European Heart Journal (2011) **32**, 2650–2659
doi:10.1093/eurheartj/ehr148

Efficacy of apolipoprotein B synthesis inhibition in subjects with mild-to-moderate hyperlipidaemia

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Received 24 August 2010; revised 7 March 2011; accepted 16 April 2011; online publish-ahead-of-print 18 May 2011

Aims Mipomersen, an apolipoprotein (apo) B synthesis inhibitor, has been shown to produce potent reductions in apoB and LDL-cholesterol levels in animal models as well as healthy human volunteers. A randomized, double-blind, placebo-controlled, dose-escalation study was designed to evaluate the efficacy and safety of mipomersen monotherapy with or without dose loading in subjects with mild-to-moderate hyperlipidaemia.

Methods and results Fifty subjects with LDL-cholesterol levels between 119 and 266 mg/dL were enrolled into five cohorts at a 4:1 randomization ratio of active to placebo. Two 13-week dose regimens were evaluated at doses ranging from 50 to 400 mg/week. Mipomersen produced dose-dependent reductions in all apoB containing lipoproteins. In the 200 and 300 mg/week dose cohorts, mean reductions from baseline in LDL cholesterol were $-45 \pm 10\%$ ($P = 0.000$) and $-61 \pm 8\%$ ($P = 0.000$), corresponding to a $-46 \pm 11\%$ ($P = 0.000$) and $-61 \pm 7\%$ ($P = 0.000$) decrease in apoB levels. Triglyceride levels were also lowered with median reductions up to 53% ($P = 0.021$). The most common adverse events were injection site reactions. Seven of 40 subjects (18%) showed consecutive *trans*-aminase elevations $>3\times$ upper limit of normal. Five of these subjects received 400 mg/week, four of whom had apoB levels below the limit of detection. As a consequence, the 400 mg/week cohort was discontinued.

Conclusions Mipomersen administered as monotherapy in subjects with mild-to-moderate hyperlipidaemia produced potent reductions in all apoB-containing lipoproteins. Higher doses were associated with hepatic *trans*-aminase increases.

This trial is registered at clinicaltrials.gov as NCT00216463.

Keywords Apolipoproteins • Lipoproteins • Hyperlipidaemia • Antisense • *Trans*-aminases

Introduction

Low-density lipoprotein (LDL) cholesterol is a primary target for lipid-lowering therapy and cardiovascular risk reduction.[1] A meta-analysis of all major statin trials comprising a total of 90 056 subjects demonstrated a 23% decrease in cardiovascular event rate for each 1 mmol/L (40 mg/dL) reduction of LDL cholesterol.[2] Intensification of LDL cholesterol-lowering treatments to achieve even lower levels has been shown to lead to further reduction in cardiovascular event rates (TNT, IDEAL).[3,4] Unfortunately, increasing the dose of a statin offers only limited incremental lowering of LDL cholesterol and in some cases these higher doses are not well tolerated.[5] Therefore, novel LDL cholesterol-lowering modalities are needed to achieve incremental reductions, particularly in high-risk patients not reaching target levels.

Direct inhibition of apolipoprotein (apo) B-100 is an attractive approach for lowering LDL cholesterol. Apolipoprotein B-100 is an essential component of LDL cholesterol and related atherogenic lipoproteins such as very-low-density lipoprotein (VLDL), intermediate-density lipoprotein, and lipoprotein (a) [Lp(a)].[6,7] Whereas to date direct inhibition of apoB-100 production has not been feasible using small molecules, this approach has been made possible with the use of an antisense inhibitor of apoB.[8,9]

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Mipomersen is a modified oligonucleotide that is complementary in sequence to the human apoB messenger RNA and distributes to the liver where apoB-100 is expressed.[8] In healthy volunteers with total cholesterol between 200 and 300 mg/dL, 4 weeks of mipomersen treatment produced a 50% reduction in circulating apoB levels and concomitant 35% reduction in LDL cholesterol by dose loading in the first week.[10] Injection site reactions were the most frequently reported adverse events (AEs). Similar efficacy and safety profiles have been shown in subsequent short-term Phase 2 studies involving patients with hyperlipidaemia on statins and other lipid-lowering therapies.[11,12] In the present study, we report the effects of mipomersen in subjects in good health with mild-to-moderate hyperlipidaemia following 13 weeks of mipomersen treatment with or without dose loading.

Methods

Study subjects

Eligible subjects were 18–65 years of age with a body mass index $\geq$25 to $\leq$32 kg/m^2. Subjects were selected with a mild-to-moderate hyperlipidaemia phenotype, with untreated LDL cholesterol levels $\geq$130 mg/dL and fasting triglycerides (TGs) $<$400 mg/dL at screening. Subjects were in good health without any clinically significant medical conditions or laboratory test abnormalities. With the exception of hyperlipidaemia, eligible subjects had no current diagnosis or history of endocrine, haematologic, renal, hepatic, metabolic, psychiatric, neurologic, pulmonary, or cardiovascular disease, including any condition that pre-disposes to secondary hyperlipidaemia, such as diabetes mellitus and hypothyroidism. Females were not of child-bearing potential. The study excluded subjects who were undergoing or had undergone treatment with another investigational drug, biological agent, or device within 90 days prior to screening; and subjects taking any lipid-lowering drug within 30 days or 5 drug half-lives (whichever was longer) prior to screening. Subjects were enrolled at a single site in Germany (PAREXEL International GmbH) between 23 August 2005 and 4 December 2006. All subjects gave written informed consent prior to enrolment.

Study design

A randomized, placebo-controlled, double-blind, dose-escalation design was used to evaluate the effects of mipomersen as a monotherapy in the selected study population. Eligible subjects were assigned to one of three parallel dose cohorts (50, 100, and 200 mg/week) or one of two subsequent and sequential dose escalation cohorts (300 or 400 mg/week). A 2-week loading dose period (200 mg dose two times per week) was evaluated in the 50 and 100 mg/week cohorts, which was followed by a 100 or 200 mg dose every other week for 11 weeks, respectively. The loading dose period was designed to rapidly achieve tissue steady-state levels based on a tissue drug half-life of $\sim$30 days.[13–15] The higher dose cohorts (200, 300, and 400 mg/week) were dosed once per week for 13 weeks at the specified dose, without a loading dose period. Subjects were randomized at a ratio of 4 : 1, active to placebo. Study drug was administered by subcutaneous injection.

The use of any prescription or alternative medication, which in the opinion of the investigator could potentially interfere with the clinical assessment of mipomersen, was not allowed during the study. The use of any other lipid-lowering drug or drug that may affect coagulation, such as warfarin, heparin, or fractionated heparin products was prohibited. No other medication was permitted during the study except for those deemed necessary by the investigator to treat AEs.

Mipomersen was supplied by Isis Pharmaceuticals Inc. (Carlsbad, CA, USA) as a lyophilized powder (100 mg/vial) for reconstitution with sterile water to a concentration of 200 mg/mL. Subjects, investigators, and study staff were blinded to the treatment assignment, except for the pharmacist who prepared the study drug. The study was approved by the local institutional review board and performed in compliance with the Declaration of Helsinki in its revised edition (Washington 2002), and the requirements of the European Clinical Trial Directive 2001/20/EC and corresponding German Drug Law.

Safety monitoring

The safety of mipomersen was assessed by determining the incidence, severity, and dose relationship of AEs and changes in laboratory parameters. Laboratory evaluations included routine haematology, blood chemistry, coagulation parameters, and urinalysis. Serum samples were analysed for antibodies to mipomersen using a validated ELISA method at PPD Development (Richmond, VA, USA). Other assessments included a physical examination, a 12-lead electrocardiogram, and vital signs. All subjects were followed up for 6 months after their last dose of study drug.

Lipid and lipoprotein analysis

Fasting blood samples were analysed for LDL cholesterol, high-density lipoprotein (HDL) cholesterol, VLDL cholesterol, total cholesterol, TGs, lipoprotein(a) [Lp(a)], apoB, and apoA1 by W & T Laboratories (Berlin, Germany). Total cholesterol, LDL cholesterol, HDL cholesterol, and TGs were measured using homogeneous enzyme-based colorimetric assays. Very-low-density lipoprotein cholesterol was measured using a combination of gel electrophoresis and densitometry (Liposcript AT). Lipoprotein (a), apoB, and apoAI were measured by rate nephelometry. Results for all lipid parameters were listed unmasked in the laboratory test reports.

Pharmacokinetic analysis

Pharmacokinetic parameters were obtained from plasma drug concentration–time profiles. Serial blood samples were collected prior to, during, and following the last dose on Day 85. Plasma trough concentrations were determined from samples collected $\sim$7 days after the previous dose throughout the 13-week treatment period. Subjects also had blood drawn during the 6-month follow-up period for determination of the drug terminal elimination half-life.

Plasma drug concentrations were determined using a validated hybridization-dependent ELISA method at PPD Development (Richmond, VA, USA). The lower limit of quantitation was 0.23 ng/mL. Pharmacokinetic properties of mipomersen were assessed by a non-compartmental method of analysis (WinNonlin Version 5.2).

Statistical analysis

Sample size was based on a standard deviation of 12% in the per cent change of LDL cholesterol and analysis of the data between five treatment groups and pooled placebo. Under these assumptions, a sample size of six per group would provide 80% power to detect a 33% difference in LDL cholesterol per cent change with a statistical significance level of 0.01.

Study endpoints were analysed on the intent-to-treat population ($n = 50$). Efficacy endpoints were analysed 14 days post last dose ($\pm$2 days). The primary efficacy endpoint was reduction in LDL-C from baseline to 14 days post last dose. Missing values were

imputed by the last observation carried forward. Baseline was defined as the average of two screening values and the pre-dose Day 1 measurement. Descriptive statistics are presented for lipid parameters by treatment group. Per cent change from baseline for each mipomersen dose group was compared with the pooled placebo group using the exact Wilcoxon rank sum test. Statistical tests were two sided with a significant level of 0.05. Software utilized for the analyses was SAS version 8.2 (SAS Institute, Cary, NC, USA). The authors had full access to the data and take responsibility for their integrity.

Results

Study subjects

Fifty subjects were enrolled from 87 candidates screened (*Figure 1*), including 45 men and 5 women (28–65 years). Forty-eight (96%) were Caucasian. None of the subjects were taking background medication at baseline, including other lipid-lowering agents. Baseline LDL cholesterol ranged from 119 to 266 mg/dL with a mean of 173 mg/dL. Demographic and baseline characteristics by treatment group are summarized in *Table 1*. Baseline parameters were similar across dose cohorts.

Thirty-six of 50 subjects completed the study protocol (*Figure 1*). Of the 14 subjects that did not complete the protocol, one subject discontinued dosing (100 mg/week) based on a serious AE (encephalitis, considered unrelated to the study drug). Three subjects withdrew voluntarily from the study, one during dosing (300 mg/week) and two in follow-up (placebo and 300 mg/week). Dosing was discontinued in all 10 subjects of the 400 mg/week cohort, including those assigned to placebo, after 10 doses due to elevated liver *trans*-aminases.

Efficacy

A dose-dependent and prolonged reduction in LDL cholesterol resulted from the treatment of subjects with mipomersen (*Figure 2*). The initial rate of LDL lowering was greater in the 50 and 100 mg/week dose groups compared to the 200 mg/week dose group. This effect is consistent with the greater drug exposure in the loading dose period. Fourteen days after the last dose, mean LDL cholesterol reductions from baseline were $-7 \pm 22\%$, $-15 \pm 13\%$ ($P = 0.027$), $-45 \pm 10\%$ ($P = 0.000$), $-61 \pm 8\%$ ($P = 0.000$), and $-71 \pm 10\%$ ($P = 0.000$) in the 50–400 mg/week dose groups, respectively. Mean apoB reductions from baseline were $-16 \pm 21\%$, $-22 \pm 7\%$ ($P = 0.000$), $-46 \pm 11\%$ ($P = 0.000$), $-61 \pm 7\%$ ($P = 0.000$), and $-71 \pm 4\%$ ($P = 0.000$). In the 400 mg/week dose group, 50% of subjects reached apoB levels below the limit of detection (35 mg/dL). Median TG reductions by dose group were -7% [interquartile range (IQR) -25, 44], -23% (IQR -33, 0), -46% (IQR -58, -32; $P = 0.034$), -48% (IQR -67, -33; $P = 0.027$), and -53% (IQR -58, -44; $P = 0.021$), respectively. This corresponded to mean VLDL cholesterol change from baseline of $4 \pm 40\%$, $-23 \pm 33\%$, $-53 \pm 17\%$ ($P = 0.025$), $-49 \pm 15\%$ ($P = 0.051$), and

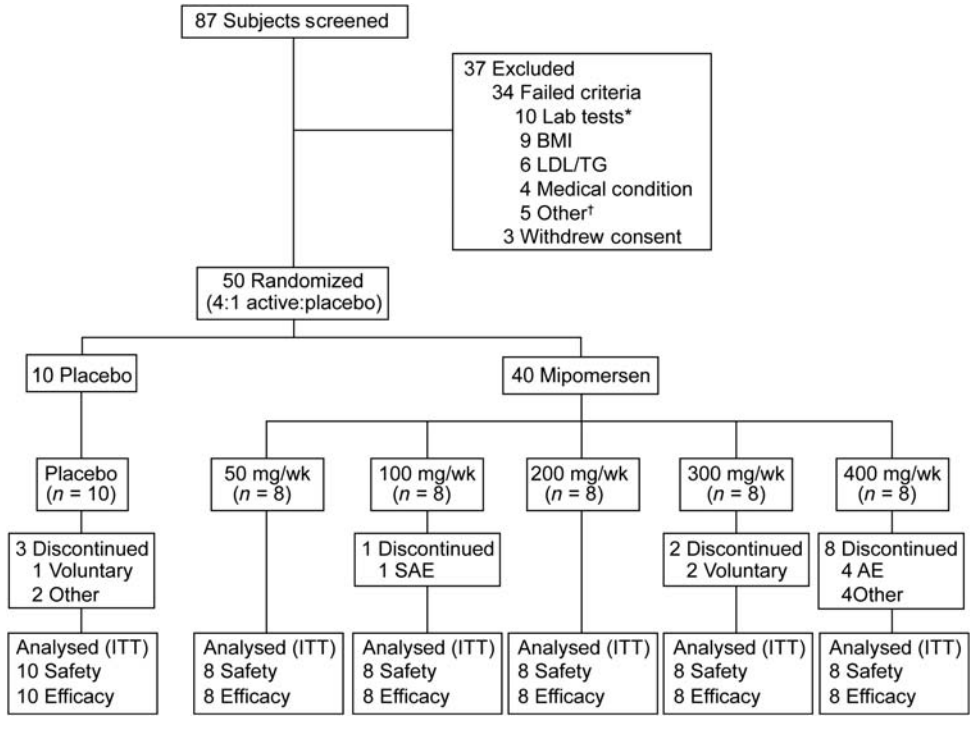

Figure 1 Flow of study participants. BMI denotes body mass index. LDL/TG denotes low-density-lipoprotein cholesterol and/or triglycerides. Asterisk represents liver function, creatine kinase, and fasting glucose tests and dagger represents blood pressure, history of current drug abuse, availability for follow-up and active infection.

Table 1 Subject demographics and baseline characteristics

	Placebo (n = 10)	50 mg/week (n = 8)	100 mg/week (n = 8)	200 mg/week (n = 8)	300 mg/week (n = 8)	400 mg/wk (n = 8)	Total (n = 50)
Gender (M : F)	8:2	7:1	7:1	7:1	8:0	8:0	45:5
Age (years)	52.3 ± 7.4	47.4 ± 7.2	40.5 ± 8.2	49.6 ± 8.5	51.6 ± 9.0	55.4 ± 9.8	49.6 ± 9.2
BMI (kg/m^2)	27.6 ± 2.4	27.1 ± 2.3	27.3 ± 2.5	26.5 ± 2.3	27.1 ± 2.5	28.7 ± 2.8	27.4 ± 2.4
BP (mmHg)							
Systolic	135.3 ± 15.2	138.8 ± 10.2	128.0 ± 12.3	130.9 ± 7.5	123.1 ± 9.0	133.6 ± 17.4	131.8 ± 12.9
Diastolic	80.9 ± 8.3	83.4 ± 7.5	77.6 ± 7.0	77.9 ± 7.2	73.8 ± 3.2	80.8 ± 9.8	79.1 ± 7.7
Pulse (b.p.m.)	69.1 ± 9.4	73.4 ± 16.6	66.5 ± 6.4	62.5 ± 9.4	68.5 ± 6.3	63.1 ± 3.3	67.3 ± 9.7
Glucose (mg/dL)	101.3 ± 17.4	97.8 ± 8.5	88.5 ± 5.2	87.2 ± 9.6	90.8 ± 4.3	99.6 ± 13.2	94.5 ± 12.0
Lipid parameters (mg/dL)							
LDL cholesterol	159.7 ± 16.5	173.4 ± 34.3	166.0 ± 41.7	171.0 ± 30.1	178.7 ± 34.6	192.3 ± 36.7	173.0 ± 32.7
VLDL cholesterol	27.1 ± 16.4	24.1 ± 15.3	20.5 ± 8.9	18.5 ± 8.9	23.1 ± 12.0	29.7 ± 12.3	24.0 ± 12.7
Non-HDL cholesterol	191.9 ± 27.9	204.6 ± 39.2	195.5 ± 41.9	190.8 ± 28.1	195.5 ± 25.6	216.5 ± 31.1	198.8 ± 32.2
HDL cholesterol	53.0 ± 7.1	55.0 ± 7.3	56.6 ± 5.5	54.4 ± 3.9	48.8 ± 6.8	50.4 ± 7.4	53.0 ± 6.7
Total cholesterol	244.8 ± 32.9	259.6 ± 41.4	252.0 ± 44.2	245.2 ± 26.4	244.3 ± 29.6	266.8 ± 35.6	251.9 ± 34.7
TGs	177 (88–474)	161 (79–330)	125 (53–295)	129 (62–200)	182 (67–240)	219 (101–293)	160 (53–474)
apoB	128.2 ± 17.0	146.0 ± 30.3	130.8 ± 27.6	124.9 ± 21.1	136.5 ± 17.8	147.3 ± 17.5	135.3 ± 22.8
Lp(a)	23.1 ± 26.7	26.6 ± 27.5	29.4 ± 29.2	31.5 ± 25.7	33.3 ± 34.7	53.2 ± 56.7	32.5 ± 34.4

Data are presented as the mean ± standard deviation. TGs are presented as the median (min−max). The display of mean vs. median is based on conventional reporting tendencies.
M, male; F, female; BMI, body mass index; BP, blood pressure; LDL, low-density lipoprotein; VLDL, very-low density lipoprotein; HDL, high-density lipoprotein.

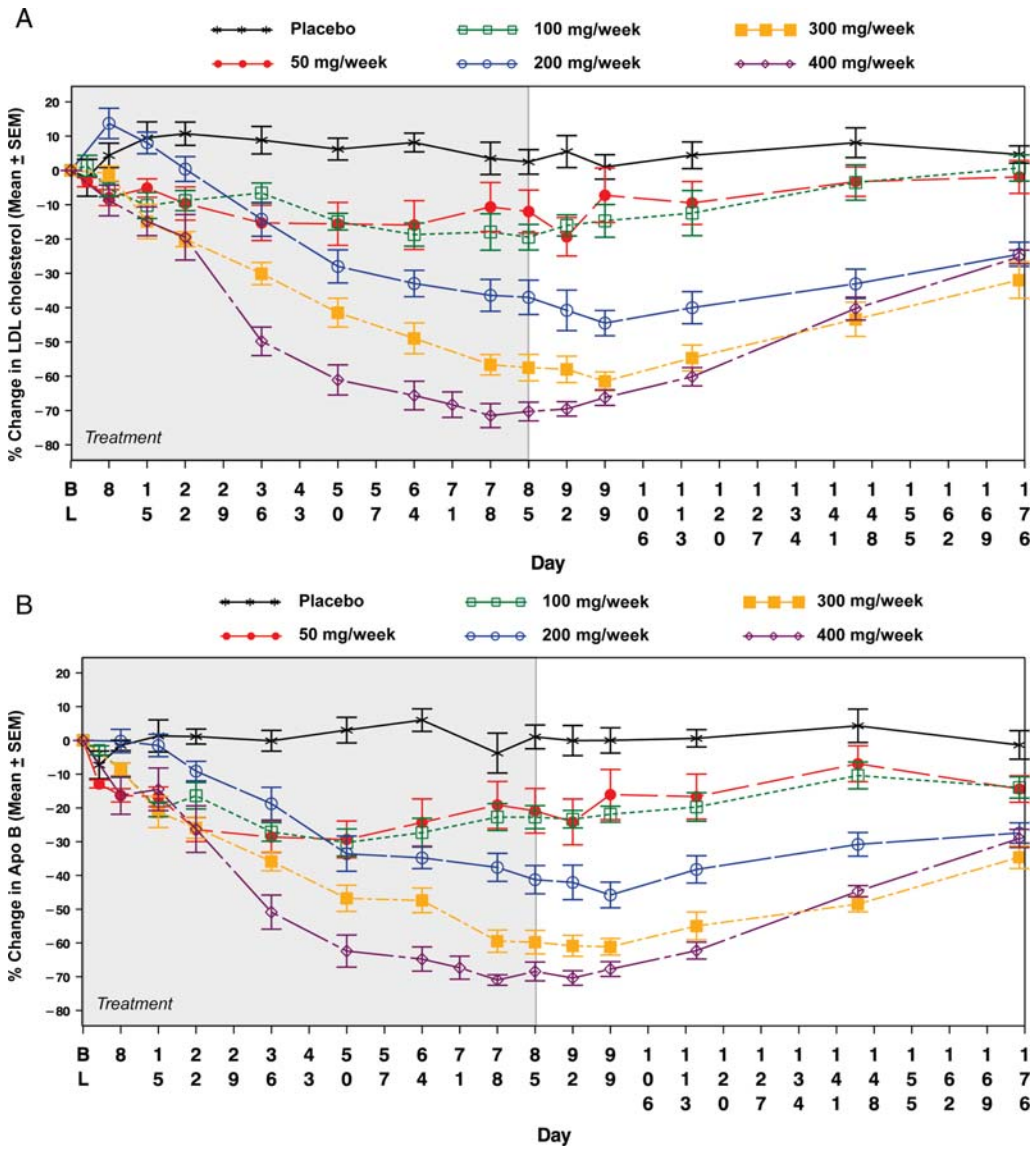

Figure 2 Dose-dependent and prolonged effect of mipomersen on LDL cholesterol and apoB. (*A*) LDL cholesterol and (*B*) apolipoprotein B are presented as mean per cent change from baseline. Error bars represent ± standard error of the mean. Time period ranges from Day 1 to Day 175.

−61 ± 16% ($P = 0.034$). The mean non-HDL cholesterol reductions were −12 ± 19%, −21 ± 6% ($P = 0.006$), −44 ± 11% ($P = 0.000$), −54 ± 7% ($P = 0.000$), and −66 ± 10% ($P = 0.000$) by dose group. Significant reductions in Lp(a) levels occurred in the 200 (median −42%, IQR −51, −24; $P = 0.021$), 300 (median −32%, IQR −41, −24; $P = 0.043$), and 400 mg/week (median −49%, IQR −56, −36; $P = 0.001$) dose groups. High-density lipoprotein cholesterol and apoAI did not change significantly.

Four of eight subjects (50%) in the 200 mg/week and seven out of eight subjects (88%) in the 300 mg/week dose group achieved LDL cholesterol levels in the <100 mg/dL range (*Table 2*). In the 400 mg/week group all subjects reached LDL cholesterol levels below the 100 mg/dL range after 10 injections.

Safety

The most common AE was a reaction at the subcutaneous injection site (ISR), characterized by mild painless erythema that occurred within 24 h after the injection. All subjects treated with mipomersen experienced at least one ISR. The amount of drug injected at a single site ranged from 100 to 200 mg, depending on dose group. ISRs did not worsen with repeated dosing and had no impact on adherence to the protocol. Other AEs that had an incidence of 10% or greater in subjects assigned to active

Table 2 Dose-dependent effects of mipomersen on lipid and lipoprotein concentrations

	Placebo ($n = 10$)	50 mg/week ($n = 8$)	100 mg/week ($n = 8$)	200 mg/week ($n = 8$)	300 mg/week ($n = 8$)	400 mg/week ($n = 8$)
LDL cholesterol						
Baseline	159.7 ± 16.5	173.4 ± 34.3	166.0 ± 41.7	171.0 ± 30.1	178.7 ± 34.6	192.3 ± 36.7
Endpoint	161.0 ± 22.9	159.5 ± 43.0	140.4 ± 34.3	96.4 ± 29.1	70.0 ± 24.2	56.5 ± 26.0
P value		0.745	0.116	0.000	0.000	0.000
n (%) <100 mg/dL	0 (0)	1 (13)	0 (0)	4 (50)	7 (88)	8 (100)
n (%) <70 mg/dL	0 (0)	0 (0)	0 (0)	2 (25)	5 (63)	6 (75)
Non-HDL cholesterol						
Baseline	191.9 ± 27.9	204.6 ± 39.2	195.5 ± 41.9	190.8 ± 28.1	195.5 ± 25.6	216.5 ± 31.1
Endpoint	187.7 ± 33.7	178.5 ± 45.0	154.9 ± 35.0	108.3 ± 31.9	89.4 ± 20.5	74.1 ± 26.4
P value		0.762	0.101	0.000	0.000	0.000
n (%) <130 mg/dL	0 (0)	1 (13)	4 (50)	7 (88)	8 (100)	8 (100)
apoB[a]						
Baseline	128.2 ± 17.0	146.0 ± 30.3	130.8 ± 27.6	124.9 ± 21.1	136.5 ± 17.8	147.3 ± 17.5
Endpoint	128.0 ± 21.5	121.3 ± 33.5	101.9 ± 21.0	69.0 ± 21.9	53.4 ± 13.8	42.9 ± 9.4
P value		0.810	0.028	0.000	0.000	0.000
n (%) <90 mg/dL	0 (0)	2 (25)	3 (38)	7 (88)	8 (100)	8 (100)

Baseline and endpoint values represent the mean ± standard deviation in mg/dL. *P* values are based on the comparison of endpoints for each mipomersen dose group and pooled-placebo group using the exact Wilcoxon rank sum test. The number of subjects (*n*) below the indicated target level at endpoint is presented by dose group, where the per cent of the total number of subjects in the group at baseline (%) is in parentheses. Endpoint was measured 14 days after the last dose.
[a]Four of eight subjects in the 400 mg/week dose cohort had apoB levels at or below the limit of detection (35 mg/dL) on Day 78. Mean endpoint was calculated using a value of 35 mg/dL for these four subjects.

treatment are listed in *Table 3*. The most frequently reported concomitant medications were anilides, predominantly paracetamol for headache. A summary of concomitant medications are provided in the Supplementary material online.

Nine of 40 subjects (23%) dosed with mipomersen had alanine aminotransferase (ALT) elevations ≥3 times the upper limit of normal (ULN = 55 IU/L). At the efficacy endpoint, median (min–max) ALT levels were 70 (22–165), 46 (19–110), 55 (38–115), 90 (44–144), and 165 (71–186) IU/L by increasing dose group and 30 (14–72) IU/L in the pooled-placebo group. Seven of 40 subjects (18%) had elevations ≥3× ULN on two or more consecutive occasions at least 7 days apart. These subjects were assigned to the upper dose ranges ($n = 1$, 200 mg/week; $n = 1$, 300 mg/week; and $n = 5$, 400 mg/week). The maximum ALT measured in subjects with consecutive elevations ≥3× ULN was 347 IU/L. Four of eight subjects in the 400 mg/week dose group discontinued treatment due to hepatic enzyme increases. Four subjects in this dose group were also noted with apoB levels below the limit of detection (35 mg/dL). Subsequently, the investigator and sponsor decided to discontinue dosing of all other subjects in the 400 mg/week cohort. No concomitant elevations in total bilirubin (>2× ULN) or changes in other measures of liver function (prothrombin time, albumin) were observed. All *trans*-aminase elevations resolved spontaneously when drug was discontinued. Liver function test results by treatment group are provided in the Supplementary material online.

One serious AE (encephalitis) was reported in the 100 mg/week dose group. This AE was considered not related to the study drug. Overall, there were no clinically significant changes in vital signs, electrocardiograms, urinalysis, serum glucose, or other safety laboratory evaluations, including blood counts. There was no evidence of abnormal changes or treatment-related effects on kidney function based on serum chemistry. There was no evidence of a specific-antibody response to mipomersen. Summary tables for vitals and specified laboratory evaluations are provided in the Supplementary materials online.

Pharmacokinetics

Mean peak plasma drug concentrations ranged from 1.2 to 4.3 μg/mL at doses that ranged from 100 to 300 mg mipomersen. Mean time to maximum plasma concentration ranged from 3.4 to 4.0 h. The maximum concentration for the 400 mg/week cohort was not measured due to discontinuation of treatment. Characterization of the terminal elimination phase following the last dose yielded a mean terminal elimination half-life of 46–48 days, which is consistent with observations in experimental models.[14,15]

Mean plasma trough concentrations ranged from 8 to 51 ng/mL, 1 week after the last dose in the 50–400 mg/week dose groups, respectively (*Figure 3*). Plasma trough concentrations reached steady state in the 50 and 100 mg/week dose groups as a result of the 2-week loading period. Subjects receiving 100 mg every other week in the following 11 weeks (50 mg/

Table 3 Treatment-emergent adverse events

Event *n* (%)	Placebo (*n* = 10)	50 mg/week (*n* = 8)	100 mg/week (*n* = 8)	200 mg/week (*n* = 8)	300 mg/week (*n* = 8)	400 mg/week (*n* = 8)	Mipomersen (*n* = 40)
Injection site reaction	6 (60)	8 (100)	8 (100)	8 (100)	8 (100)	8 (100)	40 (100)
Headache	5 (50)	4 (50)	5 (63)	3 (38)	5 (63)	4 (50)	21 (53)
Nasopharyngitis	7 (70)	2 (25)	2 (25)	4 (50)	2 (25)	1 (13)	11 (28)
Fatigue	1 (10)	1 (13)	1 (13)	1 (13)	2 (25)	2 (25)	7 (18)
Myalgia	1 (10)	0 (0)	0 (0)	0 (0)	4 (50)	2 (25)	6 (15)
Influenza-like illness	1 (10)	0 (0)	0 (0)	0 (0)	3 (38)	2 (25)	5 (13)
Hepatic enzyme increase[a]	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (63)	5 (13)
Back pain	1 (10)	1 (13)	1 (13)	0 (0)	2 (25)	0 (0)	4 (10)
Nausea	0 (0)	0 (0)	1 (13)	0 (0)	0 (0)	3 (38)	4 (10)
Listless	0 (0)	0 (0)	4 (50)	0 (0)	0 (0)	0 (0)	4 (10)

Values (*n*) represent the number of subjects with at least one event. Per cent of the total number of subjects by treatment group is within parentheses.
Only AEs with an incidence >10% in all mipomersen-treated subjects (*n* = 40) are listed.
[a]Hepatic enzyme increases were reported as an AE for five of the seven subjects who had elevated ALT levels ≥3× ULN on two or more consecutive occasions at least 7 days apart.

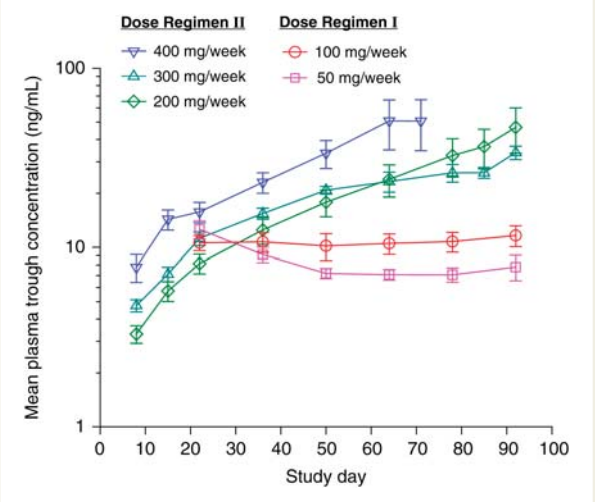

Figure 3 Drug plasma trough concentrations. Dose Regimen I was based on a 2-week dose-loading period followed by a dose schedule of every other week for 11 weeks. Dose Regimen II was based on a dose schedule of once per week for 13 weeks, except for the 400 mg/week dose group which discontinued dosing after 10 weeks.

week) did not maintain the plasma trough concentration achieved during the loading period, but instead established a lower steady-state level consistent with the downward adjustment in dose and dose interval. In subjects receiving weekly doses without a loading period (200, 300, and 400 mg/week dose groups), the plasma trough concentrations increased throughout the treatment period consistent with the drug elimination half-life and dose interval.

Discussion

This is the first study in which different dose regimens of mipomersen monotherapy were administered to subjects with mild-to-moderate hyperlipidaemia for a duration of 3 months. A dose-dependent lowering of apoB-containing atherogenic lipoproteins, including LDL cholesterol, VLDL cholesterol, and Lp(a) was observed. The most common AEs in subjects treated with mipomersen were injection site reactions. Elevations in liver *trans*-aminases were observed, but predominantly in the highest dose group.

Mipomersen resulted in significant LDL cholesterol reductions that ranged from 15% in the lowest dose group to 71% in the highest dose group. The LDL cholesterol reductions observed upon higher dosages of mipomersen exceeded the maximal LDL cholesterol lowering of 40–55%, that can be achieved by potent statins such as atorvastatin and rosuvastatin.[4,16–19] The apoB reduction of mipomersen also exceeded apoB reductions reported for statins, with maximal reductions for the latter reported between 30 and 36%.[18] ApoB reductions of >45% were seen on treatment with mipomersen at a dose of 200 mg/week or higher. This potent apoB lowering capacity of the antisense compound compared with statins is a logical consequence of its primary mode of action. Statins primarily target inhibition of cholesterol synthesis via inhibiting the rate-limiting HMG-CoA reductase enzyme, resulting in reduced production of cholesterol in the liver. This leads to upregulation of LDL receptors and preferential clearance of larger LDL particles. As a consequence, statins maximally achieve 40–55% LDL cholesterol lowering with a concomitant 20–30% reduction of apoB levels.[20] The preferential lowering of cholesterol as opposed to apoB was illustrated in the ACCESS study, where atorvastatin caused LDL cholesterol lowering from the 90th to the 25th percentile with apoB decreasing from the 90th to only the 50th percentile.[21] ApoB reduction

has been put forward as a prime target for cardiovascular prevention and retains its predictive value in patients being treated with statins.[6,22–24] The latter implies that apoB may actually be a superior treatment target and the profound impact of mipomersen on apoB levels is potentially attractive for a new treatment modality. In a separate Phase 2 study, mipomersen demonstrated an incremental dose-dependent reduction in apoB when administered to hyperlipidemic subjects on stable statin therapy.[11]

Besides LDL cholesterol and apoB reduction, mipomersen also reduced VLDL cholesterol, TGs, and Lp(a) when dosed at 200 mg/week or higher. Whereas statins also lower TG levels, this is most commonly observed in those subjects with higher baseline TGs.[25] TG lowering by statins usually ranges from $<5\%$ in subjects with normal TG levels to $\sim 25\%$ in hypertriglyceridemic subjects.[19] The fact that we observed TG reductions $>45\%$ during antisense therapy might imply that the mechanism of TG lowering may in fact differ between apoB antisense and statin therapy. Indeed, VLDL lowering during statin therapy has been shown to relate predominantly to enhanced peripheral uptake of VLDL remnants, whereas production rates of VLDL particles remain unaffected.[26] In contrast, inhibition of apoB synthesis may lower TG by directly affecting VLDL production rates, which most likely explains the greater efficacy of mipomersen on TG levels.

Inhibition of apoB synthesis is also likely the basis of the reduction in Lp(a) levels since apoB is an essential component of this lipoprotein through covalent attachment to apoprotein(a).[27]

Seven of 40 subjects assigned to mipomersen treatment had an ALT increase of $\geq 3\times$ ULN on two or more consecutive occasions. Five of these seven subjects received the highest dose of 400 mg/week mipomersen. Since direct liver toxicity of this antisense compound was not detected in pre-clinical studies, these abnormalities are possibly a consequence of the profound impact on lipid metabolism in the liver. In support, four of the five subjects in the highest-dose group with $>3\times$ ULN *trans*-aminase increases had apoB levels below the limit of detection. In absence of the capacity to secrete TGs, it is conceivable that the accumulation of liver fat may be the basis of the serum *trans*-aminase increases. Interestingly, apoB synthesis inhibition did not result in increased liver fat in a variety of animal models.[8,9] The fact that no significant changes in liver fat content was observed may in part be explained by upregulation of counter-regulatory pathways, including increased fatty acid oxidation and stearoyl-CoA desaturase-1 activity.[9] In contrast, in these same models[8] as well as in clinical trials,[28] microsomal transfer protein inhibition has elicited profound hepatic steatosis. However, it should be taken into account that even counter-regulatory pathways can be expected to fail in case of too ambitious apoB synthesis inhibition. In this respect, we recently reported the results from a randomized, placebo-controlled Phase 2 study which evaluated the effects of mipomersen on intrahepatic triglyceride (IHTG) content in patients with heterozygous familial hypercholesterolaemia administered 200 mg once per week for 13 weeks.[29] Although no significant changes in IHTG content were found in this study, a trend towards an increase in IHTG was observed. Further validation following prolonged treatment in subjects at increased risk of hepatic steatosis is needed.

The most commonly observed side effect in the present study consisted of mild erythema at the site of injection. This effect has been described previously, and although common, for the most part, did not interfere with continued dosing. The basis of injection site reactions is suspected to be a proinflammatory response to transient high concentrations of oligonucleotide at the injection site. Histologically, ISRs were characterized by prominent macrophage and neutrophil infiltration.[8] The degree of histological change within the biopsy sites correlated directly with the amount of stainable drug present. Investigation into the underlying mechanism and methods to further mitigate these reactions is in progress.

The pharmacological effects of mipomersen were consistent with the pharmacokinetics. This association was based on the use of plasma trough concentrations as a surrogate marker for liver tissue concentrations.[10–15,30] Steady-state drug levels, as reflected by plasma trough levels and pharmacological activity vs. time, were achieved in the two cohorts treated with a dosing regimen that included a 2-week drug-loading period. A loading period may not be necessary in routine clinical practice, however, since long-term use of lipid-lowering drugs is usually necessary. Plasma trough concentrations continually increased during treatment in the three cohorts that received once-per-week dosing without a load period. Based on the long half-life of mipomersen, and supporting data from pre-clinical monkey studies,[14,15] plasma trough and target tissue concentrations are predicted to reach steady-state levels in 6 months using this once weekly dose regimen.

Although further insight has been gained on the effects of mipomersen across doses up to 400 mg and in two types of dose regimens, interpretation of the study results is limited by the small sample size and length of treatment. Also to be considered was the availability of the lipid findings in the context of the laboratory test reports throughout the course of the study. The availability of these results may have biased AE reporting and supportive care.

Simultaneous lowering of all apoB-containing atherogenic lipoproteins constitutes an attractive opportunity for the treatment of patients who are at high risk for cardiovascular disease and not reaching LDL cholesterol goals as defined by the NCEP-ATP III guidelines.[31] Apart from further LDL cholesterol lowering in high-risk subjects,[32] mipomersen also holds a promise for the treatment of patients with familial hypercholesterolaemia.[33,34] In a recent Phase 3 study, mipomersen 200 mg sc weekly for 26 weeks reduced LDL cholesterol in patients with homozygous familial hypercholesterolaemia by 25% when added to maximally tolerated lipid-lowering therapy, including high-dose statins.[35] Further studies are needed to determine whether lowering of apoB by this novel mechanism may in fact predispose to liver *trans*-aminase elevations.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Acknowledgements

The authors thank Dr Jesse Kwoh, Dr Erik Stroes, Drs Maartje Visser, and Dr Shuting Xia for their critical review of the manuscript.

Funding

This study was funded by Isis Pharmaceuticals Inc.

Conflict of interest: J.J.P.K. has received research support from Isis Pharmaceuticals Inc., and serves as a consultant/advisory board member for Isis Pharmaceuticals Inc. R.F. was the principal investigator of this study as an employee of PAREXEL International GmbH. D.L.T., J.D.F., R.Y., R.G., J.S., B.F.B., and M.K.W. are/were employees of Isis Pharmaceuticals.

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CARDIOVASCULAR FLASHLIGHT

doi:10.1093/eurheartj/ehr100
Online publish-ahead-of-print 5 April 2011

Fatal late prosthetic aortic valve endocarditis

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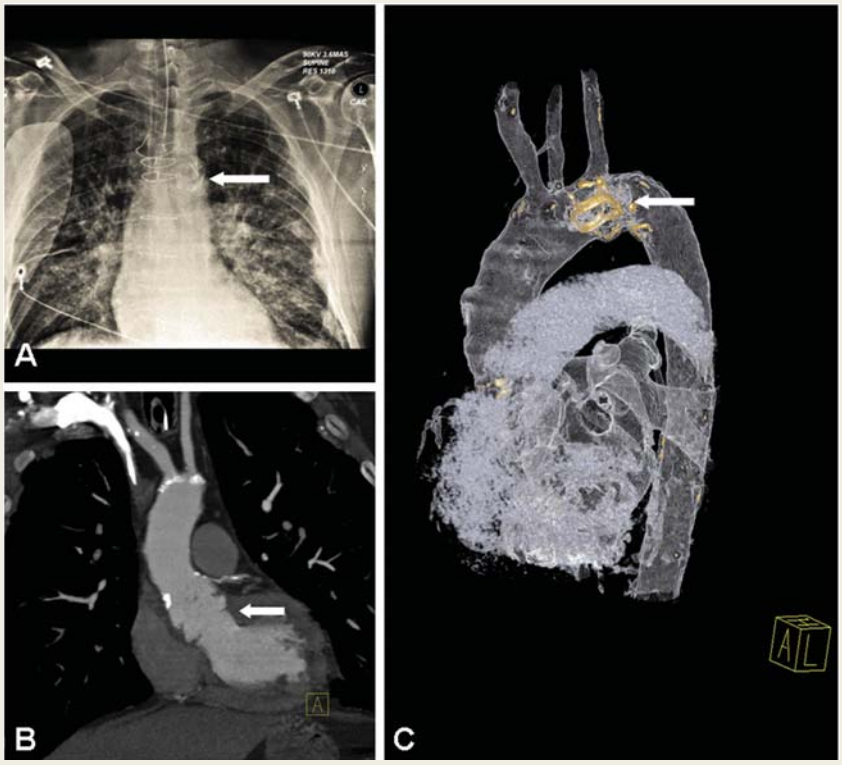

Prosthetic valve endocarditis (PVE) is a potentially devastating complication in patients who have undergone heart valve surgery. Despite the emergence of new potent antibiotics, recent improvements in diagnostic—therapeutic strategy and certain advances of surgery, PVE is still associated with high mortality between 20 and 30%. Annular extension of the infectious process is common and carries a substantial prognostic significance in determining the chances of a surgical treatment. An appropriately timed surgical intervention of the infected heart valve contributes to reduced mortality.

We report a 68-year-old man presented to the Accident and Emergency Unit, with shortness of breath, which had been treated as flu over the previous 3 weeks. The laboratory findings showed increased inflammatory markers. In the past medical history there is hypertension, hyperlipidemia, peripheral vascular disease and a tissue aortic valve replacement 3 years prior to recent admission. A transthoracic echo demonstrated an echodense appearance to the aortic root and a dissection flap could not be excluded.

While performing a chest X-ray his condition suddenly worsened with ongoing pulmonary oedema, loss of diastolic pressure at maintained systolic, which required intubation. Chest X-ray demonstrated patchy consolidation throughout both lungs and projection of a fine metallic frame structure in the aortic arch region. Computed tomography revealed no dissection but the aortic valve was found within the arch at the origin of the supraaortic vessels. The metal frame was surrounded by low-density material and the annulus appeared ragged with an absent valve. In the ensuing minutes the patient died from an uncontrollable circulatory shock.

Hereby we thank Dr James Shambrook, Consultant Cardiothoracic Radiologist, Southampton General Hospital, for his contribution to this work.

Panel A. Metallic framed foreign body visible in the aortic arch on the posteroanterior chest X-ray.

Panel B. Arrow marks the 'empty' aortic root on the chest computed tomography.

Panel C. Arrow shows the prosthetic tissue aortic valve impacted to the origin of the supraaortic vessels.