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Efficacy and Safety of Mipomersen, an Antisense Inhibitor of Apolipoprotein B, in Hypercholesterolemic Subjects Receiving Stable Statin Therapy

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Objectives The aim of this study was to evaluate the efficacy and safety of mipomersen in hypercholesterolemic subjects taking stable statin therapy.

Background Mipomersen is an apolipoprotein (apo) B synthesis inhibitor that has demonstrated significant reductions in apo B and low-density lipoprotein (LDL) cholesterol in Phase 1 clinical trials in healthy volunteers.

Methods A randomized, placebo-controlled, dose-escalation Phase 2 study was designed to evaluate the effects of mipomersen in hypercholesterolemic subjects taking stable statin therapy. Seventy-four subjects were enrolled sequentially into 1 of 6 dose cohorts at a 4:1 (active/placebo) ratio. Subjects received 7 doses of 30 to 400 mg over 5 weeks in the first 5 cohorts and 15 doses of 200 mg over 13 weeks in the sixth cohort. Pre-specified end points included percentage change from baseline in apo B and LDL cholesterol. Safety was assessed with laboratory test results and by the incidence and severity of adverse events.

Results The apo B and LDL cholesterol were reduced by 19% to 54% and 21% to 52%, respectively, at doses of 100 mg/week mipomersen and higher in the 5-week treatment cohorts. Efficacy seemed to increase upon treatment for 13 weeks at a dose of 200 mg/week. Injection site reactions (mild to moderate erythema [90%]) and hepatic transaminase increases (17%) were the most common adverse events, leading to discontinuation in 2 subjects and 1 subject, respectively. In the 13-week treatment cohort, 5 of 10 subjects (50%) had elevations ≥3× the upper limit of normal, 4 of which persisted on 2 consecutive occasions.

Conclusions Mipomersen might hold promise for treatment of patients not reaching target LDL cholesterol levels on stable statin therapy. Further studies are needed to address the mechanisms and clinical relevance of transaminase changes after mipomersen administration. (Dose-Escalating Safety Study in Subjects on Stable Statin Therapy; NCT00231569) (J Am Coll Cardiol 2010;55:1611–8) © 2010 by the American College of Cardiology Foundation

Low-density lipoprotein (LDL) cholesterol is the primary target for lipid-lowering therapy in patients at risk for cardiovascular disease (1,2). This guideline is based upon the consistent, positive association between circulating LDL cholesterol levels and the incidence of major adverse cardiovascular events observed in large population studies (3,4), genetically determined hypercholesterolemia phenotypes (5,6), as well as major clinical outcome trials with statins (7–9). Results from the latter investigations led to the recommendation for aggressive reductions in LDL cholesterol, particularly in very-high-risk patient populations (1). Despite the advent of potent statins and an increase in usage of combination therapies to support these updated guidelines, current therapies are still inadequate for optimal treatment of a substantial portion of the high-risk patient populations (10–12). Thus, there is a need for the development of therapeutic agents with complementary effects.

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Abbreviations and Acronyms

AE = adverse event
ALT = alanine aminotransferase
Apo = apolipoprotein
LDL = low-density lipoprotein
VLDL = very-low-density lipoprotein
HDL = high-density lipoprotein
TG = triglyceride
SAE = serious adverse event
ULN = upper limit of normal

Mipomersen is a second-generation antisense oligonucleotide designed to inhibit production of human apolipoprotein (apo) B-100 in the liver (13,14). Apo B-100 is an essential component of LDL cholesterol and all other metabolically related atherogenic lipoproteins. In healthy human volunteers dosed for 4 weeks, mipomersen produced dose-dependent reduction in apo B levels that resulted in reduction of LDL cholesterol levels as much as 44% from baseline (15). A drug elimination half-life of approximately 30 days resulted in a prolonged pharmacological effect. Treatment was well-tolerated and demonstrated an acceptable safety profile. In a second Phase 1 study, concomitant administration of either simvastatin or ezetimibe had no significant effect on the pharmacokinetic properties of mipomersen and vice versa (16). These results are consistent with the metabolism of mipomersen by nucleases followed by rapid clearance of the metabolites via urinary excretion (17).

In this report we describe the results from a randomized, placebo-controlled, dose-escalation Phase 2 study designed to determine the efficacy and safety of mipomersen in hypercholesterolemic subjects receiving stable statin therapy.

Methods

Study participants. Eligible participants were 18 to 65 years of age without any clinically significant medical condition at screening and with fasting LDL cholesterol between 100 and 220 mg/dl on stable statin therapy. Participants were receiving stable statin therapy at a dose of ≤40 mg/day for at least 3 months before treatment with the study drug. The study was approved by the institutional review board of the local centers, and all subjects gave written informed consent. The study was performed in compliance with the standards of Good Clinical Practice (ICH/E6/R1) and the Declaration of Helsinki (Washington 2002).

Study participants enrolled sequentially into 1 of 5 5-week dose-escalation cohorts at 4 sites in the Netherlands between October 24, 2005, and December 14, 2006. The protocol was amended on August 14, 2006, to evaluate the effects of an extended treatment period of 13 weeks in 2 dose cohorts, where eligible participants enrolled at 3 sites in the Netherlands and 1 site in the U.S. between October 16, 2006, and March 5, 2007.

Study design. This randomized, double-blind, placebo-controlled, dose-escalation study comprised 6 cohorts. Five cohorts were designed to evaluate the effects of short-term treatment over a range of doses, and 1 was designed to evaluate an extended treatment period at the 200-mg dose that is under consideration for Phase 3. In the 5 dose-escalation cohorts, the study drug was administered at a dose of 30, 100, 200, 300, or 400 mg for a total of 7 doses over a 5-week period. In the extended treatment cohort, the study drug was administered at a dose of 200 mg 3 times (qod) in the first week and then once/week for 12 weeks for a total of 15 doses.

Subjects were randomized at a ratio of 4:1 for active drug to placebo by dose cohort. In the 5-week dose-escalation cohorts, the study drug was administered by subcutaneous injection on Days 1, 8, 10, 12, 15, 22, and 29. In the 13-week treatment cohort, the study drug was administered by subcutaneous injection on Days 1, 3, 5, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, and 85. A 2-month evaluation period involving 4 visits followed the treatment period. After this evaluation period, subjects in the 5-week treatment cohorts with fasting LDL cholesterol <90% at baseline continued with monthly visits up to 24 weeks after the last dose. Subjects in the 13-week treatment cohort continued with monthly follow-up visits for an additional 4 months.

Safety end points included all adverse events (AEs) and serious adverse events (SAEs); physical examination data and vital signs; and hepatic, renal, and coagulation profiles from laboratory tests. Laboratory evaluations included routine hematology, blood chemistries, and urinalysis.

Pre-specified efficacy end points included percentage change in LDL cholesterol and apo B from baseline on Day 59 (30 days after the last dose in the 5-week treatment cohorts). Percentage change from baseline in very-low-density lipoprotein (VLDL) cholesterol, high-density lipoprotein (HDL) cholesterol, non-HDL cholesterol, total cholesterol, triglycerides (TGs), the LDL/HDL cholesterol ratio, and apo B/apo A1 ratio were also evaluated on Day 59. Post-hoc exploratory analysis of all lipid parameters except for subclasses was performed on Day 99 for the 13-week cohort.

Laboratory analysis. Fasting blood samples were analyzed for apo B, apo A1, VLDL cholesterol, LDL cholesterol, HDL cholesterol, total cholesterol, and TGs. Samples from the 5-week treatment cohorts were analyzed at Interlab GmbH (Munich, Germany), and those from the 13-week treatment cohort were analyzed at Medpace (Cincinnati, Ohio, and Belgium). In all cases, apo B and apo A1 concentrations were determined by rate nephelometry; total cholesterol and TGs were measured by standard enzyme-based colorimetric assays. For the 5-week treatment cohorts, VLDL cholesterol, LDL cholesterol, and HDL cholesterol concentrations were determined by gel electrophoresis (Hydragel 30, Sebia Electrophoresis, Durham, North Carolina); and LDL cholesterol subclass concentrations were determined by density gradient ultracentrifugation (Beckman Optima MAX-E, TLN-100 rotor, Beckman Coulter, Fullerton, California). For the 13-week

treatment cohort, VLDL and LDL cholesterol were calculated, and HDL cholesterol was determined by an enzyme-based colorimetric assay after dextran-sulfate precipitation.

Mipomersen plasma trough concentrations were measured at Pharma Bio-Research (Amsterdam, the Netherlands) with a hybridization-dependent enzyme-linked immunosorbent assay. Terminal plasma elimination half-lives were estimated by a non-compartmental method of analysis (WinNONLIN version 5).

Statistical analysis. Sample size was based upon an SD of 12% in the percentage change of LDL cholesterol and analysis of the data between 5 treatment groups and pooled placebo. Under these assumptions a sample size of 8/group would provide at least 80% power to detect a 20% difference in LDL cholesterol percentage change with a statistical significance level of 0.05. Study end points were analyzed on the intent-to-treat population, which consisted of all subjects that were randomized (n = 74). Missing lipid parameter values were imputed by the last observation carried forward method. Descriptive statistics for apo B and LDL cholesterol data are presented by dose versus time. Baseline was defined as the average of 2 screening values and the pre-dose Day 1 measurement. Percentage change from baseline for each of the dose groups was compared with the pooled placebo group with the exact Wilcoxon rank sum test. Software used for the analyses was SAS version 8.2 (SAS Institute, Cary, North Carolina).

A visit window of ±7 days was applied for the analyses of efficacy end points for the 5-week dose-escalation cohorts at Day 59. Descriptive analysis of efficacy was performed on the 13-week treatment cohort on Day 99 with a visit window of ±4 days.

Results

Study participants. One-hundred and thirty-two subjects were screened for inclusion in the study from October 2005 to February 2007 (Fig. 1). Seventy-four participants receiving stable statin therapy were randomly assigned sequentially by dose cohort to either placebo or mipomersen treatment. Demographic data and background statin therapy are summarized in Table 1 by treatment group.

Sixty-eight subjects completed the study protocol, whereas in total 6 subjects were excluded in the course of the study. In the 5-week dose-escalation cohorts, 3 subjects dropped out: 1 was lost to follow-up, 1 missed 3 consecutive visits, and 1 withdrew informed consent for personal reasons. In the 13-week treatment cohort, 3 subjects (200 mg/week) discontinued dosing due to an AE, comprising transient injection site reactions in 2 and a transient alanine aminotransferase (ALT) level increase ≥5× the upper limit of normal (ULN) (maximum 213 U/l) occurring after receiving 9 doses in 1 (see section, Safety observations).

5-week dose-escalation cohorts. The 5-week dose-escalation cohorts involved 39 men and 23 women ranging in age from 49 to 65 years (data not shown). Baseline LDL cholesterol levels ranged from 84 to 216 mg/dl with a mean of 135 mg/dl. Baseline lipid parameters are summarized in Table 2 by treatment group. Reductions in apo B and LDL cholesterol seemed to occur in a dose-dependent fashion in subjects treated with mipomersen on a background of stable statin ther-

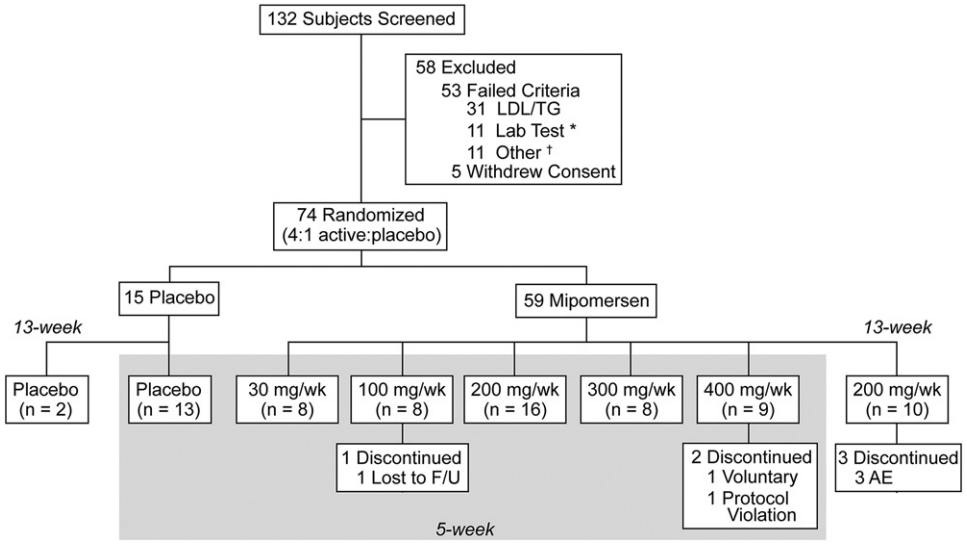

Figure 1 Flow of Study Participants

*Liver function, fasting glucose, and bilirubin tests. †Statin therapy, age, blood pressure, alcohol abuse, physical or medical condition, and hepatitis C virus positive. AE = adverse event; F/U = follow-up; LDL/TG = low-density lipoprotein cholesterol and/or triglycerides.

Table 1 Demographic Data and Background Statin Therapy

		5-Week Treatment Cohorts					13-Week Treatment Cohort	
	Placebo (n = 15)	30 mg/week (n = 8)	100 mg/week (n = 8)	200 mg/week (n = 16)	300 mg/week (n = 8)	400 mg/week (n = 9)	200 mg/week (n = 10)	Total (n = 74)
Sex (M:F)	8:7	6:2	4:4	11:5	4:4	7:2	6:4	46:28
Age (yrs)	60.8 ± 3.3	58.0 ± 3.9	57.4 ± 4.1	58.3 ± 3.8	56.9 ± 4.3	61.4 ± 3.2	59.0 ± 4.0	59.0 ± 4.1
Statin (mg/day)								
Atorvastatin								
10	0 (0)	0 (0)	1 (13)	2 (13)	2 (25)	1 (11)	2 (20)	8 (11)
20	6 (40)	2 (25)	2 (25)	6 (38)	2 (25)	2 (22)	0 (0)	20 (27)
40	1 (7)	0 (0)	2 (25)	3 (19)	0 (0)	1 (11)	3 (30)	10 (14)
Simvastatin								
5	0 (0)	0 (0)	1 (13)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)
10	3 (20)	0 (0)	1 (13)	3 (19)	1 (13)	2 (22)	1 (10)	11 (15)
20	2 (13)	1 (13)	1 (13)	2 (13)	1 (13)	2 (22)	2 (20)	11 (15)
40	2 (13)	5 (63)	0 (0)	0 (0)	2 (25)	1 (11)	2 (20)	12 (16)
Rosuvastatin								
10	1 (7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)

Statin values represent n (%) by statin type and dose. Median duration of statin therapy before dosing estimated as 4 years.

apy (Fig. 2). Percentage changes in apo B and LDL cholesterol levels from baseline were statistically significant relative to placebo on Day 59 (30 days after the 5-week treatment period) in the 100-mg/week and higher dose groups (Table 3). The maximum reduction in apo B and LDL cholesterol was observed in the 300-mg/week dose group, with reductions of 54% and 52% from baseline, respectively ($p < 0.001$ for both). In line, a maximum reduction of 52% from baseline in non-HDL cholesterol levels ($p < 0.001$) was demonstrated in the 300-mg/week dose group. Dose-dependent effects on apo B/A1 and LDL/HDL cholesterol ratios were consistent with the decreases in apo B and LDL cholesterol. Treatment at doses of 300 mg/week and 400 mg/week also produced a significant reduction in TGs of 41% ($p < 0.05$) and 31% ($p < 0.01$) from baseline. The HDL cholesterol levels were unaffected across all dose cohorts.

Seventy-three percent of the subjects in the 200-mg/week or higher dose cohorts (24 of 33) had LDL cholesterol levels below 100 mg/dl after 5 weeks of treatment (Table 4). Thirty-three percent had LDL cholesterol levels below 70 mg/dl. All but 1 of the 24 subjects with LDL cholesterol levels below 100 mg/dl also achieved non-HDL cholesterol levels below 130 mg/dl and apo B levels below 90 mg/dl. No relationship between baseline LDL cholesterol in percentage change after mipomersen therapy was observed.

13-week treatment cohort. The 13-week treatment cohort involved 12 subjects (7 men and 5 women) ranging in age from 50 to 65 years. Baseline LDL cholesterol levels ranged from 104 to 154 mg/dl with a mean of 127 mg/dl (data not shown). Ten subjects were assigned to active treatment. An incremental reduction in apo B and LDL cholesterol of 36% from baseline was demonstrated in subjects treated with 200 mg/week mipomersen, 2 weeks after the last dose of the treatment period (Table 5). These reductions were greater than that observed in the same-dose 5-week treatment group. The HDL choles-

Table 2 Baseline Lipid Parameters Among 5-Week Treatment Cohorts

Lipid Parameters (mg/dl)	Placebo (n = 13)*	30 mg/week (n = 8)*	100 mg/week (n = 8)	200 mg/week (n = 16)	300 mg/week (n = 8)	400 mg/week (n = 9)	Total (n = 62)
Apo B	105.0 ± 17.0	97.9 ± 10.8	106.0 ± 24.3	103.7 ± 17.7	138.8 ± 14.4	109.1 ± 13.0	108.8 ± 20.2
LDL cholesterol	130.4 ± 19.9	114.9 ± 18.7	135.0 ± 34.4	128.5 ± 20.2	170.9 ± 26.5	136.3 ± 18.6	134.6 ± 26.8
VLDL cholesterol	13.1 ± 6.8	13.3 ± 6.5	11.8 ± 7.7	13.8 ± 12.6	16.4 ± 8.3	11.6 ± 5.3	13.3 ± 8.6
Non-HDL cholesterol	143.5 ± 23.5	128.1 ± 16.7	146.8 ± 36.9	142.3 ± 28.7	187.3 ± 28.2	147.9 ± 20.3	147.9 ± 30.2
HDL cholesterol	65.3 ± 20.9	54.0 ± 8.6	51.5 ± 7.7	69.0 ± 21.5	64.1 ± 18.3	58.7 ± 11.7	61.9 ± 17.6
Total cholesterol	208.8 ± 21.7	182.1 ± 23.8	198.3 ± 37.5	211.3 ± 33.5	251.5 ± 35.8	206.6 ± 21.9	209.8 ± 33.9
Triglycerides	133 (93–249)	118 (76–188)	117 (85–237)	132 (65–301)	152 (128–210)	114 (70–209)	134 (65–301)
Apo B/A1	0.6 ± 0.2	0.6 ± 0.0	0.7 ± 0.2	0.6 ± 0.2	0.8 ± 0.2	1.4 ± 2.2	0.8 ± 0.8
LDL/HDL cholesterol	2.2 ± 0.7	2.1 ± 0.2	2.7 ± 0.9	2.1 ± 0.8	2.9 ± 0.9	2.4 ± 0.7	2.4 ± 0.8

Values represent the mean ± SD. Triglycerides are presented as the median and range (minimum to maximum). Multiply values by 0.0259 (cholesterol) and 0.0113 (triglycerides) for conversion to SI units (mmol/l). *Baseline lipid values for 1 subject were based on the average of 4 independent measures before the first dose; all others were based on the average of 3 independent measures before the first dose as described in the Methods section.

Apo = apolipoprotein; HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very-low-density lipoprotein.

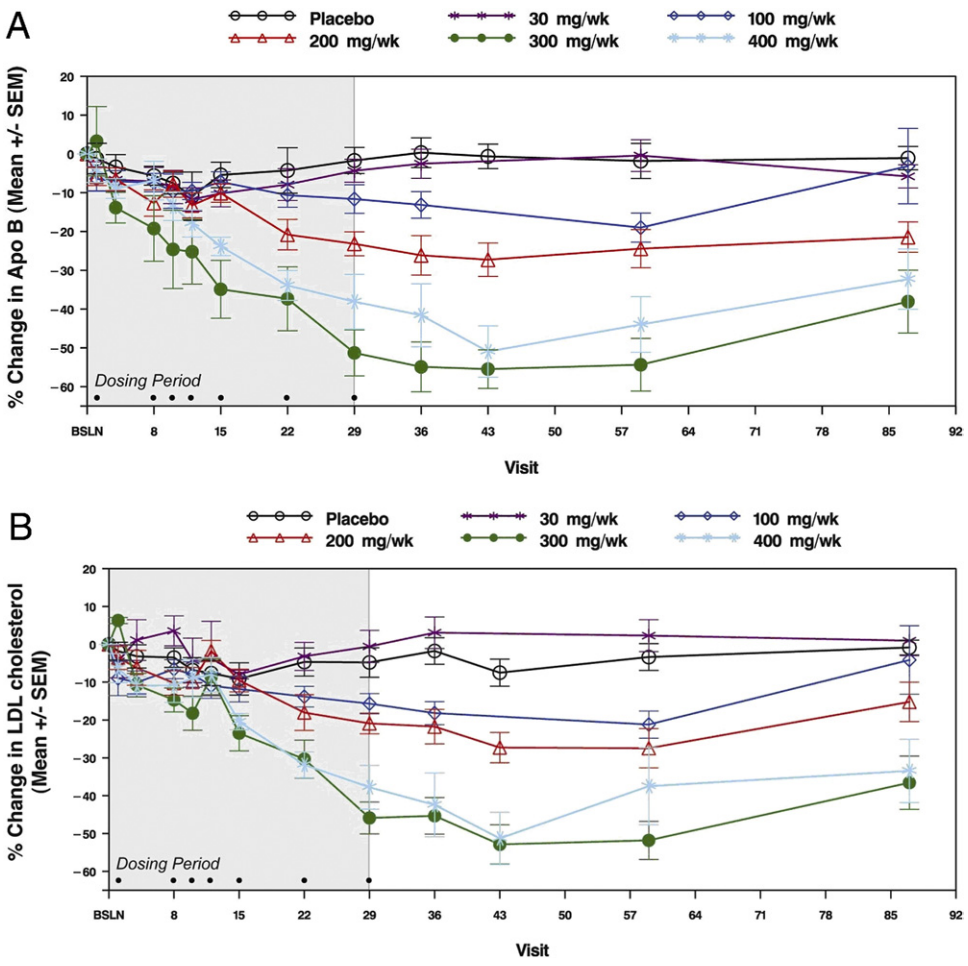

Figure 2 Dose-Dependent and Prolonged Effect of Mipomersen on Apo B and LDL Cholesterol in 5-Week Treatment Cohorts

(A) Apolipoprotein (Apo) B and **(B)** low-density lipoprotein (LDL) cholesterol presented as mean percentage change from baseline (BLSN) ± SEM. Time period ranges from Day 1 to Day 87 (end of evaluation period). **Grey-shading** indicates treatment period, and **solid circles** located above the x-axes indicate the dose schedule (7 doses). **Unshaded area** indicates the post-treatment follow-up period.

Table 3 Effects on Lipid Parameters Among 5-Week Treatment Cohorts

		Percentage Change From Baseline				
Lipid Parameters (mg/dl)	Placebo (n = 13)	30 mg/week (n = 8)	100 mg/week (n = 8)	200 mg/week (n = 16)	300 mg/week (n = 8)	400 mg/week (n = 9)
Apo B	−1.8 ± 16.3	−0.4 ± 11.6	−19.0 ± 10.6*	−24.4 ± 19.7†	−54.4 ± 19.2‡	−44.0 ± 21.5‡
LDL cholesterol	−3.3 ± 12.8	2.3 ± 11.9	−21.2 ± 10.1†	−27.4 ± 20.7‡	−51.8 ± 14.3‡	−37.5 ± 30.5*
VLDL cholesterol	1.0 ± 38.0	3.7 ± 41.7	5.6 ± 33.9	3.0 ± 84.6	−27.4 ± 87.5	−45.8 ± 40.5†
Non-HDL cholesterol	−3.3 ± 12.4	3.2 ± 12.0	−19.4 ± 9.8†	−25.0 ± 23.4†	−52.0 ± 14.9‡	−38.4 ± 30.6*
HDL cholesterol	8.0 ± 13.6	1.9 ± 12.5	−2.8 ± 11.3*	2.8 ± 13.5	2.9 ± 17.3	−1.4 ± 24.7
Total cholesterol	0.0 ± 9.3	2.7 ± 10.8	−15.0 ± 8.3†	−15.4 ± 17.7†	−38.5 ± 12.5‡	−28.4 ± 16.1‡
Triglycerides	1.0	3.5	−3.9	−23.1	−40.5*	−30.8†
Apo B/A1	241 ± 883	−1.0 ± 7.4	−16.5 ± 11.2*	−24.1 ± 19.1†	−55.6 ± 14.2‡	−42.7 ± 30.2†
LDL/HDL cholesterol	−9.7 ± 15.8	0.7 ± 13.3	−19.2 ± 11.8	−29.7 ± 17.8†	−53.7 ± 11.0‡	−19.5 ± 84.0*

Values represent the mean ± SD, 30 days after the last dose in the 5-week treatment cohorts (Day 59). Triglycerides are presented as the median. Percentage change from baseline (3 time points) for each of the dose groups was compared with the pooled placebo group. The p value was calculated with the exact Wilcoxon rank sum test. *$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$.
Abbreviations as in Table 2.

JACC Vol. 55, No. 15, 2010
April 13, 2010:1611–8

Table 4 Effects on Lipid and Lipoprotein Concentrations Among 5-Week Treatment Cohorts

Lipid Parameter	Placebo (n = 13)	Mean Values at Baseline and Day 59				
		30 mg/week (n = 8)	100 mg/week (n = 8)	200 mg/week (n = 16)	300 mg/week (n = 8)	400 mg/week (n = 9)
LDL cholesterol						
Baseline	130.4 ± 19.9	114.9 ± 18.7	135.0 ± 34.4	128.5 ± 20.2	170.9 ± 26.5	136.3 ± 18.6
Day 59	126.4 ± 26.8	117.3 ± 22.4	106.7 ± 32.4	91.6 ± 23.5	84.0 ± 36.1	86.6 ± 47.1
n (%) <100 mg/dl	4 (31)	2 (25)	4 (50)	10 (63)	7 (88)	7 (78)
n (%) <70 mg/dl	0 (0)	0 (0)	1 (13)	3 (19)	3 (38)	5 (56)
Non-HDL cholesterol						
Baseline	143.5 ± 23.5	128.1 ± 16.7	146.8 ± 36.9	142.3 ± 28.7	187.3 ± 28.2	147.9 ± 20.3
Day 59	139.3 ± 31.2	131.5 ± 18.4	118.6 ± 33.9	104.3 ± 30.6	91.1 ± 37.9	91.9 ± 48.7
n (%) <130 mg/dl	5 (38)	5 (63)	6 (75)	12 (75)	7 (88)	7 (78)
Apo B						
Baseline	105.0 ± 17.0	97.9 ± 10.8	106.0 ± 24.3	103.7 ± 17.7	138.8 ± 14.4	109.1 ± 13.0
Day 59	102.7 ± 22.2	97.2 ± 13.2	86.9 ± 27.5	77.1 ± 19.7	64.7 ± 32.4	61.8 ± 26.6
n (%) <90 mg/dl	4 (31)	2 (25)	5 (63)	11 (69)	7 (88)	7 (78)

Baseline and Day 59 values represent the mean ± SD in mg/dl. The number of subjects (n) below the indicated target level at the end point is presented by dose group, where the percentage of the total number of subjects in the group at baseline (%) is in parenthesis.

Abbreviations as in Table 2.

terol levels remained unchanged. A plasma terminal elimination half-life of approximately 30 days was observed across all dose groups. There was no significant change in the drug's half-life with extension of the dosing period from 5 to 13 weeks.

Safety observations. Three SAEs occurred in this study. One subject presented with fever (39°C) that occurred more than 12 h after receiving a first dose of 400 mg. The fever resolved spontaneously within 24 h and was considered possibly related to the study drug. The second SAE was a myocardial infarction that occurred 6 weeks after the 5-week treatment period. In view of the severe atherosclerotic disease on coronary angiography in this high-risk patient (hypercholesterolemia, obesity, smoking with 48 pack-years), this AE was considered as "unknown relationship to the study drug." The third SAE was for surgical treatment of lumbar spinal stenosis, which was considered not related to the study drug.

The most common AE in this study was a reaction at the subcutaneous injection site (Table 6). These injection site reactions were characterized by mild-to-moderate erythema, which did not worsen with repeated dosing and resolved spontaneously several days after the injection. Another frequent AE was transaminase increases. In the 5-week treatment cohorts, 5 of the 49 subjects (10%) receiving mipomersen (n = 2, 200 mg/week; n = 2, 300 mg/week; n = 1, 400 mg/week) and 1 of 13 subjects (8%) receiving placebo experienced ALT elevations ≥3× ULN. In 3 subjects (n = 1, 200 mg/week; n = 2, 300 mg/week) ALT ≥3× ULN persisted on 2 or more consecutive occasions at least 7 days apart. In the 13-week treatment cohort, 5 of 10 subjects (50%) had elevations ≥3× ULN, of which 4 had elevations ≥3× ULN on 2 consecutive occasions. None of the patients showed elevations in total bilirubin >2× ULN, and other measures of liver function, such as albumin, remained unchanged.

Other AEs that demonstrated a possible dose-dependent association were flu-like illness, fatigue, and pyrexia. There was no evidence of abnormal changes or treatment-related effects on kidney function on the basis of serum chemistry

Table 5 Effects on Lipid Parameters After 13 Weeks of Dosing at 200 mg/Week Mipomersen

Lipid Parameter (mg/dl)	Baseline (n = 10)	Day 99 (n = 10)	Change	
			Absolute	%
Apo B	109.0 ± 12.0	70.8 ± 20.3	−38.2 ± 14.2	−35.7 ± 14.1
LDL cholesterol	126.9 ± 15.1	81.3 ± 23.8	−45.6 ± 23.5	−35.8 ± 16.4
VLDL cholesterol	27.4 ± 7.9	25.4 ± 13.3	−2.0 ± 7.2	−11.0 ± 21.6
Non-HDL cholesterol	155.6 ± 15.4	111.8 ± 32.3	−43.8 ± 27.5	−28.5 ± 17.5
HDL cholesterol	46.9 ± 16.0	47.0 ± 19.1	0.1 ± 4.3	−1.1 ± 8.5
Total cholesterol	202.5 ± 18.9	158.8 ± 31.9	43.7 ± 26.1	−21.8 ± 12.9
Triglycerides	145	127	−19.7	−14.6
Apo B/A1	0.8 ± 0.2	0.5 ± 0.2	−0.2 ± 0.1	−31.1 ± 12.6
LDL/HDL cholesterol	2.9 ± 0.9	1.9 ± 0.8	−1.0 ± 0.6	−35.7 ± 17.5

Values represent the mean ± SD. Triglycerides are presented as the medians. Multiply values by 0.0259 (cholesterol) and 0.0113 (triglycerides) for conversion to SI units (mmol/l).

Abbreviations as in Table 2.

Table 6 Treatment-Emergent Adverse Events by Dose Group (Mipomersen Treated ≥10%)

		5-Week Treatment Cohorts					13-Week Treatment Cohort	
Event, n (%)	Placebo (n = 15)	30 mg/week (n = 8)	100 mg/week (n = 8)	200 mg/week (n = 16)	300 mg/week (n = 8)	400 mg/week (n = 9)	200 mg/week (n = 10)	Mipomersen (n = 59)
Injection site reaction	2 (13)	6 (75)	8 (100)	14 (88)	8 (100)	8 (89)	9 (90)	53 (90)
Headache	6 (40)	2 (25)	2 (25)	6 (38)	3 (38)	3 (33)	2 (20)	18 (31)
Influenza-like illness	1 (7)	1 (13)	2 (25)	1 (6)	2 (25)	4 (44)	5 (50)	15 (25)
Fatigue	0 (0)	0 (0)	0 (0)	2 (13)	1 (13)	4 (44)	4 (40)	11 (19)
Nasopharyngitis	1 (7)	1 (13)	2 (25)	2 (13)	2 (25)	1 (11)	2 (20)	10 (17)
Back pain	1 (7)	1 (13)	2 (25)	1 (6)	0 (0)	3 (33)	3 (30)	10 (17)
Hepatic enzyme increase	1 (7)	0 (0)	0 (0)	1 (6)	2 (25)	2 (22)	5 (50)	10 (17)
Urinary tract infection	0 (0)	2 (25)	3 (38)	1 (6)	0 (0)	0 (0)	0 (0)	6 (10)
Pyrexia	0 (0)	0 (0)	0 (0)	2 (13)	0 (0)	3 (33)	1 (10)	6 (10)

Value (n) denotes the number of subjects with at least 1 event. Percentage of the total number of subjects (%) by treatment group is in parenthesis.

and urinary protein data. There were no clinically significant effects of treatment on coagulation on the basis of prothrombin and activated partial thromboplastin times.

Discussion

In the present study, mipomersen produced significant incremental lipid-lowering effects in hypercholesterolemic subjects receiving stable statin therapy, resulting in an increased proportion of patients reaching LDL-target levels. A substantial number of patients, however, experienced transient increases in hepatic transaminase levels, particularly at higher dosages of mipomersen, without bilirubin change or change in other liver synthesis functions. Before long-term application of higher-dosed mipomersen, additional studies are needed to elucidate the mechanism as well as the clinical relevance of these transaminase increases.

Efficacy. Mipomersen seemed to reduce apo B and LDL cholesterol levels from the on-statin baseline in a dose-dependent fashion. However, because steady state levels for efficacy are calculated to be reached only at 26 weeks, it is difficult to draw conclusions on dose-dependent efficacy in the present 5-week study. Reductions in LDL cholesterol ranged from 18% to 49% relative to the placebo-control group at doses of 100 mg or higher. This response compares favorably to those reported for other lipid-lowering agents, (e.g., ezetimibe [18] and colesevelam [19]) when added to stable doses of statins in subjects with primary hypercholesterolemia.

In the current study population, mipomersen produced broad atherogenic lipid-lowering effects that were consistent with earlier phase 1 studies involving healthy volunteers (14,15). These effects included significant reductions in non-HDL cholesterol and TGs. Non-HDL cholesterol is considered a secondary target of therapy after LDL cholesterol, particularly when TGs are above 200 mg/dl (1). Elevated TG concentrations are typically associated with cardiovascular risk (20,21) and the presence of highly atherogenic, small-dense LDL particles (22). Reductions in these secondary parameters by mipomersen were within range to those reported for other agents, (e.g., ezetimibe [18] and fibrates [23]) when added to ongoing statin therapy. The HDL cholesterol levels were not significantly altered during mipomersen administration.

Safety. Three SAEs occurred in this study, of which 2 (myocardial infarction and lumbar stenosis) were deemed not related to the study drug by the investigator. The remaining SAE (fever) occurred subsequent to administration of the first 400-mg dose and was considered possibly related to the study drug. In fact, a transient temperature increase was experienced by 2 other subjects in this dose group subsequent to the first dose but with no recurrence upon continued treatment. Flu-like symptoms in general seemed to be dose-related and might have reflected a transient response upon initial exposure to a higher concentration of the drug (24).

Hepatic enzyme increases were observed in a substantial proportion of subjects, particularly in the higher dose cohorts, but without concomitant changes in other indicators of hepatic injury (e.g., increase in total bilirubin or decrease in albumin). The basis and clinical significance of the transaminase increases is not known at the present time. On the basis of the pharmacology, it is conceivable that changes in transaminase levels might reflect a change in hepatic TG accumulation. In line, inhibition of a more distal enzyme involved in apo B lipidation—the microsomal TG transfer protein—has also been associated with hepatic TG accumulation in both animal models (25,26) as well as in humans (27). In contrast, studies in both mouse and monkey models of hyperlipidemia found no evidence of persistent liver TG accumulation from antisense inhibition of apo B (13,14). The latter coincided with compensatory changes in response to antisense inhibition of apo B leading to increased fatty acid oxidation and decreased fatty acid synthase activity, which did not occur after microsomal TG transfer protein inhibition in these same animal models (14). To directly assess the impact of apo B inhibition on hepatic TG excretion, a placebo-controlled study is underway to elucidate the effects of mipomersen treatment on

hepatic TG content in subjects with varying degrees of hyperlipidemia.

Clinical implications. Results from the current Phase 2 study bear direct relevance to the treatment of patients at high risk for cardiovascular disease. In fact, mipomersen on top of statin therapy resulted in a significant, higher proportion of patients reaching target LDL (<100 mg/dl) as well as intensive LDL target (<70 mg/dl) compared with statin monotherapy. However, only a minority of patients used maximally tolerated lipid-lowering therapy in the present study. Because mipomersen is likely to be used in patients not reaching target levels despite maximum conventional therapy, further studies are needed to address the effects of mipomersen when given on top of high-dose statin such as atorvastatin 80 mg or rosuvastatin 20 to 40 mg. Given the short-term treatment period, small sample size, and narrowly defined subject population, additional clinical studies are required to confirm these results. Ongoing and planned clinical studies for mipomersen include longer-term dosing for 6 to 24 months in unmet medical need populations (e.g., those with familial hypercholesterolemia), those with high-risk cardiovascular disease that are not reaching their goal on current therapies, and those intolerant to statin therapy.

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REFERENCES

1. Grundy SM, Cleeman JI, Merz MB, et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. Circulation 2004;110:227–39.
2. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486–97.
3. Prospective Studies Collaboration. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55000 vascular deaths. Lancet 2007;370:1829–39.
4. Stamler J, Daviglus ML, Garside DB, Dyer AR, Greenland P, Neaton JD. Relationship of baseline serum cholesterol levels in 3 large cohorts of younger men to long-term coronary, cardiovascular, and all-cause mortality and to longevity. JAMA 2000;284:311–8.
5. Austin ME, Austin MA, Hutter CM, Zimmern RL, Humphries SE. Familial hypercholesterolemia and coronary heart disease: a HuGE association review. Am J Epidemiol 2004;160:421–9.
6. Goldstein J, Hobbs H, Brown M. Familial hypercholesterolemia. In: Valle D, Scriver CR, Beaudet A. The Metabolic and Molecular Bases of Inherited Disease. 8th edition. Vol 2. New York, NY: McGraw Hill, 2001:2863–913.
7. Cannon CP, Steinberg BA, Murphy SA, Mega JL, Braunwald E. Meta-analysis of cardiovascular outcomes trials comparing intensive versus moderate statin therapy. J Am Coll Cardiol 2006;48:438–45.
8. Baigent C, Keech A, Kearney PM, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet 2005; 366:1267–78.
9. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet 2002;360:7–22.
10. O'Keefe JH, Cordain L, Harris WH, Moe RM, Vogel R. Optimal low-density lipoprotein is 50 to 70 mg/dl. J Am Coll Cardiol 2004;43:2142–6.
11. LaRosa JC. Low-density lipoprotein cholesterol reduction: the end is more important than the means. Am J Cardiol 2007;100:240–2.
12. Davidson MH, Robinson JG. Safety of aggressive lipid management. J Am Coll Cardiol 2007;49:1753–62.
13. Crooke RM, Graham MJ, Lemonidis KM, Whipple CP, Koo S, Perera RJ. An apolipoprotein B antisense oligonucleotide lowers LDL cholesterol in hyperlipidemic mice without causing hepatic steatosis. J Lipid Res 2005;46:872–84.
14. Crooke R, Baker B, Wedel M. Cardiovascular therapeutic applications. In: Crooke ST, editor. Antisense Drug Technology; Principles, Strategies and Applications. 2nd edition. Boca Raton, FL: CRC Press, 2007:601–39.
15. Kastelein JJ, Wedel MK, Baker BF, et al. Potent reduction of apolipoprotein B and LDL cholesterol by short-term administration of an antisense inhibitor of apolipoprotein B. Circulation 2006;114:1729–35.
16. Yu RZ, Geary RS, Flaim J, et al. Lack of pharmacokinetic interaction for ISIS 301012, a 2'-O-methoxyethyl modified antisense oligonucleotide targeting apoB-100 mRNA, with oral lipid-lowering agents, simvastatin and ezetimibe, when co-administered in healthy human subjects. Clin Pharmacokinet 2009;48:39–50.
17. Yu RZ, Kim TW, Hong A, Watanabe TA, Gaus HJ, Geary RS. Cross-species pharmacokinetic comparison from mouse to man of a second generation antisense oligonucleotide ISIS 301012, targeting human Apo B-100. Drug Metab Dispos 2007;35:460–8.
18. Gagné C, Bays HE, Weiss SR, et al., Ezetimibe Study Group. Efficacy and safety of ezetimibe added to ongoing statin therapy for treatment of patients with primary hypercholesterolemia. Am J Cardiol 2002;90: 1084–91.
19. Bays HE, Davidson M, Jones MR, Abby SL. Effects of colesevelam hydrochloride on low-density lipoprotein cholesterol and high-sensitivity C-reactive protein when added to statins in patients with hypercholesterolemia. Am J Cardiol 2006;97:1198–205.
20. Sarwar N, Danesh J, Eiriksdottir G, et al. Triglycerides and the risk of coronary heart disease: 10,158 incident cases among 262,525 participants in 29 Western prospective studies. Circulation 2007;115:450–8.
21. McBride PE. Triglycerides and risk for coronary heart disease. JAMA 2007;298:336–8.
22. Sniderman AD, Blank D, Zakarian R, Bergeron J, Frohlich J. Triglyceride and small dense LDL: the twin Achilles heels of the Friedewald formula. Clin Biochem 2003;36:499–504.
23. Nissen SE, Nicholls SJ, Wolski K, et al. Effects of a potent and selective PPAR-alpha agonist in patients with atherogenic dyslipidemia or hypercholesterolemia: two randomized controlled trials. JAMA 2007;297:1362–73.
24. Kwoh J. An overview of the clinical safety experience of first- and second-generation antisense oligonucleotides. In: Crooke ST, editor. Antisense Drug Technology; Principles, Strategies and Applications. 2nd edition. Boca Raton, FL: CRC Press, 2008:365–99.
25. Chandler CE, Wilder DE, Pettini JL, et al. CP-346086: an MTP inhibitor that lowers plasma cholesterol and triglycerides in experimental animals and in humans. J Lipid Res 2003;44:1887–901.
26. Letteron P, Sutton A, Mansouri A, Fromenty B, Pessayre D. Inhibition of microsomal triglyceride transfer protein: another mechanism for drug-induced steatosis in mice. Hepatology 2003;38:133–40.
27. Cuchel M, Bloedon LT, Szapary PO, et al. Inhibition of microsomal triglyceride transfer protein in familial hypercholesterolemia. N Engl J Med 2007;356:148–56.

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