ORIGINAL ARTICLE

Cardiovascular and Metabolic Effects of *ANGPTL3* Antisense Oligonucleotides

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ABSTRACT

BACKGROUND

Epidemiologic and genomewide association studies have linked loss-of-function variants in *ANGPTL3*, encoding angiopoietin-like 3, with low levels of plasma lipoproteins.

METHODS

We evaluated antisense oligonucleotides (ASOs) targeting *Angptl3* messenger RNA (mRNA) for effects on plasma lipid levels, triglyceride clearance, liver triglyceride content, insulin sensitivity, and atherosclerosis in mice. Subsequently, 44 human participants (with triglyceride levels of either 90 to 150 mg per deciliter [1.0 to 1.7 mmol per liter] or >150 mg per deciliter, depending on the dose group) were randomly assigned to receive subcutaneous injections of placebo or an antisense oligonucleotide targeting *ANGPTL3* mRNA in a single dose (20, 40, or 80 mg) or multiple doses (10, 20, 40, or 60 mg per week for 6 weeks). The main end points were safety, side-effect profile, pharmacokinetic and pharmacodynamic measures, and changes in levels of lipids and lipoproteins.

RESULTS

The treated mice had dose-dependent reductions in levels of hepatic *Angptl3* mRNA, Angptl3 protein, triglycerides, and low-density lipoprotein (LDL) cholesterol, as well as reductions in liver triglyceride content and atherosclerosis progression and increases in insulin sensitivity. After 6 weeks of treatment, persons in the multiple-dose groups had reductions in levels of ANGPTL3 protein (reductions of 46.6 to 84.5% from baseline, P<0.01 for all doses vs. placebo) and in levels of triglycerides (reductions of 33.2 to 63.1%), LDL cholesterol (1.3 to 32.9%), very-low-density lipoprotein cholesterol (27.9 to 60.0%), non–high-density lipoprotein cholesterol (10.0 to 36.6%), apolipoprotein B (3.4 to 25.7%), and apolipoprotein C-III (18.9 to 58.8%). Three participants who received the antisense oligonucleotide and three who received placebo reported dizziness or headache. There were no serious adverse events.

CONCLUSIONS

Oligonucleotides targeting mouse *Angptl3* retarded the progression of atherosclerosis and reduced levels of atherogenic lipoproteins in mice. Use of the same strategy to target human *ANGPTL3* reduced levels of atherogenic lipoproteins in humans. (Funded by Ionis Pharmaceuticals; ClinicalTrials.gov number, NCT02709850.)

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Despite advances in the development of lipid-lowering therapies, clinical trials have shown that a substantial risk of cardiovascular disease persists after currently recommended medical therapy. For example, in one trial involving patients who had had an acute coronary syndrome, the lowering of low-density lipoprotein (LDL) cholesterol levels to a median of 54 mg per deciliter (1.4 mmol per liter) with the use of a statin plus ezetimibe was found to prevent only a slightly higher proportion of events than treatment with a statin alone; the difference in absolute risk associated with the two regimens was only 2 percentage points, and approximately one third of both sets of patients had a major cardiovascular event within 7 years.[1] A similar reduction in the absolute risk of coronary events was found among patients whose levels of LDL cholesterol were reduced to 30 mg per deciliter (0.78 mmol per liter) with the use of proprotein convertase subtilisin–kexin type 9 (PCSK9) inhibitors.[2]

Genomewide association and exome-sequencing studies have identified associations between loss-of-function genetic variants in the gene encoding angiopoietin-like 3 (*ANGPTL3*)[3,4] and in *ANGPTL4*[5-7] and low levels of plasma LDL cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides. Loss-of-function variants in *ANGPTL3* are also associated with beneficial metabolic effects, including increases in levels of lipoprotein lipase and endothelial lipase activity, enhancement of insulin sensitivity, and decreases in levels of free fatty acids in the serum.[8] These findings are consistent with the inhibition of lipoprotein lipase and endothelial lipase by ANGPTL3.[9,10] A complete absence of ANGPTL3 protein, effected by null variants of *ANGPTL3*, results in familial hypobetalipoproteinemia, which is characterized by a reduction in the levels of all lipoproteins except lipoprotein(a).[11] Persons who are homozygous or compound heterozygous for null variants in *ANGPTL3* have levels of plasma LDL cholesterol and triglycerides that are approximately 70% lower than those in persons without such variants. They also have enhanced insulin sensitivity without an increased prevalence of fatty liver disease or an apparent increased risk of cardiovascular disease.[11]

To evaluate *ANGPTL3* as a potential cardiometabolic therapeutic target for reducing the risk of cardiovascular disease, we conducted preclinical studies and a randomized, double-blind, placebo-controlled phase 1 trial of antisense oligonucleotides (ASOs) targeting hepatic *ANGPTL3* messenger RNA (mRNA).

Methods

Preclinical Studies

The mouse-specific *Angptl3* ASO and its *N*-acetylgalactosamine (GalNAc)–conjugated counterpart are described in the Supplementary Appendix, available with the full text of this article at NEJM.org. Details of the preclinical studies, including the pharmacokinetics and pharmacodynamics of the ASOs and the methodologic details of the protocols and of the lipid, lipoprotein, metabolic, and atherosclerosis studies, are provided in the Supplementary Appendix. We used a variety of mouse models, maintained on either standard chow or a Western diet, including wild-type C57BL/6 mice, mice with knockout of the LDL receptor ($Ldlr^{-/-}$), double-knockout mice ($Apoc3^{-/-}$ and $Ldlr^{-/-}$), heterozygous mice ($Apoc3^{+/-}$ and $Ldlr^{-/-}$), mice with diet-induced obesity, and mice overexpressing human apolipoprotein C-III. In selected experiments, the mice were treated with *Angptl3* ASO, GalNAc-conjugated *Angptl3* ASO, microsomal transfer triglyceride protein (*Mttp*) ASO, or both *Angptl3* and *Mttp* ASOs.

Studies in Humans

Sequence and Structure of Human IONIS-ANGPTL3-L_{Rx}

IONIS-ANGPTL3-L_{Rx} (hereafter referred to as ANGPTL3-L_{Rx}) is a second-generation ligand-conjugated antisense drug — specifically, a GalNAc-conjugated ASO drug targeted to a region within the human *ANGPTL3* mRNA coding sequence. ANGPTL3-L_{Rx} contains three GalNAc moieties covalently attached to its 5′ end; its 20 nucleotides are linked by 13 phosphorothioate linkages and 6 phosphodiester linkages at positions 2, 3, 4, 5, 16, and 17 (Fig. S1 in the Supplementary Appendix). Full details of the design of ANGPTL3-L_{Rx} are provided in the Supplementary Appendix.

Phase 1 Trial of ANGPTL3-L_{Rx}

We conducted a randomized, double-blind, placebo-controlled, phase 1 clinical trial designed to test the safety, side-effect profile, pharmacokinetics, and pharmacodynamics of single ascending doses and multiple ascending doses of ANGPTL3-L_{Rx} in healthy adults 18 to 65 years of age. Participants were randomly assigned in a 3:1

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ratio to receive ANGPTL3-L_{Rx} or placebo. In the single-dose groups, the participants were given a single subcutaneous injection of ANGPTL3-L_{Rx} (20, 40, or 80 mg) or placebo (0.9% sterile saline). In the multiple-dose groups, participants were given subcutaneous injections of ANGPTL3-L_{Rx} (10, 20, 40, or 60 mg) or placebo (0.9% sterile saline) once weekly for a total of six doses. The inclusion criteria included a fasting LDL cholesterol level higher than 70 mg per deciliter (1.8 mmol per liter) (for all participants), a fasting triglyceride level between 90 and 150 mg per deciliter (between 1.0 and 1.7 mmol per liter) for participants in the 40-mg and 80-mg single-dose groups, and a fasting triglyceride level of at least 150 mg per deciliter (1.7 mmol per liter) for participants in the 20-mg single-dose group and all the multiple-dose groups. Safety assessments, clinical laboratory evaluations, and pharmacokinetic analyses were performed regularly throughout the treatment and post-treatment periods. Full details of the design of this phase 1 trial are provided in the Supplementary Appendix.

This trial was conducted at the BioPharma Services phase 1 unit in Toronto from November 2015 through October 2016. A total of 158 participants were screened in order to achieve the final enrollment of 44 participants (Figs. S2 and S3 in the Supplementary Appendix). The protocol for the IONIS-ANGPTL3-L_{Rx} trial, available at NEJM.org, was approved by Health Canada as well as by a central institutional review board (Institutional Review Board Services). The study was performed in compliance with the Declaration of Helsinki (2002) and Good Clinical Practice guidelines. All participants provided written informed consent before enrollment. The sponsor prepared the randomization list. All participants, monitors, and sponsor and trial center personnel connected to the trial (except for the pharmacist who prepared the study drug and placebo and the pharmacy monitor who monitored the pharmacy records and procedures) were unaware of the study-group assignments throughout the trial.

The sponsor of the trial was responsible for the trial design, trial conduct, data collection, data analysis, data interpretation, and the writing of the manuscript. The first three authors wrote the first draft of the manuscript. All the authors interpreted the data, collaborated in the preparation of the manuscript, made the decision to submit the manuscript for publication, and vouch for the accuracy and completeness of the data and analyses and for the fidelity of trial to the protocol. The tenth and last two authors (all of whom were employees of the sponsor) had access to the full data set.

STATISTICAL ANALYSIS

A description of the statistical analysis of the preclinical data is provided in the Supplementary Appendix. The main goals of the phase 1 trial were to provide an assessment of the safety, side-effect profile, pharmacokinetics, and preliminary pharmacodynamics of ANGPTL3-L_{Rx} while minimizing unnecessary exposure of participants to the drug. All participants who received at least one dose of placebo or ANGPTL3-L_{Rx} were included in the safety population. Pharmacodynamic assessments were conducted in the safety population. For lipid, lipoprotein, and pharmacodynamic assessments, the baseline value was defined as the mean of all pretreatment measurements, including day 1 predose assessments when applicable. For other assessments, the baseline value was defined as the last measurement before the first dose. The descriptive statistics for ANGPTL3 protein and lipid measures are presented according to dose over time. The percentage change from baseline in each group was compared with the change in the pooled placebo group with the use of the exact Wilcoxon rank-sum test, with day 15 for the single-dose group and day 43 for the multiple-dose group used as the therapeutic end point for ANGPTL3 protein and lipid variables that reflects the maximal predicted reduction after the final dose, as has been shown with other ligand-conjugated antisense drugs.[12] Because this was a phase 1 trial that was designed to gauge safety and side effects, adjustment for multiple comparisons was not performed.

RESULTS

PRECLINICAL STUDIES

Angptl3 *mRNA, Angptl3 Protein, and Lipid Measures*

Mouse *Angptl3* ASO was administered to wild-type C57BL/6 mice, *Ldlr*$^{-/-}$ mice, *Apoc3*$^{-/-}$ mice, and mice that were engineered to overexpress human apolipoprotein C-III. The mice were fed standard chow or a Western diet. After administration of the ASO, hepatic *Angptl3* mRNA expression was significantly suppressed (decreases of 69 to 91%) in each of

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these mouse models, and plasma Angptl3 protein levels were decreased (by 50 to 90%) (Table S1 in the Supplementary Appendix). Quantitative polymerase-chain-reaction analysis of hepatic gene expression in *Ldlr*$^{-/-}$ mice receiving a Western diet and administered *Angptl3* ASO showed no significant changes in the level of mRNA expression of *Apoc3*, *Angptl4*, *Angptl8*, *Srebp2*, *Hmgcr*, or *Apob* (data not shown).

Administration of the *Angptl3* ASO resulted in reductions in levels of triglycerides (reductions of 35 to 85%), LDL cholesterol (7 to 64%) and HDL cholesterol (3 to 23%) (Table S1 and Fig. S4 in the Supplementary Appendix), as well as a reduction in triglycerides within very-low-density lipoprotein (VLDL), intermediate-density lipoprotein, and LDL particles (Table S2 in the Supplementary Appendix) in various mouse models, including the *Ldlr*$^{-/-}$ mice fed a Western diet, *Apoc3*$^{+/-}$/*Ldlr*$^{-/-}$ mice, and *Apoc3*$^{-/-}$/*Ldlr*$^{-/-}$ mice. These data show that anti-*Angptl3*–mediated lowering of levels of plasma triglycerides and LDL cholesterol occurs independently of the LDL receptor pathway and occurs in the absence or presence of an excess of apolipoprotein C-III, the reduction of which generally accompanies most triglyceride-lowering perturbations.[13] The *Angptl3* ASO did not cause significant changes in liver mass, aminotransferase levels, or histopathological characteristics. We observed no adverse events in the mice.

Effects of Angptl3 *ASO on Liver Triglyceride Secretion*

Administration of the *Angptl3* ASO to C57BL/6 mice that were fed standard chow or a Western diet resulted in a significant reduction in liver triglyceride secretion. Clearance of an intravenously administered ^{3}H-triglyceride emulsion was enhanced in chow-fed C57BL/6 mice treated with the *Angptl3* ASO, which was probably independent of whole lipoprotein uptake by the liver, because the rate of ^{125}I-VLDL clearance did not change in response to treatment with *Angptl3* ASO. In addition, intestinal lipid absorption was unaffected. Details regarding these results are provided in Figure S5 in the Supplementary Appendix.

Effects of Angptl3 *ASO in the Presence of Apolipoprotein C-III and on Lipoprotein Lipase*

Treatment with the *Angptl3* ASO significantly reduced plasma triglyceride levels and LDL cholesterol levels in mice overexpressing human apolipoprotein C-III (Fig. S6 in the Supplementary Appendix). Lipoprotein lipase activity measured in chow-fed C57BL/6 mice that were treated with *Angptl3* ASO for 6 weeks was 88% higher than in control animals (P=0.03), whereas hepatic lipase activity did not differ significantly between mice treated with the *Angptl3* ASO and those treated with the control ASO (Fig. S7 in the Supplementary Appendix).

Effects of Angptl3 *ASO on Insulin Resistance and Liver Triglyceride Content*

To determine the effects of the inhibition of mouse *Angptl3* on insulin resistance, the mouse *Angptl3* ASO was administered (50 mg per kilogram of body weight per week) for 6 weeks to obese mice (with obesity caused through diet). Insulin sensitivity, measured by means of intraperitoneal glucose tolerance and insulin tolerance testing, was significantly higher among mice treated with the ASO than among control mice (intraperitoneal glucose tolerance area under the curve was 28% lower than control [P=0.02] and insulin tolerance area under the curve was 36% lower than control [P=0.02]). Furthermore, liver triglyceride accumulation, assessed by biochemical quantification of liver triglyceride content, was 81% lower among mice administered the *Angptl3* ASO than among control mice (mean [±SE], 45.5±14.6 mg per gram in controls vs. 8.7±2.6 mg per gram in *Angptl3* ASO mice; P=0.03). Details are provided in Figure S8 in the Supplementary Appendix.

To further explore the mechanism of reduced hepatic triglyceride secretion and decreased hepatic triglyceride accumulation, we inhibited *Angptl3* gene expression in *Ldlr*$^{-/-}$ mice fed a Western diet (a model of atherosclerosis), in which we suppressed hepatic microsomal triglyceride transfer protein (Mttp) through administration of an *Mttp*-specific ASO (Fig. 1). Inhibition of hepatic *Mttp* is known to cause hepatic triglyceride accumulation in hyperlipidemic mouse models.[14] Individually, both the *Mttp* ASO and the *Angptl3* ASO were associated with lower plasma levels of total cholesterol, LDL cholesterol, and triglycerides, and the combination of the two ASOs potentiated these effects. ASO-mediated inhibition of *Mttp* was associated with significantly higher levels of alanine aminotransferase and higher liver triglyceride content, whereas ASO suppression of *Angptl3* was not associated with higher levels of either alanine aminotransferase or he-

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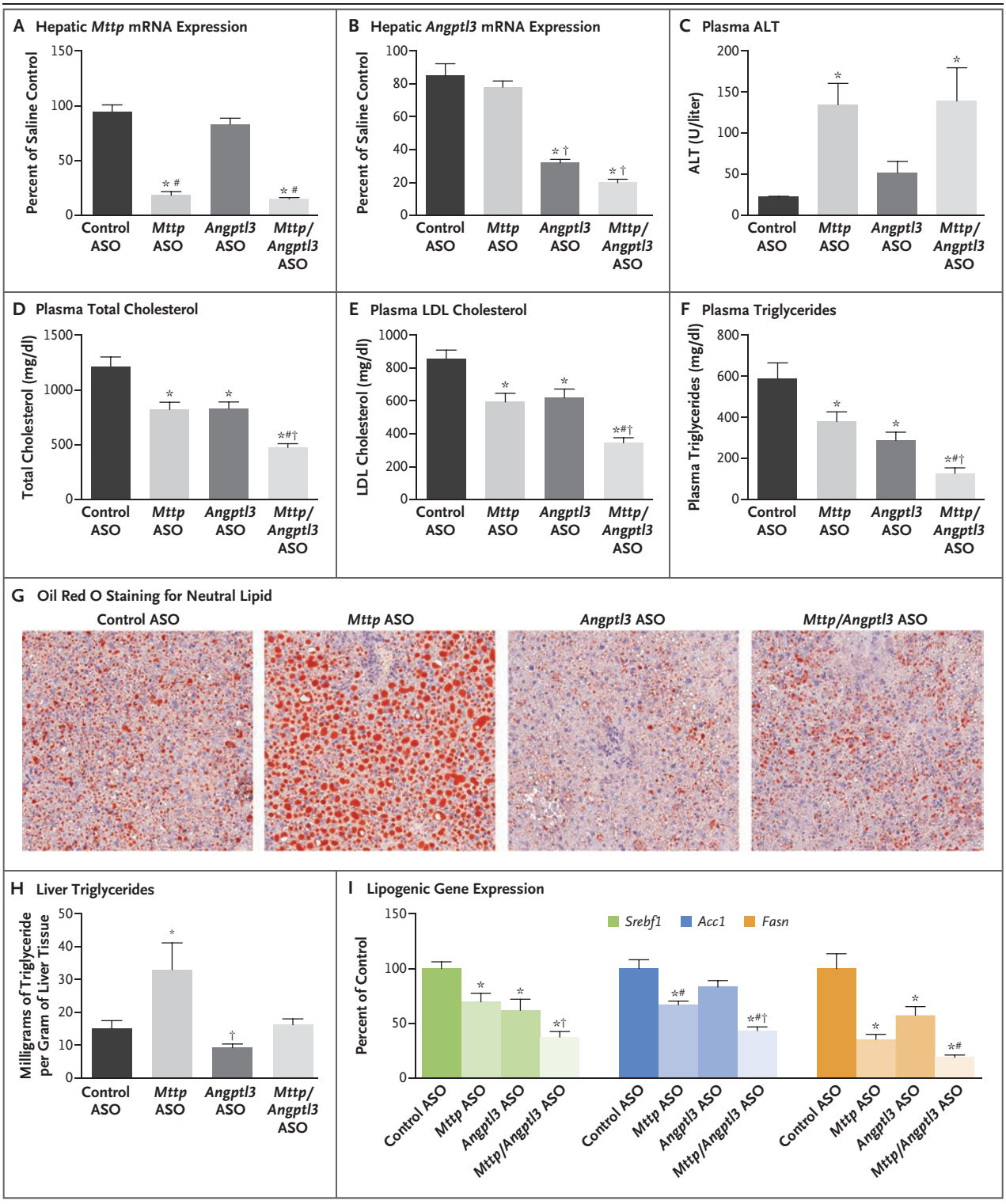

patic fat stores. A similar experiment conducted in the mouse model of diet-induced obesity yielded similar results (Table S3 in the Supplementary Appendix). Coadministration of the *Mttp* and *Angptl3* ASOs had an effect on liver triglyceride content similar to that of the control ASO and was associated with lower lipogenic gene expression than either individual ASO (Fig. 1I). No significant changes were found in *Hspa5* (*Bip*) mRNA expression (data not shown).

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Figure 1 (facing page). Effects of Second-Generation *Mttp* and *Angptl3* ASOs on Plasma Cholesterol and Triglyceride Levels, Liver Triglyceride Content, and Lipogenic Gene Expression in *Ldlr*$^{-/-}$ Mice.

Nine-week-old male *Ldlr*$^{-/-}$ mice (eight mice per group) on a Western diet were randomly assigned to one of the following treatments for 6 weeks: control antisense oligonucleotide (ASO) (50 mg per kilogram of body weight per week), *Mttp* ASO (25 mg per kilogram per week), *Angptl3* ASO (25 mg per kilogram per week), or combined *Angptl3* ASO (25 mg per kilogram per week) plus *Mttp* ASO (25 mg per kilogram per week). Panels A and B show hepatic expression of *Mttp* and *Angptl3*, respectively, in response to treatment with each type of ASO. A saline group was included to allow determination of the effects of the nonspecific, control ASO on hepatic *Mttp* and *Angptl3* mRNA expression; expression in Panels A and B is shown as a percentage of the results obtained with the saline control. Panels C through F show levels of plasma alanine aminotransferase (ALT) (Panel C), plasma total cholesterol (Panel D), plasma low-density lipoprotein (LDL) cholesterol (Panel E), and plasma triglycerides (Panel F) in response to ASO treatment. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. Hepatic lipid accumulation after ASO treatment was assessed by Oil Red O staining of histologic sections (Panel G) and biochemical quantification of triglycerides (Panel H). Hepatic lipogenic gene expression after ASO treatment was determined by quantitative polymerase chain reaction (Panel I). An asterisk denotes $P<0.05$ for the comparison with control ASO, a pound sign $P<0.05$ for the comparison with *Angptl3* ASO, and a dagger $P<0.05$ for the comparison with *Mttp* ASO. T bars denote standard errors.

Effects of Angptl3 *ASO on Atherosclerosis Progression*

Treatment of Western-diet–fed *Ldlr*$^{-/-}$ mice with *Angptl3* ASO retarded the progression of en face atherosclerosis, relative to that observed in mice that received the control ASO, by 52% (5.4±1.1% vs. 11.4±1.3%, $P=0.002$) in the group that received a dose of 50 mg per kilogram per week and by 37% (7.2±0.8% vs. 11.4±1.3%, $P=0.048$) in the group that received 12.5 mg per kilogram per week (Fig. S9 in the Supplementary Appendix).

Conjugation of the Angptl3 *ASO with GalNAc*

The ED_{50} (i.e., the effective dose required to reduce hepatic *Angptl3* mRNA levels by 50%) for the GalNAc-conjugated *Angptl3* ASO was one nineteenth that of the unconjugated *Angptl3* ASO; the GalNAc-conjugated *Angptl3* ASO was also associated with lower plasma Angptl3 protein levels than the unconjugated ASO (Fig. S10 in the Supplementary Appendix). We also found significantly lower levels of triglycerides, LDL cholesterol, and HDL cholesterol in mice that received the conjugated ASO.

IONIS-ANGPTL3-L_{Rx} PHASE 1 TRIAL IN HUMANS

Effects of ANGPTL3-L_{Rx} on Levels of ANGPTL3 Protein, Lipids, and Lipoproteins

The baseline characteristics of the trial participants in the single-dose groups are shown in Table S4 in the Supplementary Appendix; those of the participants in the multiple-dose groups are shown in Table 1. Twelve participants were randomly assigned to single-dose groups (9 to active-agent dose groups and 3 to the placebo group) and 32 were randomly assigned to multiple-dose groups (24 to active-agent dose groups and 8 to the placebo group) (Figs. S2 and S3 in the Supplementary Appendix).

Among the single-dose groups at day 15, we found lower absolute levels of ANGPTL3 protein, triglycerides, VLDL cholesterol, non-HDL cholesterol, and total cholesterol in the active-agent groups than in the placebo group, as well as greater mean percentage reductions from baseline in these measures; these effects were dose-dependent (Tables S5 and S6 in the Supplementary Appendix). These differences were not significant, probably because of the small size of the groups.

Among the multiple-dose groups, we found significantly lower absolute levels of ANGPTL3 protein at day 43 (1 week after the final dose) in the active-agent groups than in the placebo group, as well as significantly greater percentage reductions from baseline in these levels; again, the effects were dose dependent (Table 2 and Fig. 2, and Table S7 in the Supplementary Appendix). The mean percentage reductions in ANGPTL3 levels from baseline at day 43 were 46.6% in the 10-mg group ($P=0.001$ vs. placebo), 72.5% in the 20-mg group ($P=0.003$), 81.3% in the 40-mg group ($P=0.001$), and 84.5% in the 60-mg group ($P=0.001$) (Table 3). The mean percentage changes in lipids and lipoproteins from baseline to day 43 in the multiple-dose groups are provided in Table 3, and in Table S7 in the Supplementary Appendix. Triglyceride levels were reduced from baseline by a maximum of 63.1% ($P=0.01$), apolipoprotein C-III levels by a maximum of 58.8% ($P=0.001$), non-HDL cholesterol levels by a maximum of 36.6% ($P=0.001$), and apolipoprotein B levels by a maximum of 25.7% ($P=0.001$). Between the final dose and the end of

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Table 1. Baseline Characteristics of the Participants in the Multiple-Dose Groups in the IONIS-ANGPTL3-L_{Rx} Trial.*

Characteristic	Placebo (N=8)	ANGPTL3-L_{Rx}			
		10 mg (N=6)	20 mg (N=6)	40 mg (N=6)	60 mg (N=6)
Mean age (range) — yr	55 (46–64)	46 (28–64)	52 (28–65)	54 (39–62)	56 (49–62)
Sex ratio (male:female)	6:2	5:1	6:0	6:0	6:0
BMI†	28.0±3.8	27.9±3.4	27.0±4.0	26.4±3.2	28.5±2.6
ANGPTL3 — ng/ml	126.1±32.5	84.5±23.5	96.8±19.3	112.4±7.8	109.7±38.3
Triglycerides — mg/dl	201±36	212±107	196±50	212±62	168±60
LDL cholesterol — mg/dl	132±16	133±41	141±29	154±32	128±42
VLDL cholesterol — mg/dl	38±10	39±20	38±12	38±11	33±13
Apolipoprotein B100 — mg/dl	108±13	107±28	120±15	120±13	103±39
Non-HDL cholesterol — mg/dl	170±19	172±36	180±30	192±27	161±50
Total cholesterol — mg/dl	216±12	217±35	216±32	230±31	206±48
HDL cholesterol — mg/dl	46±11	46±16	37±7	38±5	45±10
Apolipoprotein AI — mg/dl	146±18	149±32	131±15	129±10	137±16
Apolipoprotein C-III — mg/dl	12.6±2.9	11.2±4.6	9.7±1.9	11.0±2.8	9.7±3.1
Lipoprotein(a) — nmol/liter	35±23	70±72	32±53	20±28	10±14

* Plus–minus values are means ±SD. 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. ANGPTL3 denotes angiopoietin-like 3, HDL high-density lipoprotein, LDL low-density lipoprotein, and VLDL very-low-density lipoprotein.
† The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters.

follow-up (day 127), the plasma levels of ANGPTL3 protein, triglycerides, non-HDL cholesterol, and apolipoprotein C-III (Fig. 2) and of LDL cholesterol, VLDL cholesterol, apolipoprotein B, total cholesterol, HDL cholesterol, and apolipoprotein AI (data not shown) slowly returned to normal.

Potency, Safety, and Side Effects of ANGPTL3-L_{Rx}

The model-estimated ED_{50} for plasma ANGPTL3 was 10.4±1.2 mg per week for ANGPTL3-L_{Rx} (Fig. S11 in the Supplementary Appendix). A summary of the pharmacokinetic properties of ANGPTL3-L_{Rx} is provided in Tables S8 and S9 in the Supplementary Appendix.

There were no serious adverse events documented during the trial. One participant in the 20-mg multiple-dose group was lost to follow-up after five doses. There were no other discontinuations during the treatment period. No injection-site reactions were reported (Table S10 in the Supplementary Appendix), and no adverse events were reported among the participants receiving the single-dose regimen (Table S11 in the Supplementary Appendix). Of those participants who received the multiple-dose regimen, three reported headache (one who received placebo and two who received ANGPTL3-L_{Rx}) and three reported dizziness (two who received placebo and one who received ANGPTL3-L_{Rx}) (Table S11 in the Supplementary Appendix). There was no clinical evidence of prothrombotic effects, bleeding episodes, significant decreases in platelet count or thrombocytopenia, or significant changes in liver or renal function.

DISCUSSION

We found that inhibition of *ANGPTL3* mRNA results in favorable cardiometabolic effects in several different mouse models and in healthy human volunteers. The preclinical studies indicated that suppression of hepatic Angptl3 protein production in mice resulted in significant reductions in levels of triglycerides, LDL cholesterol, non-HDL cholesterol, and VLDL cholesterol and that these favorable effects were associated with decreased liver triglyceride content, increases in insulin sensitivity, and a reduction

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Table 2. Absolute Levels of ANGPTL3, Lipids, and Lipoproteins at Day 43 after Initiation of ANGPTL3-L_{Rx} Treatment in the Multiple-Dose Groups.*

Measure	Placebo (N=8)	ANGPTL3-L_{Rx}			
		10 mg (N=6)	20 mg (N=5)	40 mg (N=6)	60 mg (N=6)
ANGPTL3 — ng/ml	132.5±38.9	45.3±22.9†	24.5±7.5†	21.1±5.0†	16.6±8.1†
Triglycerides — mg/dl	183±76	135±55	73±20†	93±24‡	82±27†
LDL cholesterol — mg/dl	151±18	126±29	124±24	115±31‡	85±26†
VLDL cholesterol — mg/dl	37±15	27±11	15±4†	19±5‡	16±6†
Apolipoprotein B — mg/dl	122±19	102±22	99±13‡	90±19‡	78±22†
Non-HDL cholesterol — mg/dl	188±25	153±28‡	139±26‡	133±32‡	101±31†
Total cholesterol — mg/dl	230±20	197±27‡	171±30†	168±33†	134±29†
HDL cholesterol — mg/dl	42±12	44±16	32±5	35±4	33±10
Apolipoprotein AI — mg/dl	146±15	143±36	115±15†	112±13†	105±23†
Apolipoprotein C-III — mg/dl	12.8±3.2	9.1±3.8	4.2±2.3†	5.7±3.1†	3.8±1.0†
Lipoprotein(a) — nmol/liter	32±21	71±69	13±12	18±24	5±8†

* Plus–minus values are means ±SD. The results presented are based on the safety population. The difference between each group and the pooled placebo group was evaluated by the exact Wilcoxon rank-sum test.
† The difference in comparison with the placebo group was significant ($P<0.01$).
‡ The difference in comparison with the placebo group was significant ($P<0.05$).

in atherosclerosis progression. We also found that these triglyceride-lowering and LDL cholesterol–lowering effects can occur in the absence of the LDL receptor and that the mechanisms responsible for triglyceride lowering are not dependent on the reduction of apolipoprotein C-III, although lowering of apolipoprotein C-III levels may also secondarily contribute to triglyceride lowering.[15-17] The trial in humans indicated that inhibition of hepatic ANGPTL3 led to lowering of triglycerides, LDL and VLDL cholesterol levels, and apolipoprotein C-III levels. Whereas most triglyceride-lowering perturbations and therapies are associated with increases in LDL cholesterol levels,[13,18] hepatic inhibition of ANGPTL3 led to significant decreases in levels of LDL cholesterol, as well as a decrease in the total apolipoprotein B level. These studies provide a rationale for pursuing the development of an ANGPTL3 therapeutic agent as treatment for elevated levels of triglyceride-rich lipoproteins, in order to further reduce the risk of cardiovascular disease in persons who are already taking recommended medical and preventative therapies.[19]

The preclinical studies indicated that the plasma triglyceride–lowering effects of hepatic knockdown of *ANGPTL3* mRNA can be explained by decreased hepatic VLDL triglyceride secretion, as well as by increased lipoprotein lipase activity — an explanation that is consistent with the proposed mechanism of ANGPTL3, which is to inhibit lipoprotein lipase activity.[8] We did not find an enhanced rate of clearance of VLDL–apolipoprotein B and did not study the clearance of LDL. The mechanisms that we observed are consistent with recent reports describing the effect of neutralization of plasma Angptl3 in mouse models with a monoclonal antibody[20,21]; these effects included an enhanced rate of clearance of LDL through a noncanonical pathway and HDL cholesterol lowering through enhanced activity of endothelial lipase.

An antibody-based approach might be expected to differ in some respects from an ASO-based approach. For example, in addition to lowering plasma ANGPTL3 levels, antisense drugs also inhibit ANGPTL3 synthesis within the hepatocyte. Indeed, we found a reduction of hepatic triglyceride content in several different mouse models, a finding that contrasts with the observation that hepatic triglyceride content is not affected by therapeutic anti-ANGPTL3 antibodies.[20] This was clearly demonstrated by the prevention of hepatic steatosis caused by Mttp inhibition. Hepatic tri-

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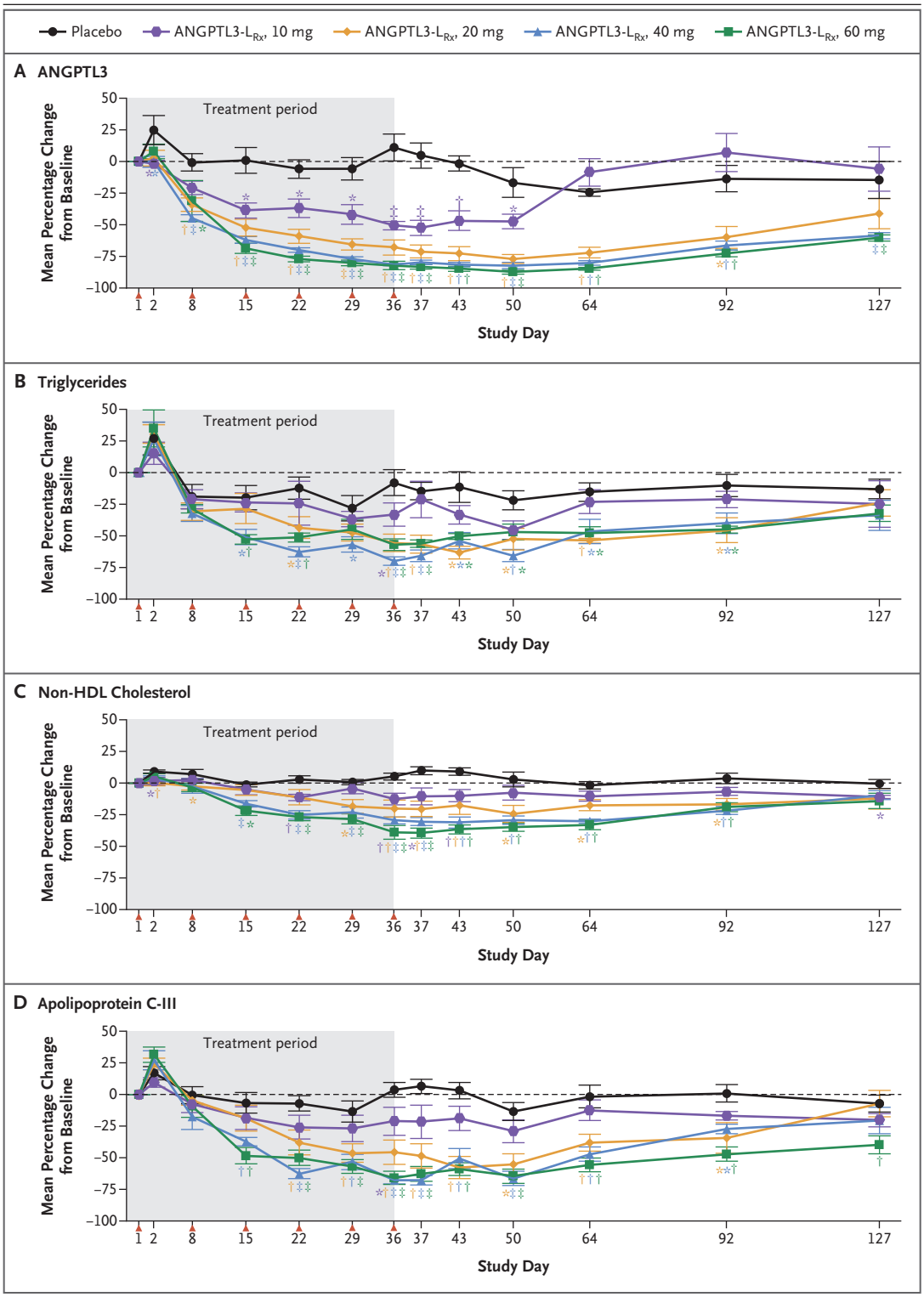

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Figure 2 (facing page). Effect of ANGPTL3-L_{Rx} on Levels of ANGPTL3 Protein, Apolipoprotein C-III, Triglycerides, and Non-HDL Cholesterol in the Multiple-Dose Groups.

The data are the mean percentage changes in levels from baseline; I bars denote standard errors. Red arrowheads indicate the timing of dosing weekly for 6 weeks; the shaded area denotes the dosing duration, and the white area the time of follow-up. Day 43 was used as the therapeutic end point for ANGPTL3 protein and lipid variables that reflects the maximal predicted reduction in levels after the final dose. P values are for the differences in efficacy at day 43 as determined by the exact Wilcoxon rank-sum test comparing ANGPTL3-L_{Rx} and placebo. An asterisk denotes P<0.05, a dagger P<0.01, and a double dagger P<0.001. HDL denotes high-density lipoprotein.

glyceride secretion was also reduced, probably owing to the inhibition of key lipogenic enzymes, although the mechanisms effecting this inhibition remain to be more completely defined. Given these findings, it is conceivable that a drug targeting ANGPTL3 would benefit people with hepatic steatosis, who frequently have varying degrees of hypertriglyceridemia and insulin resistance. Supporting this line of reasoning are the improved measures of insulin sensitivity that we found in mice with diet-induced obesity and in humans with genetically mediated ANGPTL3 deficiency.[11]

There is now a growing body of epidemiologic, genetic, and genomewide association studies[4,11,22,23] supporting the hypothesis that lowering levels of ANGPTL3 in plasma by inhibiting hepatic ANGPTL3 synthesis will be beneficial in terms of reducing plasma levels of atherogenic apolipoprotein B and in improving metabolic measures associated with dyslipidemia. Because the ASO is effectively targeted to the hepatocyte, the drug doses can be greatly reduced, and as modeled in these studies, are on average one twentieth as high as those used with nonconjugated ASO drugs. We reported similar findings with a GalNac-conjugated ASO designed to target *LPA* mRNA, encoding apolipoprotein(a), using doses ranging from 10 to 40 mg that achieved efficacy superior to that obtained with 100 to 300 mg of an unconjugated ASO also targeting *LPA* mRNA.[12]

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Table 3. Percentage Changes in ANGPTL3, Lipid, and Lipoprotein Levels at Day 43 after Initiation of ANGPTL3-L_{Rx} Treatment in the Multiple-Dose Groups.*

Measure	Placebo (N=8)	ANGPTL3-L_{Rx}			
		10 mg (N=6)	20 mg (N=6)	40 mg (N=6)	60 mg (N=6)
		percentage change from baseline			
ANGPTL3	−1.6±15.4	−46.6±18.5†	−72.5±12.4†	−81.3±4.0†	−84.5±5.1†
Triglycerides	−11.4±31.9	−33.2±17.8	−63.1±10.9‡	−53.8±15.6‡	−50.4±5.9‡
LDL cholesterol	13.6±12.1	−1.3±23.6	−4.3±18.5	−25.4±16.5†	−32.9±10.4†
VLDL cholesterol	−4.0±30.8	−27.9±18.3	−60.0±15.5†	−48.5±14.3†	−48.7±8.5‡
Apolipoprotein B	11.0±7.7	−3.4±17.0	−13.3±15.0†	−25.7±10.8†	−22.2±12.7†
Non-HDL cholesterol	9.1±8.2	−10.0±11.6†	−17.6±16.2†	−31.1±11.0†	−36.6±8.9†
Apolipoprotein C-III	3.1±17.0	−18.9±23.4	−57.8±19.4†	−50.7±19.3†	−58.8±12.3†

* Plus–minus values are means ±SD. The results presented are based on the safety population. Values are the percentage change from baseline to day 43, 1 week after the final dose. Baseline values were defined as the mean of all pretreatment measurements, including the day 1 predose measurement. The difference between percentage change in each dose group and that in the pooled placebo group was evaluated by the exact Wilcoxon rank-sum test.
† The difference in comparison with the placebo group was significant (P<0.01).
‡ The difference in comparison with the placebo group was significant (P<0.05).

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