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Safety and efficacy of mipomersen in patients with heterozygous familial hypercholesterolemia

Laurens F. Reeskamp[a,*], John J.P. Kastelein[a], Patrick M. Moriarty[b], P. Barton Duell[c], Alberico L. Catapano[d,e], Raul D. Santos[f,g], Christie M. Ballantyne[h]

[a] Department of Vascular Medicine, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands
[b] Division of Clinical Pharmacology, Department of Internal Medicine, University of Kansas Medical Center, Kansas City, KS, USA
[c] Knight Cardiovascular Institute, Oregon Health and Science University, Portland, OR, USA
[d] Dipartimento Dieccellenza Scienze Farmacologiche e Biomolecolari, Milano, Italy
[e] IRCCS Multimedica, Milano, Italy
[f] Lipid Clinic Heart Institute (InCor), University of Sao Paulo Medical School, University of Sao Paulo, Sao Paulo, Brazil
[g] Hospital Israelita Albert Einstein, Sao Paulo, Brazil
[h] Sections of Cardiology and Cardiovascular Research, Baylor College of Medicine, Houston, TX, USA

HIGHLIGHTS

- Mipomersen 200 mg once weekly and 70 mg thrice weekly are effective in lowering apoB-containing lipoproteins in patients with heterozygous FH.
- This effectiveness is counterbalanced by limited tolerability and increased hepatic transaminase levels in about 21% of patients.
- Thrice-weekly dosing is associated with less flu-like symptoms and lower discontinuation rates, but otherwise had no major benefits.

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ABSTRACT

Background and aims: Heterozygous familial hypercholesterolemia (HeFH) is a common genetic disorder characterized by elevated low-density lipoprotein cholesterol (LDL-C) and increased cardiovascular disease risk. Despite multiple LDL-C–lowering therapies, many HeFH patients do not reach LDL-C targets. Mipomersen, an antisense oligonucleotide against apolipoprotein B (apoB), might further lower LDL-C in HeFH patients. We assessed the efficacy and safety of two mipomersen dosing regimens in HeFH patients and explored whether thrice-weekly dosing improves the benefit-risk profile.

Methods: In this double-blind trial, HeFH patients (LDL-C > 160 mg/dL) on maximal tolerated LDL-lowering therapy were randomized to mipomersen 200 mg once weekly (n = 104), mipomersen 70 mg thrice weekly (n = 102), or placebo in matching frequency (n = 103) for 60 weeks. Main outcomes were LDL-C, apoB, and lipoprotein(a) levels after 60 weeks of treatment.

Results: Mipomersen 200 mg once weekly and mipomersen 70 mg thrice weekly significantly lowered LDL-C compared with placebo by 21.0% and 18.8%, respectively, and apoB by 22.1% and 21.7% (all $p < 0.001$). Lipoprotein(a) was significantly lowered by 27.7% ($p < 0.001$) with thrice-weekly dosing. Injection-site reactions and flu-like symptoms led to discontinuation in 21.2% (200 mg), 17.6% (70 mg), and 5.8% (placebo) of participants. Alanine transaminase was elevated (≥3× upper limit of normal at least once) in 21.2%, 21.6%, and 1.0% of subjects, respectively.

Conclusions: Mipomersen 200 mg once weekly and 70 mg thrice weekly are effective in lowering apoB-containing lipoproteins in HeFH patients. This is counterbalanced by limited tolerability and increased hepatic transaminase levels in about 21% of patients. The thrice-weekly dosing regimen was associated with lower frequency of flu-like symptoms, which might help avert discontinuation in some patients, but otherwise had no major benefits.

* Corresponding author. Department of Vascular Medicine, Amsterdam UMC, Meibergdreef 9, room F4-140, 1105AZ, Amsterdam, the Netherlands.
E-mail address: l.f.reeskamp@amc.uva.nl (L.F. Reeskamp).

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1. Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant disorder characterized by elevated plasma levels of low-density lipoprotein cholesterol (LDL-C), consequently leading to an increased risk for premature cardiovascular disease (CVD) [1]. Heterozygous FH (HeFH) has a prevalence of 1/200–1/500 [1], and typically leads to CVD in men before the age of 55 and in women before the age of 60 [2,3]. Most commonly, FH is caused by mutations in the LDL receptor gene (*LDLR*); however, a much smaller proportion of FH patients have mutations in the apolipoprotein B (*APOB*) or proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene, leading to impaired LDL-C uptake or increased LDL receptor degradation, respectively [4].

Current treatment of HeFH patients consists of high doses of high-intensity statins, often in combination with ezetimibe, with LDL-C goals of < 100 mg/dL (< 2.6 mmol/L) in primary prevention or < 70 mg/dL (< 1.8 mmol/L) after a CVD event [5]. However, those goals are often not reached with this therapeutic regimen [6], underlining the need of additional LDL-lowering options. One of these is PCSK9 inhibition, with promising results of a robust additional LDL-C reduction and relevant reduction in major adverse cardiac events (MACE) even in individuals with FH [7,8].

Another novel lipid-lowering strategy is mipomersen, an antisense oligonucleotide directed against apolipoprotein B100 (apoB100) mRNA in the liver, ultimately resulting in decreased levels of apoB100-containing lipoproteins such as LDL and lipoprotein(a) [Lp(a)] [9]. Mipomersen has showed LDL-C–lowering efficacy in patients with homozygous FH (HoFH), severe HeFH, severe hypercholesterolemia at high CVD risk, and statin intolerance [10–14,18]. Since mipomersen exerts its lipid-modifying effect independently of the function of the LDL receptor, it is considered an additional or alternative treatment option to traditional cholesterol-lowering drugs targeting the LDL receptor pathway. Mipomersen was approved in 2013 by the U.S. Food and Drug Administration (FDA) for HoFH patients in the United States. However, its use is associated with some frequently occurring side effects: injection-site reactions, flu-like symptoms, and, inherent to its pharmacological mechanism, hepatic fat accumulation and transaminase elevation [15]. A study of different doses and frequencies of mipomersen has shown a potential beneficial side effect profile regarding injection site reactions for a thrice weekly dosing regimen compared to once weekly dosing [16].

Therefore, the aim of this long-term double-blind study was to assess the efficacy and safety of two different dosing regimens of mipomersen (200 mg subcutaneously [SQ] once weekly or 70 mg SQ thrice weekly) in a large population of patients with HeFH on stable maximally tolerated lipid-lowering regimens, and to explore whether the thrice-weekly dosing regimen improved the benefit–risk profile compared to the FDA-approved weekly dosing regimen.

2. Patients and methods

2.1. Study subjects

The study cohort comprised two populations of very high risk HeFH patients, 18 years and older, with persistent severe hypercholesterolemia (LDL-C > 160 mg/dL [> 4.14 mmol/L]) on maximally tolerated LDL-lowering therapy. Cohort 1 (severe HeFH) consisted of subjects with fasting LDL-C at screening ≥300 mg/dL (7.77 mmol/L) or LDL-C ≥200 mg/dL (5.18 mmol/L) plus documented coronary heart disease (CHD)/CHD risk equivalents. Cohort 2 (high-risk HeFH) consisted of HeFH subjects with LDL-C levels between 160 mg/dL (4.14 mmol/L) and 200 mg/dL (5.18 mmol/L) plus documented CHD/CHD risk equivalents. A diagnosis of HeFH in cohort 2 had to be made with the Simon Broom, US MedPED, or Dutch Lipid Clinic Network criteria. Patients had to be on a stable, maximally tolerated, lipid-lowering regimen for at least 12 weeks prior to screening. Furthermore, a body mass index (BMI) ≤40 kg/m^2 with stable weight and triglyceride (TG) levels below 350 mg/dL (3.95 mmol/L) were required. Females could not be pregnant and required contraceptive regimens up to 24 weeks after the last mipomersen dose. Males had to be either surgically sterile or they and their partners had to be willing to use effective contraceptive methods.

Exclusion criteria included CVD events within 24 weeks prior to screening, apheresis within 3 months prior to screening or expected apheresis during study treatment, insulin-dependent diabetes mellitus (type 1) or a glycated haemoglobin > 8%, New York Heart Association class III and IV heart failure, uncontrolled hypertension (i.e., systolic and diastolic blood pressures ≥160/95 mmHg), a positive test for human immunodeficiency virus or hepatitis B/C/E, uncontrolled hypothyroidism, history of malignancy (other than skin cancer), hepatic disease (e.g., non-alcoholic steatohepatitis), and renal disease. Patients were excluded when their laboratory values at screening showed elevated creatine kinase (≥3 × upper limit of normal [ULN]), alanine transaminase (ALT; ≥1.5 × ULN) or aspartate transaminase (AST; ≥1.5 × ULN), creatinine (> 8.8 μmol/L above ULN in women; > 17.7 μmol/L above ULN in men), proteinuria (> 1000 mg protein/g creatinine), or total bilirubin > 1.5 × ULN.

2.2. Study design

A multicenter, randomized, double-blind, placebo-controlled, parallel-group study was performed at 131 sites in 30 countries to assess the efficacy and safety of mipomersen in the selected populations. Patients with severe HeFH (cohort 1) or high-risk HeFH (cohort 2) were randomized and stratified by geographic region at a 1:1 ratio to a regimen of mipomersen 200 mg SQ or placebo once weekly (n = 155) or a regimen of mipomersen 70 mg SQ or placebo thrice weekly (n = 154). See the consort diagram (Fig. 1) for a study overview. Patients were then stratified by gender and use of statins and randomized at a 2:1 ratio to receive mipomersen or placebo. The total study duration consisted of a 60-week blinded treatment phase followed by a 24-week post-treatment safety follow-up period. Patients could choose to enter a 26-week open-label continuation period before entering the post-treatment safety follow-up period. During the open-label extension, participants received mipomersen according to their assigned dose regimen, either continuing previously blinded treatment with mipomersen or changing from placebo to mipomersen at the same dosing regimen. Patients, investigators, and study staff were blinded to treatment allocation and lipid data of the patients during the entire blinded treatment period. During the first 8 weeks of the study, patients received 50% of the assigned dose of investigational product, consisting of dosing once every other week for mipomersen 200 mg (or placebo) and thrice every other week for mipomersen 70 mg (or placebo). From week 9 and onwards the dose frequency was increased to weekly. The study was approved by all local Institutional Review Boards of the participating sites and performed in compliance with the Declaration of Helsinki (revised edition, Washington 2002). All participants gave informed consent prior to participation in this trial. An independent Data Monitoring Committee was established to provide an expert review of unblinded safety, efficacy, and tolerability data and assure safety of study subjects. An independent Cardiovascular Adjudication Committee was established to apply uniform criteria for the adjudication of cardiovascular events in a blinded, uniform manner.

2.3. Safety monitoring

The safety of mipomersen was assessed by recording the incidence of (serious) adverse events ([S]AE), physical examinations, 12-lead ECGs, measurement of routine laboratory analytes (chemistry, coagulation, hematology, and urinalysis), inflammatory markers, anti-mipomersen antibodies, and serial hepatic magnetic resonance imaging to assess the percent hepatic fat fraction. ALT and AST levels were

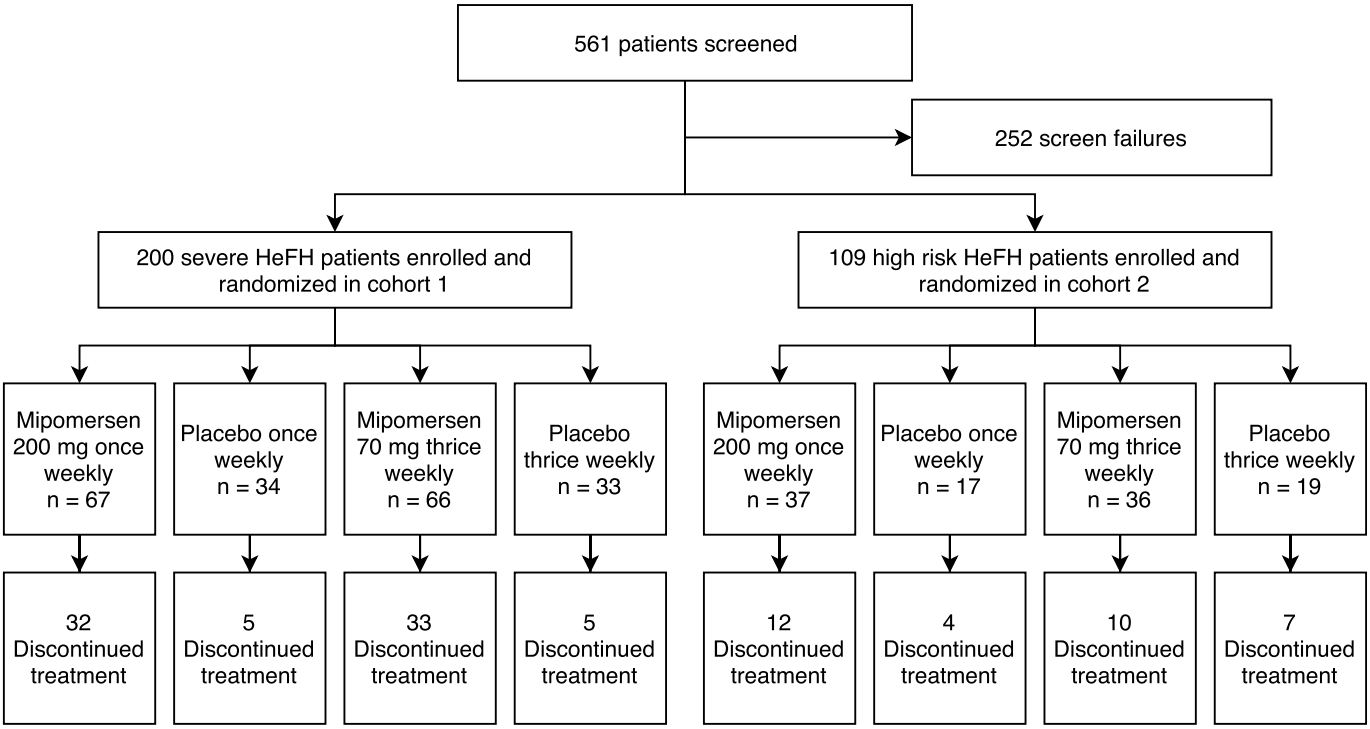

Fig. 1. Consort diagram.

measured regularly, and if elevated, dosing adjustment or temporary discontinuation of mipomersen was considered based on predetermined "liver chemistry stopping rules" (Supplementary Table 1).

2.4. Lipid assessments

Fasting blood samples were analyzed for LDL-C, very low density lipoprotein cholesterol (VLDL-C), TG, high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), Lp(a), apoB100, and apolipoprotein A1 (apoA1). TC, HDL-C, and TG were measured with enzymatic colorimetry, apoB100 and apoA1 with rate nephelometry, and Lp(a) with a standardized isoform-independent assay. LDL-C was calculated with the Friedewald formula, or measured through ultracentrifugation if TG levels exceeded 400 mg/dL.

2.5. Outcome parameters

The primary outcome parameter was the percent change in LDL-C levels from baseline for both dose regimens in cohort 1 compared with placebo. Secondary outcome parameters were percent change in apoB100 and Lp(a) levels in both cohorts, and LDL-C change in cohort 2. Furthermore, other lipid parameters and differences in the safety and tolerability profiles of the two dosing regimens were determined in both cohorts of HeFH patients.

2.6. Statistical analysis

For the sample size determination, the standard deviation for the percent change in LDL-C was estimated at 22%. With a 2:1 mipomersen to placebo randomization, the inclusion of 60 patients per regimen (40 mipomersen, 20 placebo) yielded a 90% power to detect a 20% difference between treatment groups using a 2-sided alpha of 5%.

The efficacy analysis for primary and secondary outcome parameters was executed by an intention to treat analysis and per protocol analysis. A mixed model for repeated measures with terms for baseline lipid value, geographic region, gender, statin use, treatment group, study visit, and study visit–by–treatment group interaction was used to assess the mean treatment difference at week 61 for the primary and secondary outcome parameters. Statistical significance was defined as $p \leq 0.05$. Sequential testing was used to control for multiplicity associated with testing the primary and secondary efficacy outcome parameters.

3. Results

3.1. Study subjects

A total of 309 subjects were enrolled in this study and randomized among 4 different treatment arms within severe HeFH and high-risk HeFH patients; 104 subjects received mipomersen 200 mg once weekly, 51 received placebo once weekly, 102 subjects received mipomersen 70 mg thrice weekly, and 52 subjects received placebo thrice weekly (see Fig. 1 for consort diagram). Table 1 summarizes demographic and baseline characteristics of the enrolled patients by treatment group. A breakdown per cohort and treatment arm can be found in Supplementary Table 2. Rates of prior CHD were similar between mipomersen regimen groups and placebo; 77.7% of subjects in the pooled placebo group had a history of CHD compared to 73.5% and 77.9% in the mipomersen 200 mg once weekly and 70 mg thrice weekly groups, respectively. In the severe HeFH group (cohort 1), mean baseline LDL-C levels were 255 mg/dL and 262 mg/dL in the placebo and mipomersen 200 mg once weekly groups, respectively, and 263 mg/dL and 274 mg/dL in the placebo and mipomersen 70 mg thrice weekly groups, respectively. In the high-risk HeFH group (cohort 2), mean baseline LDL-C levels were 179 mg/dL and 177 mg/dL in the once-weekly placebo and mipomersen 200 mg groups, respectively, and 169 mg/dL and 178 mg/dL in the thrice-weekly placebo and mipomersen 70 mg groups, respectively.

A total of 201 patients (65.0%) completed the 60-week blinded treatment phase of the study. Most discontinuations were due to AEs. After 60 weeks of treatment, 60 subjects (57.5%) allocated to mipomersen 200 mg and 59 subjects (57.8%) allocated to mipomersen 70 mg were still on active treatment, while 82 participants (79.6%) of the pooled placebo group finished the blinded treatment period.

Table 1
Baseline characteristics.

	Mipomersen 200 mg once weekly (n = 104)	Mipomersen 70 mg thrice weekly (n = 102)	Pooled placebo (n = 103)
Gender male, n (%)	46 (44.2)	48 (47.1)	47 (45.6)
Age (years), mean (SD)	56.4 (9.8)	53.1 (11.92)	54.9 (10.18)
Weight (kg), mean (SD)	78.43 (15.6)	79.88 (16.73)	79.29 (16.28)
BMI (kg/m^2), mean (SD)	28.25 (4.65)	28.08 (4.6)	28.25 (4.32)
Race, n (%)			
White	83 (79.8)	91 (89.2)	90 (87.4)
Black	3 (2.9)	2 (2.0)	3 (2.9)
Asian	13 (12.5)	7 (6.9)	7 (6.8)
Other	4 (3.8)	2 (2.0)	3 (2.9)
Ethnicity, n (%)			
Hispanic or Latino	4 (3.8)	4 (3.9)	10 (9.7)
Not Hispanic or Latino	99 (95.2)	95 (93.1)	91 (88.3)
Unknown	1 (0.9)	3 (2.9)	2 (1.9)
Tobacco users, n (%)			
Current	16 (15.4)	23 (22.5)	22 (21.4)
Former	30 (28.8)	29 (28.4)	27 (26.2)
Never	57 (54.8)	50 (49.0)	64 (62.1)
Alcohol, n (%)			
Current	40 (28.8)	45 (44.1)	37 (35.9)
Former	6 (5.8)	14 (13.7)	14 (13.6)
Never	58 (55.8)	43 (42.2)	52 (50.5)
HoFH, n (%)	1 (1.0)	2 (2.0)	4 (3.8)
CHD, n (%)	81 (77.9)	75 (73.5)	80 (77.7)
MI n (%)	36 (34.6)	35 (34.3)	35 (34.0)
CABG	23 (22.1)	22 (21.6)	23 (22.3)
PCI or alternative revascularization	36 (34.6)	29 (28.4)	37 (35.9)
Other atherosclerotic disease (peripheral, carotid, abdominal aortic aneurysm), n (%)	32 (30.8)	36 (35.3)	43 (41.7)
Hypertension, n (%)	72 (69.2)	60 (58.8)	66 (64.1)
Diabetes, n (%)	23 (22.1)	11 (10.8)	15 (14.6)
Statin use, n (%)	91 (87.5)	89 (87.3)	85 (82.5)

Baseline characteristics of all participants. The pooled placebo group consists of patients receiving placebo once weekly and thrice weekly.
BMI = body-mass index, HoFH = homozygous familial hypercholesterolemia, CHD = coronary heart disease, MI = myocardial infarction, CABG = coronary artery bypass grafting, PCI = percutaneous coronary intervention.

3.2. Efficacy

Mipomersen 200 mg once weekly and mipomersen 70 mg thrice weekly significantly lowered LDL-C by 21.0% and 18.8%, respectively, compared to placebo ($p < 0.001$). The time course of changes in LDL-C levels during the 60-week blinded treatment period is shown in Fig. 2 per treatment arm and per cohort. ApoB decreased by 22.1% with mipomersen 200 mg once weekly and 21.7% with mipomersen 70 mg thrice weekly compared to placebo ($p < 0.001$). Lp(a) was significantly lowered after 60 weeks of treatment with mipomersen 70 mg thrice weekly but not with the once-weekly dosing regimen. The efficacy of mipomersen on LDL-C, apoB, and Lp(a) is summarized in Table 2 for both cohorts combined. The active treatment regimens did not differ significantly from each other in their efficacy of lowering LDL-C and apoB.

In cohort 1 (severe HeFH) and cohort 2 (high-risk HeFH), mipomersen 200 mg once weekly significantly lowered LDL-C, apoB, TC, and VLDL-C compared with placebo after 60 weeks of treatment (Supplementary Tables 3 and 4). Lp(a) levels were not significantly changed by this mipomersen regimen compared with placebo in both cohorts. Mipomersen 70 mg thrice weekly did not significantly reduce LDL-C compared with placebo in severe HeFH, although the reduction in apoB was nominally significant ($p = 0.020$). In contrast, in the high-risk HeFH cohort, this regimen was effective in lowering LDL-C, apoB, and TC. Mipomersen 70 mg lowered Lp(a) by 17.1% and 54.4% compared to placebo in the severe and high-risk HeFH cohorts, respectively.

3.3. Safety

A total of 632 AEs occurred in 91 (87.5%) patients receiving mipomersen 200 mg once weekly, 554 events in 87 (85.3%) patients receiving mipomersen 70 mg thrice weekly, and 459 events in 81 (78.6%) patients on placebo (Table 3). Flu-like symptoms were the most frequently reported AE, with an incidence of 42.3% in the mipomersen 200 mg group, 25.5% in the mipomersen 70 mg group, and 19.4% in the pooled placebo group. The most frequent injection-site reactions were erythema, pain, swelling, and pruritus and were more frequent in patients receiving active treatment than placebo (Table 3). The occurrence of flu-like symptoms or injection-site reactions led to discontinuation in 22 of 104 (21.2%) patients on mipomersen 200 mg once weekly, compared to 18 of 103 (17.6%) subjects in the 70 mg thrice-weekly group, and 7 of 102 (6.8%) subjects in the pooled placebo group.

A total of 41 SAEs occurred among all treatment arms. The number of MACE was low in the three treatment groups: 6 subjects out of 103 on placebo (5.8%), 8 subjects out of 102 (7.8%) on mipomersen 70 mg thrice weekly, and 0 out of 104 subjects on mipomersen 200 mg once weekly had one or multiple MACE (Table 3). Five subjects in the placebo group and one subject on mipomersen 200 mg developed neoplasms during the blinded treatment period; the latter subject reported a hemangioma. Two subjects died during the blinded treatment period; one of a lymphoma (receiving placebo once weekly) and one of an unknown cause (receiving mipomersen 200 mg once weekly).

Hepatobiliary disorders included cholelithiasis (1 placebo, 2 mipomersen 200 mg, 1 mipomersen 70 mg), chronic cholecystitis (1 placebo), gallbladder polyp (1 mipomersen 200 mg), hepatomegaly (1 mipomersen 200 mg), hyperbilirubinemia (1 placebo), and jaundice (1 placebo). Hepatic steatosis was diagnosed on serial magnetic resonance imaging in 10 (9.6%) patients receiving mipomersen 200 mg once weekly, in 9 (8.8%) patients receiving mipomersen 70 mg thrice weekly, and in 2 (1.9%) patients receiving placebo.

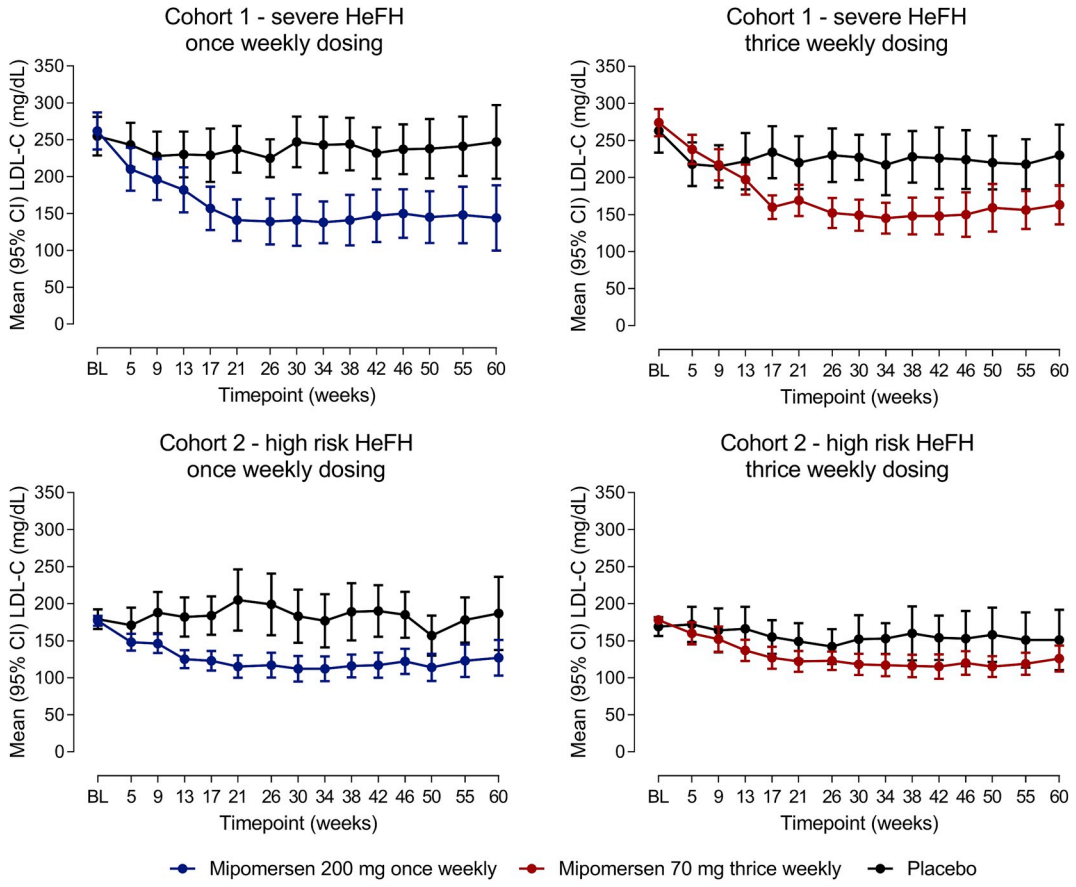

Fig. 2. Mean LDL-C over time per cohort and mipomersen regimen.
Mean LDL-C levels over time per cohort and per treatment regimen. Error bars represent 95% confidence interval.

3.4. Laboratory assessments

Mean platelet levels remained stable during the study period in all patient groups. A platelet count of < 100,000 occurred in 1 patient receiving mipomersen 200 mg and in 1 patient receiving mipomersen 70 mg. Another subject in the mipomersen 200 mg group had a platelet count of < 50,000 during the study treatment period.

Hepatic transaminase elevations were more common in subjects receiving mipomersen 200 mg and mipomersen 70 mg than in those receiving placebo. Maximum ALT levels of ≥3 × ULN at least once were seen in 22 patients in both mipomersen groups (21.2% and 21.6%, respectively) compared to 1 (1.0%) in the placebo group. Maximum AST levels reached ≥3 × ULN at least once in 13 patients (12.5%) on the mipomersen 200 mg regimen and 9 patients (8.8%) on the

Table 2
Change in key lipids from baseline to primary efficacy time point.

	Once weekly dosing					
	Mipomersen (n = 104)		**Placebo** (n = 51)		**Difference mipomersen *vs.* placebo**	
Lipid	Baseline	Mean % change	Baseline	Mean % change	Difference % change	*p*-value
LDL-C	232 (93.1)	−29.06 (4.358)	229 (72.3)	−8.10 (5.278)	−20.96 (5.117)	< 0.001
ApoB	157 (47.0)	−27.34 (3.834)	158 (34.6)	−5.23 (4.700)	−22.11 (4.685)	< 0.001
Lp(a)	32 (12, 64)	−22.50 (5.899)	30 (13, 70)	−17.83 (7.445)	−4.67 (7.988)	0.560
	Thrice weekly dosing					
	Mipomersen (n = 102)		**Placebo** (n = 52)		**Difference mipomersen *vs.* placebo**	
LDL-C	240 (76.7)	−27.49 (4.263)	229 (81.4)	−8.69 (4.765)	−18.80 (5.104)	< 0.001
ApoB	165 (38.7)	−25.78 (3.736)	157 (44.3)	−4.07 (4.283)	−21.71 (4.705)	< 0.001
Lp(a)	29 (12, 66)	−27.79 (5.421)	25 (9, 73)	−0.14 (6.543)	−27.65 (7.586)	< 0.001

Efficacy of mipomersen 200 mg once weekly subcutaneously (SQ) and mipomersen 70 mg thrice weekly SQ on LDL-C, apoB, and Lp(a) after a 60-week blinded treatment period for all subjects enrolled in this trial. Baseline values are in mg/dL and expressed as mean (SD), except for Lp(a) which is expressed as median (IQR). Mean % (SE) change between baseline and PET and Difference % change (SE) between mipomersen and placebo are calculated with a mixed model for repeated measurements with terms for baseline value, geopgraphic region, gender, statin use, treatment group, study visit, and study visit by treatment group interaction. Including patients who at least received one treatment dose but discontinued treatment early.
LDL-C = low density lipoprotein cholesterol, apoB = apolipoprotein B, Lp(a) = lipoprotein (a), SD = standard deviation, IQR = interquartile range, SE = standard error.

Table 3
Adverse events and discontinuation during blinded treatment period.

	Mipomersen 200 mg once weekly (n = 104)	Mipomersen 70 mg thrice weekly (n = 102)	Pooled placebo (n = 103)
Patients with AEs, n (%)	91 (87.5)	87 (85.3)	81 (78.6)
Serious AEs, n (%)	9 (8.7)	17 (16.7)	15 (14.6)
AEs leading to death, n (%)	1 (1.0)	0	1 (0.9)
Discontinuations due to AEs, n (%)	37 (35.6)	24 (23.5)	13 (12.6)
Most common AEs (> 5%), n (%)			
Flu like symptoms	44 (42.3)	26 (25.5)	20 (19.4)
Injection site erythema	8 (7.7)	13 (12.7)	2 (1.9)
Injection site pain	13 (12.5)	7 (6.9)	1 (1.0)
Injection site swelling	4 (3.8)	8 (7.8)	2 (1.9)
Injection site pruritis	3 (2.9)	7 (6.9)	2 (1.9)
Nasopharyngitis	7 (6.7)	25 (8.1)	11 (10.7)
Influenza	5 (4.8)	9 (8.8)	8 (7.8)
Upper respiratory tract infection	2 (1.9)	7 (6.9)	6 (5.8)
Bronchitis	5 (4.8)	3 (2.9)	6 (5.8)
Nausea	9 (8.7)	7 (6.9)	5 (4.9)
Lower abdominal pain	0	0	4 (3.9)
Myalgia	8 (7.7)	5 (4.9)	3 (2.9)
Pain in extremity	5 (4.8)	3 (2.9)	3 (2.9)
Headache	2 (3.9)	9 (8.8)	12 (15.5)
Dizziness	8 (7.7)	3 (2.9)	2 (1.9)
Cough	5 (4.8)	5 (4.9)	3 (2.9)
Hypertension	3 (2.9)	4 (3.9)	8 (7.8)
Hepatic steatosis	10 (9.6)	9 (8.8)	2 (1.9)
Injection site reactions or flu like symptoms leading to discontinuation	22 (21.2)	18 (17.6)	6 (5.8)
MACE events, any n (%)			
CV death	0	0	0
(Acute) myocardial infarction	0	3 (2.9)	2 (1.9)
Ischaemic stroke	0	1 (1.0)	0
Unstable angina	0	4 (3.9)	4 (3.9)

Overview of adverse events during the 60-week blinded treatment period for all participants. The pooled placebo group consists of patients receiving placebo once weekly and thrice weekly.
AE = adverse event, MACE = major adverse cardiac event, CV death = cardiovascular death.

mipomersen 70 mg regimen (Table 4) compared to 2 (1.9%) in the placebo group. Two consecutive ALT levels of ≥3 × ULN, at least 7 days apart, occurred in 21 (20.2%) patients on mipomersen 200 mg and 17 (16.7%) patients on mipomersen 70 mg compared to 0 patients in the placebo group. AST levels of ≥3 × ULN at two consecutive measurements was seen in 7 (6.7%), 3 (2.9%), and 0 patients in the respective groups. No cases of hepatic injury defined by Hy's Law were observed (elevated ALT levels [≥3 × ULN] in combination with elevated total bilirubin levels [defined as ≥1.5 × ULN]). Following the predetermined liver chemistry stopping rules, elevated liver enzymes led to adjusting of study medication doses in a total of 28 patients, 15 on mipomersen 200 mg and 13 on mipomersen 70 mg. Mipomersen 200 mg was temporarily stopped in 12 patients and permanently stopped in 4 patients because of liver rule events. Mipomersen 70 mg was temporarily stopped in 5 patients and permanently in 2 patients.

4. Discussion

The FOCUS-FH study is the largest and longest randomized, placebo-controlled trial with mipomersen in FH patients to date. Two different regimens of mipomersen SQ (200 mg once weekly and 70 mg thrice weekly) and placebo were tested for 60 weeks in 309 patients with severe HeFH or with HeFH and a history of CVD. Mipomersen 200 mg once weekly and mipomersen 70 mg thrice weekly both produced significant LDL-C and apoB lowering of around 20% on top of maximally tolerated standard lipid-lowering regimens in both cohorts combined. Lp(a) was significantly lowered only with the thrice-weekly dosage for unclear reasons. The lipid-lowering effects of the two

Table 4
Liver function test abnormalities.

	Mipomersen 200 mg once weekly (n = 104)	Mipomersen 70 mg thrice weekly (n = 102)	Pooled placebo (n = 103)
ALT ≥3× ULN, n (%)	22 (21.2)	22 (21.6)	1 (1.0)
ALT ≥5× ULN, n (%)	6 (5.8)	3 (2.9)	0
ALT ≥8× ULN, n (%)	1 (1.0)	0	0
ALT ≥3 × ULN, two consecutive results (≥7 days apart), n (%)	21 (20.2)	17 (16.7)	0
AST ≥3× ULN, n (%)	13 (12.5)	9 (8.8)	2 (1.9)
AST ≥5× ULN, n (%)	2 (1.9)	1 (1.0)	1 (1.0)
AST ≥8× ULN, n (%)	1 (1.0)	1 (1.0)	0
AST ≥3 × ULN, two consecutive results (≥7 days apart), n (%)	7 (6.7)	3 (2.9)	0

Liver function tests during the blinded treatment period of all enrolled participants. The pooled placebo group consists of patients receiving placebo once weekly and thrice weekly. Numbers depict participants with at least one elevation of transaminases above a certain threshold (e.g., ≥3× ULN). ALT = alanine transaminase, AST = aspartate transaminase, ULN = upper limit of normal.

regimens per cohort were heterogeneous. The efficacy of mipomersen 200 mg once weekly in lowering multiple lipid parameters was consistent in severe and high-risk HeFH patients and ranged from 15% to 30% reductions. However, mipomersen 70 mg did not show significant LDL-C–lowering effects in severe HeFH, but did in high-risk HeFH for unclear reasons. In general, active treatment with mipomersen was less well tolerated than placebo. As previously described [15], the most common AEs were injection-site reactions and flu like symptoms, of which the latter was less frequently present in the mipomersen 70 mg treatment arm. Elevated liver enzymes were more frequently observed in both active treatment arms compared to placebo.

Previous research has shown that every decrease of 1 mmol/L (39 mg/dL) in LDL-C levels results in a 19% reduction in coronary mortality as well as a 22% reduction in MACE [17]. This in combination with the fact that many FH patients do not reach their LDL-C guideline-recommended goals with standard lipid-lowering therapies clearly shows the need for additional or alternative LDL-C–lowering therapies for FH patients. Mipomersen may fulfil this role, as the 200 mg dose was an effective LDL-C–lowering agent in this study and others [11,12,15]. However, this might not be the case for mipomersen 70 mg thrice weekly because of its inconsistent effect on LDL-C lowering in the current study, although it is possible that noncompliance related to the higher frequency of injections may have interfered with LDL-lowering efficacy.

Since significant Lp(a) lowering during treatment with mipomersen 200 mg weekly has been documented in previous clinical trials, it is surprising that Lp(a) was lowered significantly only in subjects treated with mipomersen 70 mg thrice weekly and not in those treated with mipomersen 200 mg once weekly. The discrepancy in efficacy between the two regimens might be explained by a lack of statistical power in the current study and a wide range in Lp(a) levels within the included subjects. In contrast, a pooled analysis of four randomized clinical trials has shown a 26.4% (95% confidence interval −42.8, −5.4) decrease in Lp(a) levels by mipomersen 200 mg once weekly vs. placebo [20]. Lp(a) has been shown to be an independent risk factor for the development of CVD [21], and until recently, few therapeutic options for lowering plasma levels of Lp(a) were available. The potential Lp(a)-lowering efficacy of mipomersen adds to effective treatment options for high Lp (a) levels and could theoretically contribute to lowering CVD risk in these vulnerable patients.

Despite the efficacy of mipomersen on atherogenic lipid levels, the benefits are tempered by lower tolerability compared with placebo. One concern is the increased occurrence of hepatic steatosis and elevated liver enzyme levels during mipomersen treatment, which are mechanism-based side effects. Elevated liver enzymes can be attenuated by reducing the mipomersen dose, reducing dosing frequency, or temporarily stopping mipomersen, as documented in this study and others [12,15,19]. However, 6 out of 205 participants stopped permanently because of increased liver enzymes in the current study in accordance with prespecified liver safety rules. While the frequency of ALT and AST elevation was not different between the two mipomersen dosing regimens, temporary and permanent discontinuations of treatment occurred less frequently in subjects treated with mipomersen 70 mg thrice weekly compared to 200 mg weekly. Whether increased ALT/AST levels and hepatic steatosis are a major problem for patients treated with mipomersen for longer periods remains to be elucidated, although data from previous studies suggested that the level of hepatic steatosis and occurrence of transaminase elevations stabilized after the first year during up to 4 years of follow-up [15,22]. Despite the relatively higher risk of steatosis in patients on mipomersen [23], mipomersen has not been associated with fibrosis in the small number of subjects studied with liver biopsy [14,15,24]. Elevated AST and ALT levels have been shown to stabilize over time and trend towards baseline in a long-term follow-up study after 2 years of treatment with mipomersen 200 mg once weekly [15].

Approximately 42% of the mipomersen-treated participants, compared with 20% of the placebo group, discontinued treatment independently of dose frequency before the end of the blinded treatment period at 60 weeks. Most discontinuations were due to adverse events. Interim results of an open-label extension study have shown that 55% of patients treated with mipomersen discontinued treatment within the first 2 years [15]. Hence, one might conclude that the majority of discontinuations occur within the first year of treatment. Injection-site reactions and flu-like symptoms were the main reasons for patients to discontinue treatment. In the current study, approximately 50% of discontinuations of treatment were attributed to these AEs. The mipomersen 70 mg thrice weekly regimen led to fewer discontinuations because of flu-like symptoms compared with the 200 mg once weekly regimen. In contrast, discontinuations due to injection-site reactions were higher in the mipomersen 70 mg thrice-weekly regimen compared with the 200 mg once-weekly regimen, possibly as a consequence of the 3-fold higher number of injections. The use of newer formulations of antisense oligonucleotides or short-interfering RNAs (siRNAs) specifically targeting the liver may attenuate injection-site reactions and flu-like symptoms and could thus improve tolerability. However, because of mipomersen's mode of action, targeting apoB production, elevated liver enzymes and hepatic fat accumulation would remain a concern. Mipomersen is currently not available for clinical use.

This study was sufficiently powered to draw conclusions about the efficacy and safety of mipomersen in HeFH patients in regards to the effects on lipids and lipoproteins. Its strengths are the relatively long blinded-treatment period of 60 weeks and the large number of FH subjects. However, some potential limitations of this study are present. First, the current results are mostly applicable to Caucasians, who comprised the majority of subjects in this study (80–90%, Table 1). Further research is needed to see if the effects of mipomersen in other ethnic groups with HeFH are the same. Second, it is unknown what proportion of subjects maintained blinded treatment during the study. Despite the double-blinded study design, a 1-year duration of blinded lipid profile testing is very long among subjects with very high CHD risk. For this reason, it is possible that subjects may have inadvertently had surreptitious lipid profile testing performed through their primary care provider. In this situation, the mean on-treatment LDL-C concentration of roughly 200 mg/dL during the study could have contributed to premature study discontinuation from AEs. Third, since PCSK9 inhibitors are currently more frequently prescribed than mipomersen, it is uncertain what the effect of mipomersen will be when coadministered with PCSK9 inhibitors. One could speculate that there could still be a sufficient additional LDL-C–lowering effect of mipomersen, since it is believed to reduce production of apoB-containing lipoproteins, in contrast to drugs targeting increased clearance through up-regulation of LDL receptors (e.g., statins and PCSK9 inhibitors). Fourth, this study was not designed or powered to show benefit of treatment with mipomersen on cardiovascular outcomes. Therefore, longer and larger studies are needed to see if treatment with mipomersen reduces CVD risk and mortality, although the results of a recent prospective before-and-after analysis suggested that mipomersen may reduce the incidence of MACE during 2 years of treatment compared to 2 years prior to study entry [25]. Lastly, long-term follow-up studies are needed to evaluate mipomersen's long-term adverse effects, especially hepatic steatosis, and its long-term clinical course.

In conclusion, mipomersen 200 mg SQ once weekly and 70 mg SQ thrice weekly showed similar LDL-C–lowering effects; however, more consistent and significant efficacy was seen with the standard FDA-approved once-weekly dosage. Mipomersen is currently approved only for patients with HoFH; however, this and other studies demonstrate the LDL-C–lowering efficacy of mipomersen in patients with HeFH and persistent LDL-C elevation. The benefit of mipomersen is counterbalanced by limited tolerability in some subjects and the requirement for hepatic monitoring for transaminase elevations. In this light, a thrice-weekly regimen with mipomersen 70 mg might provide minor benefits, such as a lower prevalence of flu-like symptoms and slightly

lower requirement for dosage adjustments due to elevated liver enzymes, although more injection-site reactions were seen with this regimen.

Conflicts of interest

Laurens F. Reeskamp has nothing to disclose.

John J.P. Kastelein was a consultant to Ionis Pharmaceuticals during the FOCUS-FH study and his institution received the research grants for the clinical study.

Patrick M. Moriarty reports: Regeneron: Speaker Fee—Consultant, Speaker; Sanofi: Speaker Fee—Speaker Bureau, Consultant; Amgen: Speaker Fee—Speaker, Consultant; Duke: Consultant Fee—Consultant; Esperion: Consultant Fee—Consultant, Executive Committee; Eliaz Therapeutics: Stock Options—Advisory Board; Kaneka: Consult Fee—Consultant; RegenXBio: Consulting—Consultant; Kastle Therapeutics: Consulting—Consultant; Amarin: Speaker Fee—Speaker; Gemphire Therapeutics: Consulting—Consultant; Aegerion: Advisory Board; Stage II Innovations: Consulting—Consultant; Ambry Genetics: Speaker Fee—Speaker; Novartis: Consulting—Consultant; Regeneron, Sanofi, Amgen, Ionis, Pfizer, Novartis, Kaneka, Stage 2 Innovations, University of Penn, Zydus Discovery, Gemphire, Kowa, Akcea, FH Foundation: Grant Research Support—Research.

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Author Contributions

Reeskamp: data analysis, interpreting results, and writing manuscript.

Kastelein: interpreting results, study design, and revising manuscript.

Moriarty: interpreting results, study design, and revising manuscript.

Duell: interpreting results, study design, and revising manuscript.

Catapano: interpreting results, study design, and revising manuscript.

Santos: interpreting results, study design, and revising manuscript.

Ballantyne: interpreting results, study design, and revising manuscript.

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Appendix A. Supplementary data

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