baseline to the end of treatment (Panel A) and the absolute triglyceride levels at the end of treatment (Panel B) are shown in the cohort that received ISIS 304801 monotherapy or placebo. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129.

mean HDL cholesterol levels across all dose groups. However, the clinical consequences of these increases in HDL cholesterol levels are uncertain at this time.

The basis for the dose-dependent increase in LDL cholesterol in the monotherapy cohort may be multifactorial. First, it may reflect an increase in the conversion of VLDL to LDL that occurred as a result of enhanced lipoprotein lipase activity and subsequent lipolysis, consequent to the decrease in APOC3. Second, it may be due to

remodeling of lipoprotein content by cholesterol ester transfer protein (CETP). Third, it may be the result of changes in the secretion and catabolism of the LDL particle.[13] The relative contributions of these potential underlying mechanisms have yet to be determined. From a clinical perspective, the increase in levels of LDL cholesterol resulting from antisense inhibition of APOC3 could potentially be offset by treatment with statins or fibrates.[44,45] Indeed, in the ISIS 304801–fibrate cohort, the increases in LDL cholesterol as a consequence of triglyceride lowering associated with ISIS 304801 treatment were less pronounced than in the monotherapy cohort. In general, fibrates have been reported to lower LDL cholesterol in patients who have triglyceride levels below 400 mg per deciliter (4.5 mmol per liter).[46] No drug–drug interactions are expected between ISIS 304801 and statins or fibrates on the basis of the known mechanisms of drug metabolism.[47]

No treatment-associated safety issues were identified in the current study. The rate of drug discontinuation due to adverse events was within the range (5 to 7%) that was observed in a 12-week dose-ranging evaluation of oral n–3 fatty acids in a similar but larger study population.[11] Local injection-site reactions were comparatively few in this 13-week dose-ranging phase 2 evaluation of ISIS 304801.[48] The limitations of our study include the small number of patients, the fact that the study population comprised mostly white men, and the short-term assessment for the treatment of a chronic condition.

In conclusion, we found that treatment with ISIS 304801 resulted in dose-dependent lowering of triglyceride levels among patients with a broad range of baseline levels (200 to 1400 mg per deciliter, or 2.3 to 15.8 mmol per liter). Selective antisense inhibition of APOC3 synthesis provides evidence for a causal relationship between APOC3 and triglyceride metabolism. The results of our study support the continued development of ISIS 304801 for the treatment of patients who remain at risk for cardiovascular events and pancreatitis because of very high triglyceride levels.

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