Dyslipidemia, sense, antisense or nonsense?

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Submitted for publication
Chapter 5

Mipomersen, an apolipoprotein B synthesis inhibitor, lowers LDL cholesterol in high risk-statin intolerant patients; a randomized, double-blind, placebo-controlled trial

Abstract

Background
A randomized, double-blind, placebo-controlled study was conducted to investigate the safety and efficacy of mipomersen, an apolipoprotein B-100 (apoB) synthesis inhibitor, in patients who are statin-intolerant and at high risk for cardiovascular disease (CVD).

Methods
Thirty-three subjects, not receiving statin therapy because of statin intolerance, received a weekly subcutaneous dose of 200 mg mipomersen or placebo (2:1 randomization) for 26 weeks. The primary endpoint was percent change in LDL-c from baseline to Week 28. Other efficacy endpoints were percent change in apoB and Lp(a). Safety was determined using the incidence of treatment-emergent adverse events (AE) and clinical laboratory evaluations.

Results
After 26 weeks of mipomersen administration, LDL-c was reduced by 47 ± 18% (p<0.001 vs placebo). ApoB and Lp(a) were also significantly reduced by 46% and 27%, respectively (p<0.001 vs placebo). Four mipomersen (19%) and 2 placebo subjects (17%) discontinued dosing prematurely due to AEs. Persistent liver transaminase increases ≥ 3 x the upper limit of normal were observed in 7 (33%) subjects assigned to mipomersen. In selected subjects liver fat content was assessed, during and after treatment, using magnetic resonance spectroscopy. Liver fat content in these patients ranged from 0.8 to 47.3%. Liver needle biopsy was performed in two of these subjects, confirming hepatic steatosis with minimal inflammation or fibrosis.

Conclusion
The present data suggest that mipomersen is an effective therapeutic option in statin intolerant patients at high risk for CVD, although longer-term follow-up of liver safety is required.
Introduction

Statin-induced low-density lipoprotein cholesterol (LDL-c) lowering is the first-line treatment in patients at increased risk for cardiovascular disease (CVD)\textsuperscript{1}. Whereas statins are well tolerated, adverse events such as liver transaminase increases, myalgia and, in rare cases, rhabdomyolysis may occur. In fact, myalgia, the most common adverse event following statin treatment, has been reported in up to 10\% of patients\textsuperscript{2,3}. In a minority of these patients, the severity of statin-induced side effects may lead to discontinuation of therapy. The incidence of statin ‘intolerance’ is rising, most likely reflecting the use of higher statin doses required to achieve more stringent LDL-c targets\textsuperscript{1} whereas adverse effects of statins are dose-dependent\textsuperscript{4}. Currently available alternatives to lower LDL-c levels in statin intolerant patients include switching to other statins, non-daily or low-dosing regimens\textsuperscript{4}, and the use of non-statin LDL-lowering drugs such as ezetemibe and bile acid-binding resins\textsuperscript{5}. The efficacy of these therapeutic strategies is, however, limited. LDL-aphaeresis may be an option where available.

Mipomersen is a second generation antisense oligonucleotide which inhibits the synthesis of apolipoprotein B-100 (apoB)\textsuperscript{6}. ApoB is the main structural component of all atherogenic lipid particles and is required for the secretion of very-low-density lipoprotein (VLDL) from the liver\textsuperscript{7,8}. In previous clinical trials, mipomersen has been shown to induce dose-dependent reductions in LDL-c and all other apoB containing lipoproteins in patients with various extents of hypercholesterolemia including patients with homozygous and heterozygous familial hypercholesterolemia (FH), either as monotherapy or on top of statins\textsuperscript{9-12}. Mild to moderate injection site reactions and flu-like symptoms are the adverse events most commonly observed with mipomersen. In addition, liver transaminase increases have been observed. Since previous attempts to inhibit VLDL production with microsomal transport protein (MTP) inhibitors were complicated by profound increases in intrahepatic triglyceride (IHTG)-content\textsuperscript{13}, safety concerns regarding mipomersen have focused on the liver\textsuperscript{14}.

In the present report we describe the results of a randomized, double-blind, placebo-controlled Phase II study designed to evaluate the safety and efficacy of mipomersen administration for 26 weeks in statin-intolerant subjects at high risk for CVD.
Methods

Study participants

Thirty-four hypercholesterolemic subjects, who were statin intolerant and at high risk for CVD events, were asked to participate. High risk was defined as meeting National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria, i.e., coronary heart disease (CHD) or a CHD risk equivalent ≥20% 10-year risk for CHD\textsuperscript{1}. Heterozygous Familial hypercholesterolemia (FH) subjects at or above a certain age (≥30 for men and ≥45 for women) were also classified as high risk\textsuperscript{15-17}. FH was diagnosed either by genotyping or by fulfilling the criteria for the diagnosis of FH as outlined by the World Health Organization (FH: report of the World Health Organization, 1998).

Statin intolerance was defined as the inability to tolerate any dose of at least 2 different statins due to serious side effects of any kind. Side effects were of such severity that either the treating physician discontinued treatment or the subject declined continuation of statin therapy. Subjects with known intolerances to other lipid-lowering drugs were excluded from participation. Participants had discontinued statins at least 6 weeks prior to the screening visit. Participants did not use other lipid lowering drugs unless the dose had been stable for >8 weeks prior to screening. At screening, fasting LDL-c was ≥3.4 mmol/L (130 mg/dL) and plasma triglyceride (TG) levels <2.3 mmol/L (210 mg/dL). HbA1c was ≤8.0, ALT ≤1.5 x upper limit of normal (ULN) and serum creatine phosphokinase (CPK) <3 x ULN. Alcohol consumption had to be ≤3 units (30 g) per day and ≤12 units (120 g) per week for male subjects, and ≤2 units (20 g) per day and ≤8 units (80 g) per week for female subjects. All study participants were enrolled at one site in the Netherlands. The study protocol was approved by the local institutional review board. All subjects gave written informed consent. The study was performed in compliance with the standards of Good Clinical Practice (CPMP/ICH/135/95) and the declaration of Helsinki (Washington 2002). During the study, the protocol was amended in order to allow the inclusion of subjects with FH as well as subjects with controlled type 2 diabetes mellitus.

Study Design

Statin intolerant subjects at high CVD risk were selected to investigate the safety and efficacy of mipomersen 200 mg/wk for 26 weeks. Participants were randomized at a 2:1 ratio, active to placebo. Participants, investigators and study staff were blinded to the treatment assignment with the exception of the personnel who prepared the study drug. Study drug was administered subcutaneously weekly from Week 1 until Week 26. The 200 mg/wk dose was selected based
on safety and efficacy data from previous clinical trials\textsuperscript{14}. Pre-specified efficacy endpoints included percent change in LDL-c from baseline to 2 weeks after the last dose (Week 28 for those who completed 26 weeks of treatment). Other endpoints included percent change in total cholesterol, apoB, HDL-C, TG, non-HDL-C, VLDL, LDL/HDL ratio, Apo A1 and lipoprotein a (Lp(a)) concentrations as well as change in particle size and number from baseline to 2 weeks after the last dose. Safety was determined using the incidence of treatment-emergent adverse events (AEs), clinical laboratory evaluations, vital signs, electrocardiograms (ECGs), and physical examination findings. Due to the long half-life of mipomersen, the treatment period was followed by a 6 month evaluation period with visits at Weeks 28, 32, 40, and 50. Subjects withdrawn from the study before receiving 11 doses of study drug were replaced.

Lipid and lipoprotein analysis

Fasting blood and urine samples were taken after at least 10 hours of fasting at visit during weeks 1, 3, 5, 7, 9, 11, 13, 17, 21, 25, 28, 32, 40 and 50. Fasting blood samples were analyzed for lipids and lipoproteins by MedPace (Cincinnati, OH). ApoB, apoA1 and Lp(a) concentrations were determined by rate nephelometry; and total cholesterol (TC) and TG were measured by standard enzyme-based colorimetric assays. HDL-C was determined by an enzyme-based colorimetric assay after dextran-sulfate precipitation. LDL-c and non-HDL-C were calculated. Lipoprotein particles were analyzed by nuclear magnetic resonance spectroscopy as described previously\textsuperscript{18}.

Safety Monitoring

Safety and tolerability of mipomersen was assessed by determining the incidence, severity and possible relationship to the study drug of adverse events and laboratory parameters, including blood chemistry, routine hematology, coagulation and urinalysis. Vital signs were recorded at Weeks 1, 2, 3, 5, 7, 9, 11, 13, 15, 17, 21, 25, 28, 32, 40 and 50. Full physical examination was performed at Screening, Week 13, Week 28 and Week 50. A 12-lead electrocardiogram was recorded at Screening and at Week 28.

Liver assessment

3-Tesla proton magnetic resonance spectroscopy (MRS) was used to quantify IHTG-concentration. Initially, MRS was recommended for subjects with persistent liver transaminase levels ≥3 x ULN. Since MRS measurements were not prespecified in the original protocol, pretreatment baseline MRS measurements were not performed in this study. Following the observation of moderate hepatic
steatosis in one patient, MRS measurement was performed in all subjects who had experienced ALT levels $\geq 2 \times$ ULN at any time during treatment. If IHTG content was $\geq 10\%$, MRS measurements were repeated around Week 28 and Week 50. In case hepatic steatosis persisted, MRS was repeated until IHTG-content was $< 10\%$ or stabilized.

MRS was performed by placing 2 voxels in the right lobe of the liver. MRS is a non-invasive and highly reproducible technique (ICC > 0.99) by which liver triglyceride concentrations can be quantified\textsuperscript{19}. This technique has been shown to have a good correlation with liver biopsy data in healthy individuals and patients with hepatic steatosis\textsuperscript{20,21}. IHTG-concentration $> 5.6\%$ was defined as reflecting hepatic steatosis\textsuperscript{22}. IHTG-content values were quantified by one assessor who was masked to treatment assignment.

Subjects with persistent transaminase increases $\geq 2 \times$ ULN and an IHTG-content of $\geq 20\%$ during treatment were referred to a hepatologist for further evaluation. In patients requiring liver biopsy, hepatic macrovesicular steatosis and steatohepatitis score was determined according to Kleiner et al.\textsuperscript{23}. A Kleiner score of $\geq 5$ was considered compatible with steatohepatitis.

**Statistical analysis and calculations**

A sample size of 30 patients (20 mipomersen and 10 placebo) was planned for this study based upon the assumption that the standard deviation (SD) of the percent change in LDL-c was $\leq 20\%$. A 2-sided t-test with an alpha level of 0.05 was expected to provide $\geq 90\%$ power to detect a 30% difference in LDL-c percent reduction between the 2 groups (35% reduction for the mipomersen group and 5% reduction for the placebo group).

Study endpoints were analyzed on the intention-to-treat population, consisting of all 33 subjects randomized and treated. Demographic and baseline characteristics were summarized using descriptive statistics. For the efficacy parameters baseline was defined as the mean of the value at screening and the last value prior to the first dose. For the safety parameters baseline was defined as the last value prior to the first dose. Primary efficacy timepoint (PET) was defined as the visit closest to 2 weeks after the last dose of study treatment.

Percentage change from baseline for lipid parameters were compared between the 2 treatment groups using the t-test or the Wilcoxon Rank Sum test for data with a skewed distribution. Since baseline values for IHTG-content were absent and hepatic steatosis is prevalent in the general population, we used the difference between the highest and lowest IHTG-content measured during follow up in each individual from the active treatment group to estimate the increase in IHTG-content attributable to mipomersen. In a post-hoc analysis, comparison of each patient’s
highest and lowest IHTG-content was tested using the Wilcoxon signed rank test. Spearman’s rank correlation coefficients were calculated to assess the relationship between ALT increases, change in IHTG-content and apoB levels. Software utilized for the analyses was SAS version 9.2 (SAS Institute, Cary, North Carolina, USA). Data were expressed as mean ± SD unless specified otherwise.

Results

Study subjects

Thirty-four subjects with high CVD risk were enrolled from 42 candidates screened (figure 1). Screened candidates were excluded because of TG ≥2.3 mmol/L and 2 because of ALT levels >1.5 x ULN. One subject assigned to mipomersen was not eligible to participate because of corticosteroid use. This subject was excluded from participation before the start of treatment. Twenty-seven of the 33 subjects (82%) treated completed the study protocol and 33 subjects were analyzed. Four mipomersen treated subjects discontinued treatment, due to a stopping rule (n=1 [4%]) or an adverse event (n=3 [14%]), whereas 2 (17%) placebo treated subjects discontinued treatment due to adverse events (figure 1). Three subjects discontinued treatment before Week 11, 1 from the mipomersen and 2 from the placebo treatment group, and were replaced.

Of the 33 subjects who were analyzed, 12 had a history of CVD, 2 subjects had type 2 diabetes mellitus and 19 subjects fulfilled the inclusion criteria for FH. Subjects were randomly assigned to either mipomersen (n=22) or placebo (n=12). Demographics and baseline lipid-lowering therapy by treatment group are

Figure 1 Flow of study participants

![Flow of study participants](image-url)
summarized in table 1. Baseline lipid parameters were comparable between the treatment groups. All subjects using lipid lowering drugs were on stable therapy from 8 weeks prior to screening and throughout the study.

### Table 1 Patient demographics and baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Mipomersen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td>n=12</td>
<td>n=21</td>
</tr>
<tr>
<td>Gender (M:F), n (%)</td>
<td>4 (33)</td>
<td>11 (52)</td>
</tr>
<tr>
<td>Age a (years)</td>
<td>52 (39-68)</td>
<td>55 (46-69)</td>
</tr>
<tr>
<td>BMI a (kg/m²)</td>
<td>26 (22-29)</td>
<td>27 (21-32)</td>
</tr>
<tr>
<td>Metabolic syndrome, n (%)</td>
<td>8 (67)</td>
<td>9 (43)</td>
</tr>
<tr>
<td>FH, n (%)</td>
<td>8 (67)</td>
<td>11 (52)</td>
</tr>
<tr>
<td>DMII, n (%)</td>
<td>1 (8)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>CVD, n (%)</td>
<td>5 (42)</td>
<td>7 (33)</td>
</tr>
<tr>
<td>Lipid lowering therapy, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any lipid lowering medication</td>
<td>6 (50)</td>
<td>12 (57)</td>
</tr>
<tr>
<td>Ezetimibe</td>
<td>3 (25)</td>
<td>7 (33)</td>
</tr>
<tr>
<td>Colesevelam</td>
<td>0 (0)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Ciprofibrate</td>
<td>1 (8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>2 (17)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Fish oil or Omega-3 triglycerides</td>
<td>2 (17)</td>
<td>4 (19)</td>
</tr>
<tr>
<td>Serum aminotransferase activity, U/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALTb</td>
<td>25.0 ± 6.7</td>
<td>26.5 ± 11.8</td>
</tr>
<tr>
<td>ASTb</td>
<td>23.8 ± 4.0</td>
<td>25.5 ± 11.6</td>
</tr>
</tbody>
</table>

*a*Data are expressed as median (min-max). *b*Data are expressed as mean ± SD. M denotes male; F, female; FH, familial hypercholesterolemia; DMII, type 2 diabetes; CVD, cardiovascular disease.

### Efficacy

Efficacy results are summarized in table 2 and figure 2. Treatment with mipomersen 200 mg/wk resulted in significant reductions in LDL-c of 47% (±18) (p<0.001) with a range of -19% to -77%. Seven of the 21 (33%) subjects in the mipomersen treatment group achieved LDL-c levels <2.6 mmol/L (100 mg/dl), whereas 5 (24%) subjects achieved LDL-c levels <1.8 mmol/L (70 mg/dl). The observed reductions in LDL-c corresponded to mean apoB reductions of 46% (±20) (p<0.001) with a mean apoB of 1.0 (±0.5) g/L (100 mg/dL) at endpoint. Mipomersen treatment also significantly lowered total cholesterol, triglycerides and Lp(a) but did not affect HDL-c and apoA1. Mipomersen differentially lowered LDL-particle numbers with largest reductions in the small LDL particles (-56%±47; p=0.001) and more modest reductions in the large LDL particles (-4%±116; p<0.017) (table 3 and figure 3).
Table 2 Lipid concentrations at baseline and primary efficacy timepoint

<table>
<thead>
<tr>
<th>Lipid Parameter</th>
<th>Placebo (n = 12)</th>
<th>Mipomersen 200 mg (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline PET % Change</td>
<td>Baseline PET % Change</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>6.3 ± 1.7 -2 ± 8</td>
<td>6.3 ± 2.4 3.3 ± 1.9 -47 ± 18b</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
<td>1.8 ± 0.4 -4 ± 8</td>
<td>1.8 ± 0.5 1.0 ± 0.5 -46 ± 20b</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>8.3 ± 1.7 -2 ± 6</td>
<td>8.3 ± 2.4 5.2 ± 2.0 -37 ± 15b</td>
</tr>
<tr>
<td>Non-HDL-cholesterol</td>
<td>7.1 ± 1.7 -2 ± 7</td>
<td>7.0 ± 2.4 3.8 ± 2.1 -46 ± 18b</td>
</tr>
<tr>
<td>Triglyceridea</td>
<td>1.5 (1.2, 1.9) 6 (-10, 22)</td>
<td>1.5 (1.1, 2.2) 1.0 (0.8, 1.3) -28 (-50, -10)c</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>0.4 ± 0.8 0 ± 9</td>
<td>0.5 ± 0.5 0.4 ± 0.5 -27 ± 31c</td>
</tr>
<tr>
<td>VLDL-cholesterol</td>
<td>0.8 ± 0.3 4 ± 27</td>
<td>0.7 ± 0.3 0.5 ± 0.2 -27 ± 31c</td>
</tr>
<tr>
<td>LDL/HDLa</td>
<td>4.8 (3.8, 6.2) 4 (10, 11)</td>
<td>5.0 (3.8, 6.0) 2.5 (1.4, 2.9) -48 (-69, -37)b</td>
</tr>
<tr>
<td>ApoA1</td>
<td>1.5 ± 0.2 -1 ± 11</td>
<td>1.5 ± 0.3 1.5 ± 0.2 0 ± 12</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>1.3 ± 0.3 -2 ± 13</td>
<td>1.3 ± 0.3 1.4 ± 0.3 8 ± 17</td>
</tr>
</tbody>
</table>

Primary efficacy timepoint (PET) was Week 28 or the visit closest to 14 days after the last dose for subjects who discontinued dosing early. Data are expressed as mean±SD. To convert cholesterol and triglyceride values to mg/dL multiply by 38.67 and 88.57, respectively. p-values are for the difference between percentage change from baseline for mipomersen and placebo using the two-sided t-test when the change from baseline had a normal distribution, or otherwise by using the Wilcoxon rank-sum test. a Data are expressed as median (interquartile range). b p value <0.001, c p value <0.01.

Figure 2 Effect of mipomersen on apoB (a) and LDL-c (b) over time

LDL-c is presented as the mean percent change from baseline ± 95%CI. Dotted line represents end of treatment period.

Safety

Two serious adverse events (SAEs) were reported during the study. One was an on-treatment SAE of acute myocardial infarction in the placebo treatment group, which led to discontinuation of study drug. A second SAE of coronary artery re-stenosis was reported during the post-treatment follow-up period in the mipomersen treatment group. Both SAEs resolved by the end of study.
In the active treatment group, 4 subjects discontinued treatment due to one or more adverse events. These events included flu-like symptoms (n=1), malaise (n=1) and myalgia (n=1). One subject from the active treatment group met a stopping rule for liver transaminase increases of ≥5 x ULN. In this subject ALT increased ≥10 x ULN in Week 8. Further evaluation with MRS showed an IHTG-content of 0.8% in Week 9, which is not compatible with hepatic steatosis (ULN=5.6%). Serology for viral hepatitis or auto-immune hepatitis was negative. Liver transaminase levels returned to normal within 4 weeks. One subject from the placebo treatment group discontinued treatment due to diarrhoea.

The most common adverse events were injection site reactions (ISR) following subcutaneous administration of the study drug. Twenty (95%) subjects treated with mipomersen compared to 10 (83%) subjects on placebo treatment experienced at least one ISR.

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### Table 3 LDL particle numbers and size at baseline and primary efficacy timepoint

<table>
<thead>
<tr>
<th>LDL Particle</th>
<th>Placebo (n=12)</th>
<th>PET (n=12)</th>
<th>% Change (n=12)</th>
<th>Mipomersen 200 mg (n=20)</th>
<th>PET (n=20)</th>
<th>% Change (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number, nmol/L</td>
<td>2347 ± 742</td>
<td>2293 ± 594</td>
<td>-0.7 ± 11</td>
<td>2207 ± 962</td>
<td>1086 ± 875</td>
<td>-49 ± 22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IDL</td>
<td>135 ± 64</td>
<td>134 ± 91</td>
<td>14 ± 71</td>
<td>75 ± 78</td>
<td>47 ± 44</td>
<td>32 ± 212</td>
</tr>
<tr>
<td>Large LDL</td>
<td>807 ± 459</td>
<td>800 ± 417</td>
<td>15 ± 64</td>
<td>917 ± 439</td>
<td>563 ± 210</td>
<td>-4 ± 116&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Small LDL</td>
<td>1406 ± 854</td>
<td>1359 ± 665</td>
<td>13 ± 48</td>
<td>1215 ± 1033</td>
<td>476 ± 807</td>
<td>-56 ± 47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Particle size, nm</td>
<td>21.0 ± 1.0</td>
<td>20.9 ± 0.8</td>
<td>-0.1 ± 2.5</td>
<td>21.2 ± 0.9</td>
<td>21.9 ± 0.8</td>
<td>3.3 ± 3.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Primary efficacy timepoint (PET) was Week 28 or the visit closest to 14 days after the last dose for subjects who discontinued dosing early. Data are expressed as mean ± SD. Total LDL particle number includes IDL (23-27 nm), Large LDL (21.2-23 nm) and Small LDL (18-21.2 nm) subclasses. P-values are for the difference between change from baseline for mipomersen and placebo using the Exact Wilcoxon Rank Sum test. <sup>a</sup>p-value <0.001, <sup>b</sup>p-value <0.05.

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**Figure 3 Effect of mipomersen on LDL particle subclass distribution**

Mipomersen differentially lowered LDL-particle numbers with largest reductions in the small LDL particles and more modest reductions in the large LDL particles. Small LDL includes medium small LDL (19.8-21.2 nm), and very small LDL (18-19.8 nm).
Increases in ALT above the ULN were more common in the mipomersen treatment group (n=17 [81%]) compared to the placebo treatment group (n=3 [25%]). Persistent increases in ALT (≥3x ULN on 2 consecutive occasions at least 7 days apart) were observed in 7 subjects (33%) from the active treatment group. In the mipomersen treatment group, ALT concentrations at PET were found to correlate to apoB concentrations at PET (r=-0.644; p=0.002) but not to change in apoB concentrations from baseline to PET (r=0.309; p=0.173). ALT increases were often accompanied by lesser AST increases but not by increases in total bilirubin, alkaline phosphatase, prothrombin time or by decreases in albumin. There were no symptoms or other clinical signs suggestive of impaired hepatic function. After discontinuation of treatment, transaminases returned to normal (<1.5 x ULN) in all subjects. All persistent increases in ALT >3 x ULN were considered to be probably related to the study drug by the investigators.

Vital signs, electrocardiography and urinalysis did not show any clinically significant changes. There were no significant differences in percentage change in CRP over time between the active and placebo treatment group (data not shown).

**Hepatic MRS**

MRS was performed in 14 of 21 subjects from the active treatment group and in 1 of 12 subjects from the placebo treatment group, because of an increase in ALT of at least ≥2 x ULN. Pretreatment baseline MRS measurements were not performed. Results for on-treatment and post-treatment MRS are shown in figure 4. Highest IHTG-contents were measured around Week 28. In 2 subjects the first MRS was performed a few weeks after completion of treatment in Week 28. The median of the highest IHTG-content measured in all 14 mipomersen treated subjects was 24.4% ranging from 0.8 to 47.3%. Hepatic steatosis (IHTG-content >5.6%) was detected in 12 of the 14 subjects treated with mipomersen and in 1 of 1 placebo-treated subjects. The median absolute change from highest IHTG-content to lowest IHTG-content at follow-up was -17.7% (-6.4 to -38.0; n=12, p=0.0005). This change was correlated to apoB levels at PET (r=0.601; p=0.04, n=12). During follow-up subjects showed either stabilization or reduction in IHTG-content (figure 4).

**Liver biopsy**

Four subjects characterized by IHTG-content >20% with concomitant ALT ≥2 x ULN on 2 consecutive visits were referred to a hepatologist for further evaluation. In the 2 subjects with rapidly progressive steatosis on MRS a liver biopsy was performed. The first biopsy was performed in a subject with an ALT ≥2 x ULN and IHTG-content that increased from 17.8% in Week 4 to 34.7% in Week 18.
with a maximum of 42.0% in Week 26. Liver biopsy was performed in Week 22 and showed severe macrovesicular steatosis in >66% of the hepatocytes, with minimal lobular inflammation, few ballooning cells and minimal fibrosis (NAS activity score: 5/8, fibrosis grade 1). This subject discontinued treatment in Week 23 because of flu like symptoms. A liver biopsy was performed in a second subject with an IHTG-content of 23.7% in Week 10, increasing up to 47.3% in Week 30 with concomitant ALT elevations >3 x ULN on 2 consecutive visits. Needle biopsy was performed in Week 21 and showed severe macrovesicular hepatic steatosis and minimal lobular inflammation, few ballooning cells, but no significant fibrosis (NAS activity score 5/8, fibrosis grade 0).

**Discussion**

In subjects intolerant to statins and at high risk for CVD, mipomersen achieved robust reductions in both LDL-c and apoB and was overall well tolerated. A significantly elevated IHTG-content was observed predominantly in subjects with concomitant ALT increases ≥2 x ULN, which was reversible after discontinuation of treatment. Liver biopsies performed in 2 cases confirmed hepatic steatosis, with no indication of severe steatohepatitis. Pending long-term safety data, these findings suggest that mipomersen is an effective therapeutic strategy in patients who are statin-intolerant and at high risk for CVD.

**Efficacy**

LDL-c reductions achieved in the present study were comparable to those achieved by high doses of potent statins such as atorvastatin and rosuvastatin\(^24\);\(^25\). These profound reductions in LDL-c exceeded those achieved in most previous
clinical trials with mipomersen 200 mg/wk on top of lipid lowering therapy\textsuperscript{9,11;12,26-28}, whereas equipotent reductions were observed following mipomersen administration in a Phase II clinical trial in patients with mild to moderate hyperlipidemia not using statins\textsuperscript{10}. In addition, results from previous studies suggest that LDL-c reductions in patients with FH are less profound compared to patients with moderate hypercholesterolemia. Although these observations may imply a role for the LDL receptor in contributing to the LDL-c-lowering effect of mipomersen, studies in LDL-receptor knock-out mice did not show attenuation of mipomersen's efficacy\textsuperscript{29}.

Based on its mechanism of action, mipomersen also achieved a significant reduction in apoB (-46%). Since apoB provides a reliable prediction of all atherogenic lipoproteins and, in line, has been shown to be a better predictor of CVD-risk compared to LDL-c\textsuperscript{1,30}, the potent reduction of apoB by apoB synthesis inhibition can even be expected to offer additional cardiovascular benefit on top of its LDL-c lowering effects\textsuperscript{31}. In support, mipomersen differentially lowered LDL particle numbers, with largest reductions in the number of small LDL particles. The predominance of small-dense LDL particles contributes importantly to the atherogenicity of the LDL fraction\textsuperscript{32}. The latter may bear particular relevance for patients characterized by elevated levels of small-dense LDL-c such as patients with type 2 diabetes mellitus and the metabolic syndrome\textsuperscript{33}.

**Safety**

In the present study ISRs and flu like symptoms were amongst the most common adverse events. Markedly, these adverse events did not interfere with continued dosing. In fact, overall adherence to mipomersen exceeded 80% at the end of the study. These data compare favorably to adherence rates in statin intolerant patients using alternative dosing regiments of different statins (ranging from 50 to 80%) whereas LDL-c reductions in these trials were much less profound\textsuperscript{34-36}.

Persistent increases in liver transaminases ≥3 x ULN were observed in 33% of the subjects whereas any ALT increase was observed in >80% of the subjects assigned to mipomersen. These numbers exceed those reported in earlier studies, in which persistent ALT increases ≥3 x ULN were observed in 6 to 15% of the subjects\textsuperscript{9,11,12,26,27,37}. ALT increases following mipomersen treatment may result from a direct pharmacologic effect or may be related to hepatic fat accumulation. In support of the latter, hepatic steatosis was observed in a substantial proportion of subjects from the mipomersen treatment group who had ALT increases ≥2x ULN (12 of 14).
Results from earlier trials did not provide consistent evidence for the induction of hepatic steatosis. In preclinical animal model studies, no increases in hepatic transaminases were observed. In these models, impaired hepatic VLDL excretion did not result in IHTG accumulation due to compensatory mechanisms including downregulation of hepatic fatty acid synthase (i.e., de novo lipogenesis) combined with increased fatty acid oxidation rate due to AMP-kinase upregulation\textsuperscript{29,38}. In subsequent Phase II clinical trials, hepatic steatosis was reported only incidentally, predominantly in subjects with persistent elevations of liver transaminases\textsuperscript{12,26}. In a dedicated study, we evaluated the impact of mipomersen treatment during 12 weeks on IHTG-content in subjects with FH. Mipomersen results in a trend towards increased IHTG-content\textsuperscript{37}. Preliminary data from Phase III data in heterozygous FH patients reported an absolute median increase in percent liver fat of 4.9\% (IQR 1.3-13.3\%) following 26 weeks of mipomersen administration\textsuperscript{27}.

Several factors may have contributed to the apparently high incidence of hepatic steatosis in the present study. First, a recent post-hoc analysis of the GREACE study, a prospective, randomized, controlled trail with atorvastatin versus usual care, reported that statins reduce transaminase increases in patients with suspected NAFLD\textsuperscript{39}. Similarly, others have reported that statins may also reverse ultrasonographic evidence of NAFLD\textsuperscript{40,41}. Since the subjects in the present study did not tolerate statin therapy, this may have resulted in a higher susceptibility for hepatic fat accumulation compared to participants in most previous trials in which statin therapy was mandatory\textsuperscript{9,11,12,26}. Second, we observed more profound reductions in apoB in the present study compared to previous clinical trials with mipomersen, at similar dose and with similar treatment duration (-26\% to -37\% compared to -47\% in the present study)\textsuperscript{11,12,27,28,42}. Interestingly, liver fat changes correlated with the level of apoB at PET, compatible with the concept that impaired excretion of VLDL may enhance accumulation of triglycerides in the liver. Thus, the higher reductions in apoB in the present study may have contributed to the higher incidence of hepatic steatosis. Third, it should be noted that in most previously published Phase II clinical trials with mipomersen, assessment of liver fat was performed only if ALT levels increased ≥3 x ULN on 2 consecutive occasion. In the present study we already observed steatotic changes in subjects with milder ALT elevations. As a consequence hepatic steatosis following prolonged treatment with mipomersen may have previously been underreported in those subjects with only modest ALT increases.

**Biopsies**

Results from 2 liver biopsies confirmed the presence of significant hepatic steatosis with only minimal inflammatory changes. LDL-c levels were reduced
substantially up to 60% from baseline in both subjects with minimum levels measured at 85 and 51 mg/dL. Whereas minimal fibrosis was observed in 1 biopsy, the lack of a baseline biopsy makes it difficult to interpret this finding. Since the biopsy was performed after 21 weeks of treatment, the observed fibrosis could theoretically be related to the study drug. However, IHTG-content was already elevated in Week 4 in this particular subject, therefore these changes may also reflect preexistent abnormalities.

Steatosis observed in non alcoholic fatty liver disease (NAFLD), the most common cause of hepatic fat accumulation in the general population, remains stable in most patients. However, approximately 25-30% of patients with NALFD progress to non-alcoholic steatohepatitis (NASH)\textsuperscript{43}. NASH patients progress to more severe liver injury including fibrosis and cirrhosis in 30% and 25% of cases respectively\textsuperscript{44}. Interestingly, evidence is accumulating to show that hepatic steatosis following apoB synthesis inhibition may differ from NAFLD. For example, the majority of patients with familial hypobetalipoproteinemia (FHBL), the “genetic homologue” of apoB synthesis inhibition, are also characterized by hepatic steatosis\textsuperscript{37,45,46}. By contrast, reports on fibrosis and cirrhosis in these patients are scarce\textsuperscript{46,47}. Our recent finding that hepatic steatosis in FHBL was not associated with insulin resistance lends further support to the concept that liver fat accumulation associated with apoB inhibition is without metabolic sequelae and therefore distinctly differs from NAFLD (Chapter 7)\textsuperscript{48}. This concept needs to be validated in long-term safety studies following prolonged mipomersen administration.

\textbf{Limitations}

Several aspects of our study deserve closer attention. First, determination of IHTG-content was only performed in subjects with liver transaminase increases and not at predetermined timepoints. As a consequence baseline and highest values as well as values for IHTG-content in subjects without liver transaminase increases were missing. Therefore, the effect of mipomersen on IHTG-content in subjects without liver transaminase remains incomplete. Second, conclusions based on the biopsy findings are hampered by its small sample size, the short treatment duration and the lack of pretreatment biopsy. Therefore, in future clinical trials with mipomersen, measurement of IHTG-content should be performed in all participants, at baseline and at predefined time points during long-term treatment. In addition more liver biopsies may be required following prolonged treatment to fully explore the effects of apoB synthesis inhibition on liver tissue.
Summary

In summary, in subjects who are statin-intolerant and at high risk for CVD, mipomersen effectively lowered LDL-c and apoB and was overall well tolerated. Whereas mipomersen treatment, in some patients, was associated with persistent transaminase increases and intrahepatic fat accumulation, inflammatory changes in liver tissue appeared to be minimal, indicating no evidence of severe steatohepatitis. Therefore, pending long term safety data, apoB synthesis inhibition may offer an effective therapeutic strategy for patients at high risk for CVD with statin intolerance for whom currently no alternative option is available to effectively lower LDL-c.
Reference List


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