

Changes in Mipomersen Dosing Regimen Provide Similar Exposure With Improved Tolerability in Randomized Placebo-Controlled Study of Healthy Volunteers

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Background—Mipomersen, an apolipoprotein B synthesis inhibitor, demonstrated significant reductions in low-density lipoprotein (LDL) cholesterol, non-high density lipoprotein cholesterol, and apolipoprotein B in 4 phase 3 studies at the FDA-approved subcutaneous dose of 200 mg once weekly.

Methods and Results—A short-term phase 1 study in healthy volunteers was conducted to evaluate the relative bioavailability, safety, and tolerability of mipomersen in 2 test dose regimens in reference to the 200 mg weekly dose regimen. Eighty-four adults were randomized to 1 of 3 cohorts (30 mg once daily, 70 mg 3 times weekly, or 200 mg once weekly) and then mipomersen or placebo (3:1 ratio) for 3 weeks of treatment. Comparable mipomersen post-distribution phase plasma concentrations were observed across the 3 dose regimens suggesting similar tissue exposure. Injection site reactions were reported, but did not lead to treatment discontinuation. The median incidence of these responses per injection was decreased by lowering the dose. Signals from a diverse panel of systemic inflammation markers were essentially indistinguishable between dose regimens and placebo treatment. The one exception was a modest transient post-dose elevation of C-reactive protein (CRP) in the mipomersen 200 mg weekly group. This elevation was not associated with an increase in other proinflammatory markers.

Conclusions—This study demonstrated a similar drug exposure and overall safety profile between the 3 dosing regimens. Exploratory assessment of a diverse panel of biomarkers found no indication of a systemic inflammatory response to mipomersen treatment. These results support assessment of alternative dose regimens in longer-term studies.

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Key Words: dosing • inhibitor • pharmacokinetics • randomized controlled trial • safety

Mipomersen is an apolipoprotein B (apoB) synthesis inhibitor approved for use as an adjunct to maximum tolerated lipid-lowering therapy in patients with homozygous familial hypercholesterolemia (FH).¹ In support of this indication, mipomersen has demonstrated significant reductions in low-density lipoprotein (LDL) cholesterol, non-high-density

lipoprotein (non-HDL) cholesterol, apoB, and lipoprotein(a) (Lp(a)) in 4 randomized placebo-controlled phase 3 studies involving patients with homozygous FH,² heterozygous FH,^{3,4} and patients with high cholesterol who are at high risk for cardiovascular events, including those with type 2 diabetes mellitus.⁵ In these studies mipomersen was administered by subcutaneous (SC) injection for 26 weeks at the FDA-approved dosage of 200 mg once weekly. Injection site reactions (ISR) and flu-like symptoms (FLS) were the most common adverse events, and in some cases resulted in discontinuation from treatment. In earlier phase 1 and 2 studies these symptoms appeared to be dose-dependent and associated with the SC injection.^{6–8} Pharmacokinetic (PK) modeling predicts that more frequent administration of mipomersen at lower doses should result in lower peak plasma concentration but similar post-distribution plasma concentration—the latter a proven surrogate for tissue exposure.^{9,10} Based on this premise, more frequent administration of

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mipomersen at lower doses may lead to attenuation of drug-related side effects while maintaining efficacy.

A primary aim of this short-term phase 1 study in healthy volunteers was to determine if 2 alternative dose regimens (70 mg 3 times a week [TIW], or 30 mg once daily [QD]) produce comparable post-distribution phase concentrations relative to the 200 mg once weekly (QW) regimen, and to evaluate the safety and tolerability profile of each dosing regimen. Mipomersen post-distribution plasma concentration, and not peak or total plasma exposure measures, is the best predictor of target tissue liver exposure and respective pharmacology upon repeat dose administration.^{10,11} Relative bioavailability results were evaluated using an average bioequivalence approach with observed and dose-normalized PK exposure measures.

Safety data included adverse event (AE) profiles, ISR data, routine laboratory tests and a panel of 10 biomarkers to determine if there is a systemic inflammatory response to SC dosing with mipomersen and/or placebo under the different dosing regimens. Biomarkers were selected on the basis of relevance to putative proatherogenic inflammatory processes,^{12–14} findings from preclinical animal model studies of mipomersen and other antisense drugs,^{15,16} association with proinflammatory signal transduction pathways or systemic immune responses,^{17–20} and findings from other drugs administered by SC injection.²¹ This study is the first to systematically examine the effects of mipomersen treatment on a diverse panel of proinflammatory and immune modulation biomarkers.

Methods

Study Design

The objectives of this study were to evaluate the relative bioavailability, pharmacokinetics (PK), safety, and tolerability of SC dosing with different regimens of mipomersen in healthy volunteers. A randomized, double-blind, placebo-controlled, parallel-group phase 1 study involving 84 healthy adult volunteers was conducted at a single site (Anapharm in Montreal, Quebec, Canada) from December 2009 to June 2010. The study consisted of a 6-week screening period, a 3-week treatment period, and a 12-week safety follow-up period (Figure 1A). Study subjects were randomized equally among 3 dose cohorts (A, B, or C) and then further randomized at a ratio of 3:1 mipomersen to placebo within each cohort. A blocked randomization list was prepared by Isis Pharmaceuticals, Inc. All subjects, monitors, study center personnel, and the Sponsor were blinded during the course of the study—the exception to this was the pharmacist who allocated the study drug. The study was approved by an independent institutional review board (Institutional Review

Board Services, Ontario, Canada); and conducted in compliance with the International Conference on Harmonisation (ICH), Good Clinical Practice (GCP) guidelines, and all national, state, and local laws of the appropriate regulatory authorities.

Study Participants

Male and nonpregnant, nonlactating female subjects between the ages of 18 and 75 years and in good health with a body weight >50 kg, body mass index (BMI) <32 kg/m², and a skin type I-III based on the Fitzpatrick scale were eligible for this study. Subjects with any clinically significant abnormality in medical history, physical examination findings, electrocardiogram (ECG), vital signs, or laboratory tests were excluded. All subjects gave informed consent to participate in the study.

Dosing and Treatments

Mipomersen (200 mg/mL) or matching volume placebo was administered by SC injection. Injections were rotated among the upper arm, the thigh, and the abdomen. All injections were administered at the phase 1 unit by a health professional or trained personnel. Subjects in Cohort A received 30 mg mipomersen QD (Day 1 to 21; 630 mg total) or placebo; subjects in Cohort B received 70 mg mipomersen TIW (Day 1, 3, 5, 8, 10, 12, 15, 17, and 19; 630 mg total) or placebo; and subjects in Cohort C received 200 mg mipomersen QW (Day 1, 8, and 15; 600 mg total) or placebo. Study drug was supplied as a 1-mL solution of 200 mg mipomersen by Isis Pharmaceuticals, or placebo (0.9% saline plus 0.004 mg riboflavin supplied by Pyramid Laboratories Inc), in a 2-mL stoppered glass vial.

PK Sampling and Analysis

Plasma samples were drawn at serial time points following the first and last dose of mipomersen and during the post-dose follow-up period. For Cohort A, plasma samples were collected on Day 1 (pre-dose and 1, 2, 3, 4, 6, 8, and 12 hours post-dose), Day 2 (≈24 hours post first dose), Day 21 (pre-dose and 1, 2, 3, 4, 6, 8, and 12 hours post last dose), Day 22 (≈24 hours post last dose), Days 23, 28, 35, 49, 77, and 105. For Cohort B, plasma samples were collected on Day 1 (pre-dose and 1, 2, 3, 4, 6, 8, and 12 hours post-dose), Day 2 (≈24 hours post first dose), Day 19 (pre-dose and 1, 2, 3, 4, 6, 8, and 12 hours post last dose), Day 20 (≈24 hours post last dose), Days 21, 26, 28, 35, 49, 77, and 105. For Cohort C, plasma samples were collected on Day 1 (pre-dose and 1, 2, 3, 4, 6, 8, and 12 hours post-dose), Day 2 (≈24 hours post first dose), Day 15 (pre-dose and 1, 2, 3, 4, 6, 8, and 12 hours post last dose), Day 16 (≈24 hours post last dose), Days 17, 22, 28, 35, 49, 77, and 105.

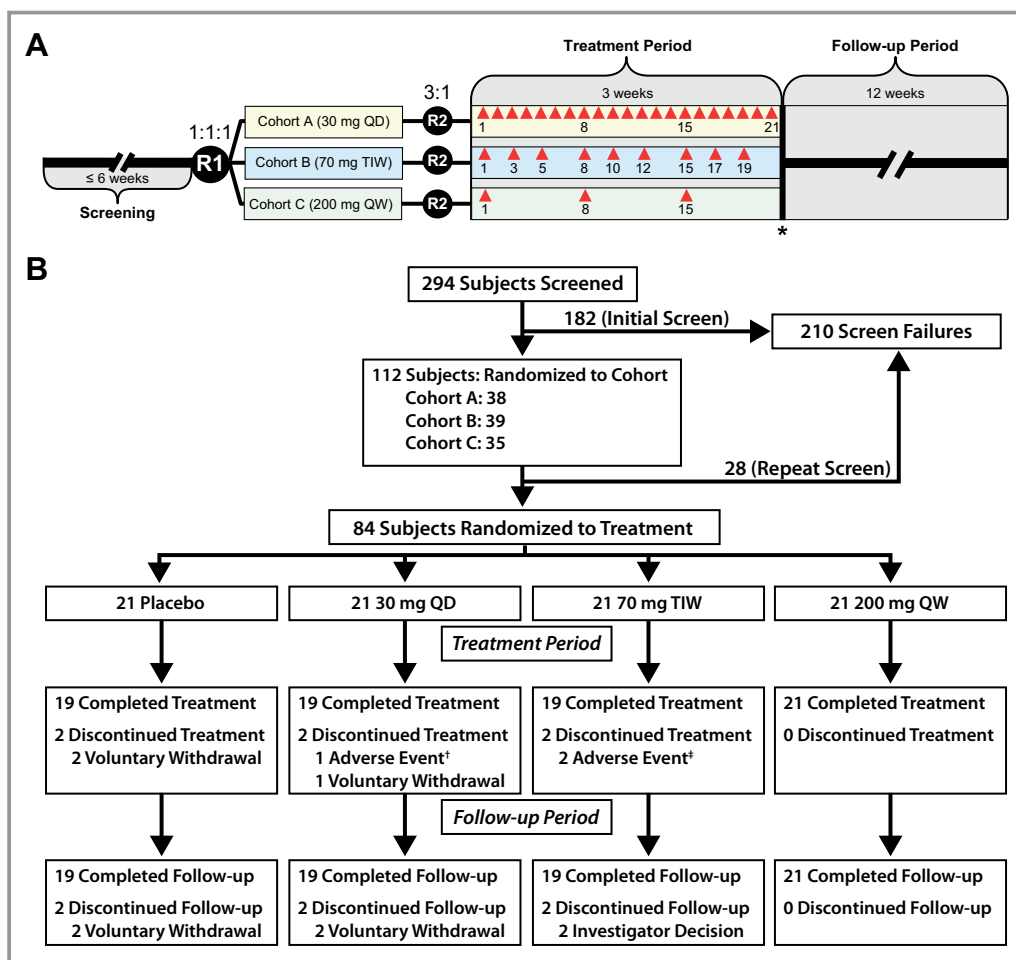


Figure 1. Evaluation of mipomersen in alternative dose regimens. Study design (A) and flow of participants (B) through the study. In the study design diagram, R denotes randomization, ratios are indicated above by cohort (R1) and active to placebo (R2), and the asterisk (*) indicates the time point for exploratory efficacy measurements, Day 28. [†]One subject discontinued dosing in the 30 mg group for asthenia and atrial flutter—both events were considered unlikely related to study drug. [‡]Two subjects discontinued dosing in the 70 mg group: one subject for gastroesophageal reflux disease, this event was considered possibly related to study drug; and one subject for ALT and AST $\geq 3 \times$ ULN, muscle tightness, increase in lactate dehydrogenase and creatine kinase, these events were considered unrelated to study drug. ALT indicates alanine transaminase; AST, aspartate transaminase; QD, once daily; QW, once weekly; TIW, 3 times a week; ULN, upper limit of normal.

Plasma drug concentrations were determined using a validated hybridization-based enzyme-linked immunosorbent assay (PPD, LLC).²² The lower limit of quantification was 0.228 ng/mL. Non-compartmental PK analysis of mipomersen was carried out on each individual subject data set using WinNonlin Professional Version 5.2 or higher (Pharsight Corp). Calculated PK parameters included maximum observed plasma concentration (C_{max}), time to maximum observed plasma concentration (T_{max}), and area under the plasma concentration time curve from 0 to 24 hours ($AUC_{0-24\ h}$) following the first and last dose of study drug. Plasma concentrations at Day 28 ($C_{Day\ 28}$) and at 7 days from last dose ($C_{7\ days\ from\ last\ dose}$) were also determined, as well as the apparent terminal elimination half-life ($t_{1/2\lambda z}$) following the last dose.

Safety Evaluation

The safety variables included AEs, physical examination findings, ECG results, vital signs, and routine laboratory tests. Dosing was discontinued if a subject met one of the following protocol-specified criteria: ALT or AST $\geq 8 \times$ ULN; ALT or AST $\geq 5 \times$ ULN at 2 consecutive weekly measurements (≥ 7 days); or ALT or AST $\geq 3 \times$ ULN with total bilirubin $> 1.5 \times$ ULN or international normalized ratio (INR) > 1.5 .

Inflammation Markers

The selected panel of markers for assessment of systemic inflammation included interferons alpha and beta (IFN- α ,

IFN- β), interleukins -1 beta, -13, and -6 (IL-1 β , IL-13, IL-6), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1 alpha (MIP-1 α), C-reactive protein (CRP), complement split products (Bb and C5a). Interferons are induced at an early stage in infection by both DNA and RNA viruses.²³ Common side effects of interferons include fever, malaise, fatigue, and muscle pains.²⁴ IL-1 β , IL-6, and IL-13 are signals of inflammasome, toll-like receptor (TLR) and mast cell activation, respectively.^{17–19} MCP-1 and MIP-1 α are leukocyte chemoattractant factors.^{25,26} CRP is an acute phase protein which is produced in response to IL-6 and IL-1 β signal transduction.^{20,27} Complement split product Bb is generated in the first stage of the alternative complement pathway and C5a is generated in the end stage of all complement pathways.^{28,29} C5a also functions as a chemokine and activator of proinflammatory signals at sites of complement activation.

Blood samples for analysis of circulating inflammation markers were drawn at serial time points prior to and following the first and last dose for each cohort; and on Day 8, 9, 10, and 11 (all cohorts), and Day 15, 16, 17, and 18 (30 mg QD and 70 mg TIW cohorts). A high-sensitivity CRP assay was used to measure CRP concentrations by MedPace, Inc. Markers of complement activation (Bb and C5a) were measured by National Jewish Health, and cytokines and chemokines (IFN- α , IFN- β , IL-1 β , IL-6, IL-13, MCP-1, and MIP-1 α) by Aushon BioSystems, Inc. Limit of detection levels for the cytokine and chemokine markers were IFN- α =0.8, IFN- β =2.3, IL-1 β =0.4, IL-6=0.4, IL-13=0.4, MCP-1=19.5, and MIP-1 α =6.2 pg/mL.

Exploratory Efficacy Evaluation

Efficacy variables included LDL cholesterol and apoB. Fasting bloods samples were analyzed for cholesterol and triglycerides by enzyme-based colorimetric assays (Medpace, Inc). HDL cholesterol was isolated by dextran-sulfate precipitation. LDL cholesterol was calculated using the Friedewald formula. ApoB was measured by rate nephelometry. Percent change in lipid parameters from baseline to end-of-treatment was calculated and compared among the treatment groups. Baseline was defined as the average of the screening and Day 1 pre-dose measurements.

Statistical Analysis

PK, safety and exploratory efficacy parameters were assessed for all subjects who received at least 1 injection of study drug, ie, an intent-to-treat analysis. Subjects assigned to placebo were pooled for analyses of disposition, safety, and efficacy assessments. Plasma concentrations and PK parameters of mipomersen were summarized by treat-

ment group using descriptive statistics. Geometric mean ratios and 90% confidence intervals were determined to establish bioequivalence between dose regimens as outlined by the FDA.³⁰ ANOVA was applied to compare the change from baseline of lipid parameters in the mipomersen-treated groups relative to the pooled-placebo group. Limit of detection values were used in calculation of descriptive statistics for left-censored data obtained from the inflammation marker assays. Post-hoc analysis included calculation of the Pearson correlation coefficient to determine the relationship between changes in CRP and IL-6 levels. Analysis of data was performed using SAS v9.1 or higher software (SAS Institute, Inc). All statistical tests were 2-sided with a significance level of 0.05.

This was a phase 1 relative bioavailability study that was not designed or powered to definitively determine bioequivalence (when present) of the evaluated plasma exposure measures between the test and reference treatments. A sample size of 18 mipomersen-treated subjects in each parallel group dose cohort was estimated to provide at least 90% power to detect whether post-distribution plasma concentrations of a test regimen (30 mg QD or 70 mg TIW) were within 30% of the referent (200 mg QW) assuming a coefficient of variation of 30%, equivalence between regimens, and a significance level of 0.1 (2 sided).

Results

Subjects

Eighty-four subjects (22 females; 62 males) ranging in age from 19 to 70 years were randomized to 1 of 3 dose cohorts and then further randomized at a ratio of 3:1 mipomersen to placebo (Figure 1, Table 1). Seventy-eight of 84 (93%) subjects completed the treatment period. Three subjects discontinued dosing due to an AE (n=1, 30 mg QD; n=2, 70 mg TIW) and 3 discontinued dosing due to withdrawal of consent (n=1, 30 mg QD; n=2, placebo). Seventy-eight (93%) of 84 subjects completed the post-treatment follow-up period. The 6 subjects who discontinued the follow-up period were the same as those who discontinued treatment.

Pharmacokinetics

Following SC administration at doses of 30, 70, or 200 mg, mipomersen was absorbed rapidly into the systemic circulation, with maximum plasma concentrations typically observed 3 to 4 hours post dose (Figure 2A). After reaching peak levels, mean plasma concentrations of mipomersen declined with time in a multi-phasic fashion for all doses. Each plasma concentration profile was characterized by a relatively rapid initial distribution phase, followed by a very slow terminal

Table 1. Subject Demographics and Baseline Characteristics

	Placebo (n=21)	30 mg QD (n=21)	70 mg TIW (n=21)	200 mg QW (n=21)	Total (N=84)
Gender, M:F	14:7	18:3	14:7	16:5	62:22
Age, y					
Median	48	47	50	52	49
IQR	36, 58	39, 56	40, 60	43, 58	39, 58
Min, max	22, 70	28, 61	22, 69	19, 70	19, 70
BMI, kg/m ²					
Median	26.9	27.5	24.7	27.4	27.0
IQR	24.3, 28.8	25.9, 29.9	23.8, 28.6	24.8, 29.7	24.3, 29.3
Min, max	18.3, 31.9	22.7, 31.8	19.3, 31.4	20.5, 31.5	18.3, 31.9

BMI indicates body mass index; IQR, interquartile range; M:F, male:female; QD, once daily; QW, once weekly; TIW, 3 times a week.

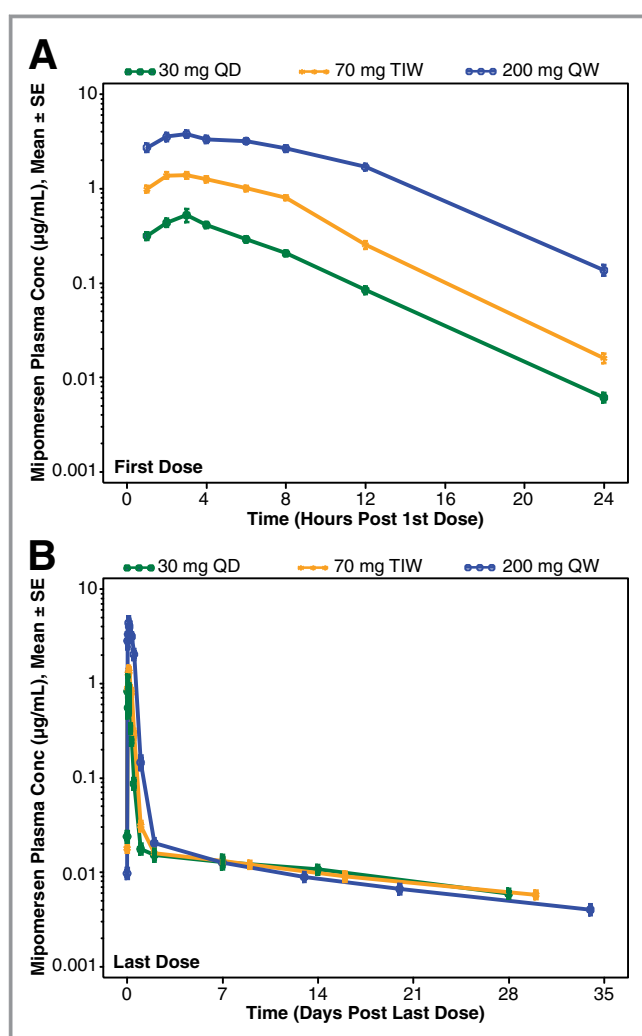


Figure 2. Mean mipomersen plasma concentrations over time by dose regimen. A, 0 to 24 hours after the first dose. B, 0 to 35 days after the last dose. QD indicates once daily; QW, once weekly; TIW, 3 times a week.

elimination phase 2 to 3 days post dose (Figure 2B). The mean apparent terminal elimination half-life values ranged from 32.6 to 49.8 days for the evaluated dose cohorts.

As expected, the geometric mean maximum observed plasma concentration (C_{max}) and area under the plasma concentration time curve from 0 to 24 hours ($AUC_{0-24\ h}$) values were dose-dependent following single and multiple SC doses of 30, 70, and 200 mg mipomersen (Table 2). These values were also similar following the first and last dose of each regimen, indicating little accumulation in peak and total plasma exposure measures and time-invariant kinetics. Based on the post-distribution phase concentrations, the relative bioavailability of the 30-mg QD and 70-mg TIW cohorts was similar to that of the reference 200-mg QW cohort (Table 2). This latter observation reflects a comparable total exposure to mipomersen across cohorts, is consistent with the post-distribution plasma concentrations being in equilibrium with tissue concentrations, and suggests similar tissue concentrations were achieved with each dosing regimen.

Safety and Tolerability

There were no serious adverse events in this short-term study comprised of a 3-week treatment period followed by a 12-week safety follow-up period. The overall safety profile of each dosing regimen was similar across mipomersen-treated groups. Three subjects discontinued dosing with mipomersen due to an adverse event (1 subject 30 mg QD; 2 subjects 70 mg TIW), with all except one AE considered unrelated to the study drug (Figure 1B). All AEs leading to dose discontinuation resolved. Injection site reactions (ISRs) were the most frequently reported adverse event across all treatment groups, including pooled-placebo (Table 3). These events were largely characterized by mild erythema. A stepwise reduction in the incidence, size, and duration of injection site erythema was observed at the lower mipomersen doses of

Table 2. Summary of Plasma Exposure Measures and Relative Bioavailability Statistics for Mipomersen

	Cohort A—30 mg QD (N=21)		Cohort B—70 mg TIW (N=21)		Cohort C—200 mg QW (N=21)	
	First Dose	Last Dose	First Dose	Last Dose	First Dose	Last Dose
Plasma exposure*						
C _{max} , µg/mL	0.51 (46.9)	0.65 (77.6)	1.53 (28.9)	1.43 (28.7)	3.82 (34.4)	4.44 (49.5)
AUC _{0-24 h} , µg·h/mL	3.69 (24.8)	4.64 (46.1)	11.9 (21.3)	12.0 (21.9)	42.2 (24.5)	48.5 (27.5)
C _{Day 28} , ng/mL	N/A	11.3 (54.8)	N/A	11.5 (32.6)	N/A	8.38 (38.8)
C _{7 days from last dose} , ng/mL	N/A	11.3 (54.8)	N/A	12.5 (35.1)	N/A	11.9 (36.9)
Dose-normalized plasma exposure*†						
C _{max} , µg/mL	0.0171 (46.9)	0.0218 (77.6)	0.0219 (28.9)	0.0205 (28.7)	0.0191 (34.4)	0.0222 (49.5)
AUC _{0-24 h} , µg·h/mL	0.123 (24.8)	0.155 (46.1)	0.171 (21.3)	0.172 (21.9)	0.211 (24.5)	0.242 (27.5)
C _{Day 28} , ng/mL	N/A	11.0 (50.1)	N/A	11.2 (30.7)	N/A	8.38 (38.8)
C _{7 days from last dose} , ng/mL	N/A	11.0 (50.1)	N/A	12.1 (33.2)	N/A	11.9 (36.9)
Relative bioavailability‡						
C _{max} , %	13.4 (11.2, 16.2)	14.7 (11.3, 19.2)	40.0 (33.2, 48.2)	32.3 (24.7, 42.2)	N/A	N/A
AUC _{0-24 h} , %	8.76 (7.77, 9.87)	9.58 (8.08, 11.4)	28.3 (25.1, 31.9)	24.9 (21.0, 29.5)	N/A	N/A
C _{Day 28} , %	N/A	135 (108, 167)	N/A	137 (110, 170)	N/A	N/A
C _{7 days from last dose} , %	N/A	94.7 (76.3, 118)	N/A	105 (84.4, 130)	N/A	N/A

AUC indicates area under the plasma concentration time curve; C, concentration; N/A, not applicable; PK, pharmacokinetic; QD, once daily; QW, once weekly; TIW, 3 times a week.

*Values represent the geometric mean (coefficient of variance%).

†C_{max} and AUC_{0-24 h} were dose normalized by dividing the untransformed value for each subject by the respective single dose amount (ie, 30, 70, or 200 mg). C_{Day 28} and C_{7 days from last dose} were dose normalized to a common total administered dose of 600 mg. Untransformed values for each subject were multiplied by the ratio of 600 mg/630 mg (0.95238) for Cohorts A and B, and by the ratio of 600 mg/600 mg (1.0) for Cohort C. Dose-normalized calculations were adjusted for those subjects who discontinued dosing early (n=2, 30 mg QD; n=2, 70 mg TIW).

‡Values represent the geometric mean ratio (90% confidence interval) as the percent. Geometric mean ratios are based on the observed PK parameter values of the test dosing regimen (Cohort A or B) relative to the reference dosing regimen (Cohort C, 200 mg QW).

Table 3. Treatment-Emergent Adverse Events (≥10% in Total Mipomersen)

Preferred MedDRA Term	Placebo (n=21)	Mipomersen		
		30 mg QD (n=21)	70 mg TIW (n=21)	200 mg QW (n=21)
Injection site reaction*	10 (50%)	21 (100%)	20 (95%)	21 (100%)
Contusion	5 (24%)	8 (38%)	5 (24%)	5 (24%)
Excoriation	1 (5%)	5 (24%)	3 (14%)	2 (10%)
C-reactive protein increase	1 (5%)	4 (19%)	2 (10%)	8 (38%)
Headache	3 (14%)	3 (14%)	5 (24%)	1 (5%)

Values represent the number of subjects who reported the adverse event on at least one occasion. The percent of the total number by treatment group is shown in parentheses.

QD indicates once daily; QW, once weekly; TIW, 3 times a week.

*Injection site reaction includes any one of the preferred Medical Dictionary for Regulatory Activities (MedDRA) terms for injection site: erythema, pain, swelling, hematoma, induration, warmth, discoloration, or pruritis.

30 and 70 mg compared with the 200 mg dose (Table 4). The incidence of flu-like symptoms, eg, fever, fatigue, or muscle aches, was low (<10% total mipomersen). Routine laboratory tests were unremarkable, including liver function tests. There were no clinically relevant differences between treatment groups with respect to hematology or coagulation parameters.

Transient post-dose elevations of CRP levels were observed in the 200 mg mipomersen QW group (Figure 3, Table 5). These elevations tended to decrease with continued dosing as indicated by a pre- to post-dose median change of +3.8 mg/L in week 1 and +2.3 mg/L in week 3. Transient increases in IL-6 levels also occurred across treatments, including placebo (Figure 3, Table 6). These increases were not associated with

Table 4. Incidence and Characteristics of Most Common Injection Site Reactions

	Cohort A—QD		Cohort B—TIW		Cohort C—QW	
	Placebo (n=7)	30 mg (n=21)	Placebo (n=7)	70 mg (n=21)	Placebo (n=7)	200 mg (n=21)
Erythema, % injections	0 (0, 4.8)	29 (14, 38)	0 (0, 0)	56 (33, 78)	0 (0, 0)	67 (33, 100)
Size \geq quarter,* % injections	0 (0, 0)	14 (5, 24)	0 (0, 0)	33 (22, 56)	0 (0, 0)	67 (33, 100)
Duration, days	0.0 (0.0, 2.0)	3.5 (2.6, 5.0)	0.0 (0.0, 0.0)	4.3 (3.0, 9.2)	0.0 (0.0, 0.0)	6.8 (2.5, 10.5)
Pain, % injections	0 (0, 5)	10 (0, 19)	0 (0, 0)	13 (0, 43)	0 (0, 33)	33 (0, 67)
Swelling, % injections	0.0 (0, 0)	5 (0, 18)	0 (0, 0)	22 (11, 44)	0 (0, 0)	33 (0, 67)

Values shown are the median (interquartile range). QD indicates once daily; QW, once weekly; TIW, 3 times a week.

*A quarter coin has a diameter of \approx 2.5 cm.

changes in CRP levels in subjects treated with mipomersen, overall ($n=63$, $r=0.004$, $P=0.978$) or in the 200 mg QW cohort ($n=21$, $r=0.022$, $P=0.925$) after the first dose, as well as the last dose. Changes from baseline levels of the type 1 interferons (IFN- α and IFN- β , Figure 4A), chemokines (MIP-1 α and MCP-1, Figure 4B), immune activation markers (IL-1 β and IL-13, Figure 4C), and complement split products (Bb and C5a, Figure 4D) were similar across dosing regimens and respective treatment groups over time, including placebo.

Exploratory Efficacy Evaluation

Mean baseline LDL cholesterol level of the study population was 3.1 mmol/L (120 mg/dL). The mean percent change in LDL cholesterol from baseline to end of treatment was -9.5% , -2.1% , and -18% for the mipomersen 30 mg QD, 70 mg TIW, and 200 mg QW groups, respectively, compared with -1.2% for the pooled-placebo group (Table 7). Parallel reductions from baseline were observed in apoB levels. Due to the long half-life of mipomersen, maximum lipid lowering would not be expected until after 3 to 6 months of treatment. Consequently, the effects observed are based on pre-steady state concentrations, which may impart additional variability.

Discussion

Similar post-distribution phase plasma exposures to mipomersen were confirmed for the alternative SC dosing regimens in this short-term placebo-controlled study involving healthy volunteers. These results suggest a comparable exposure between dosing regimens. Mipomersen was well tolerated. There were no dose discontinuations due to tolerability AEs. As expected, mild local ISRs were the most common AE experienced by mipomersen-treated subjects. A systematic examination of the effects of treatment on a diverse panel of proinflammatory and immune modulation biomarkers (IFN- α , IFN- β , IL-1 β , IL-6, IL-13, MCP-1, MIP-1 α , Bb, C5a, and CRP) found no evidence of a systemic inflammatory response to

mipomersen treatment. The transient post-dose increases in CRP levels, which discretely occurred in the mipomersen 200 mg QW group, were not associated with any other proinflammatory signals.

Consistent with estimations from PK modeling, similar post-distribution phase plasma concentrations of mipomersen were observed following the final mipomersen dose for the 3 different dosing regimens. In line with this observation, a reduction in LDL-C from baseline occurred by the end of treatment with mipomersen for each dosing regimen. These results collectively suggest that the 2 test dosing regimens (30 mg QD and 70 mg TIW) provide comparable tissue exposure relative to the reference regimen of mipomersen 200 mg QW. Maximum reductions in apoB-containing lipoproteins with these dose regimens are expected upon achievement of steady-state tissue concentrations after 3 to 6 months of treatment.^{2-5,11}

Local injection site reactions are a common side effect of SC injected drugs and can influence patient tolerability to treatment.³¹⁻³³ Tolerability is often reflected by the related rate of treatment discontinuation. In this regard, there were no dose discontinuations due to ISRs in the current study of mipomersen. This result may be due to the study design, particularly the short-term treatment period and small sample size. However, dose discontinuations due to ISRs were relatively infrequent in the placebo-controlled phase 3 studies where subjects received weekly SC doses of 200 mg mipomersen for 6 months.²⁻⁵ Assessment of ISRs by injection, rather than by dose discontinuation rate or by number of subjects with at least one event, provided a means to further explore the effects of dosing regimen in this study. This analysis indicated that ISRs may be dose dependent. As with all other studies that evaluate mipomersen, ISRs were typically characterized by self-limiting mild erythema. In this regard, within the bounds of the current study, the incidence, size, and duration of injection site erythema decreased by lowering the dose.

The sequence and structure of an oligonucleotide are key factors that determine both its propensity to elicit an immune

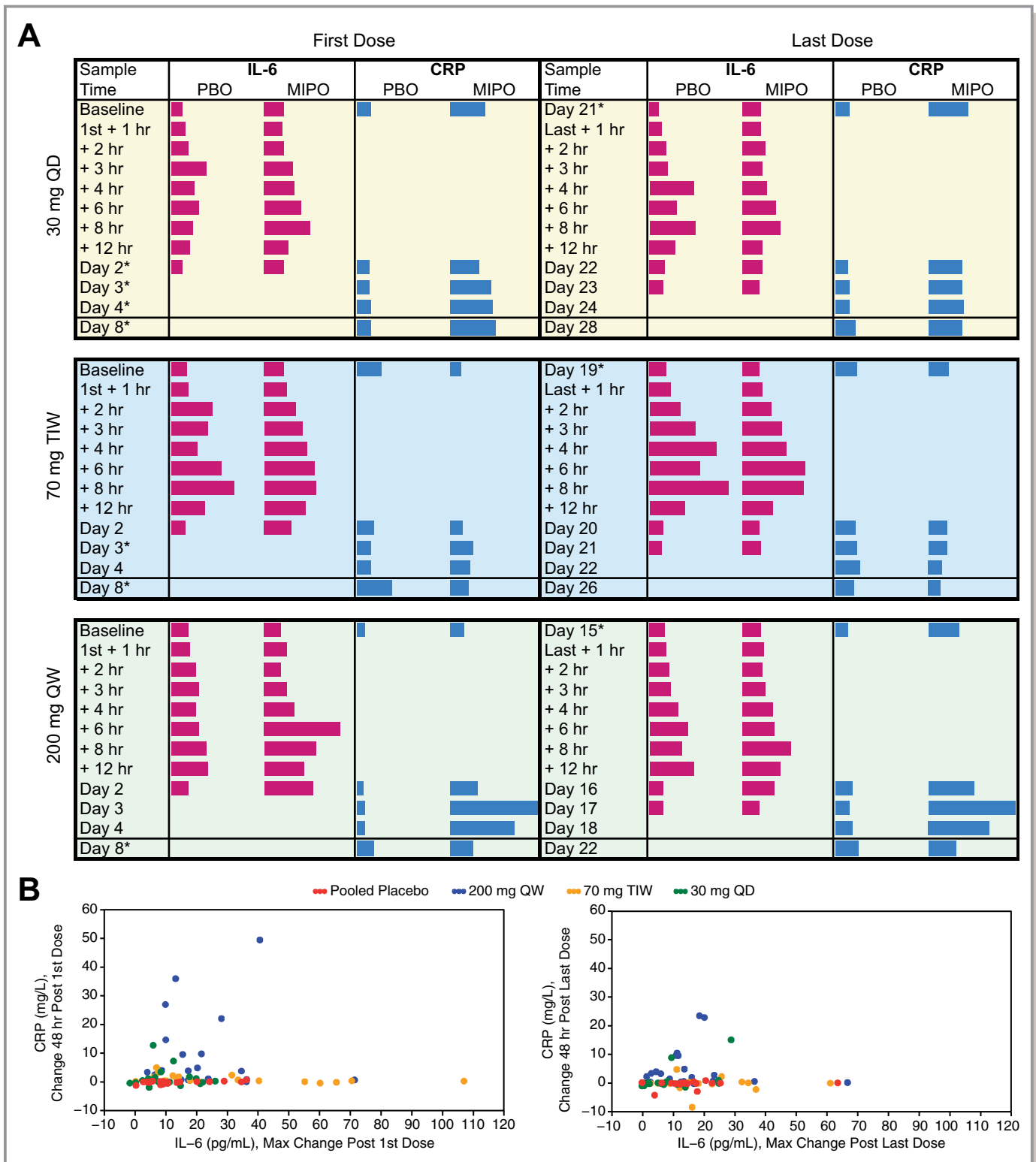


Figure 3. Effect of SC dosing regimen on serum IL-6 and CRP levels. A, Relative median levels after first and last dose-by-dose regimen cohort. B, Individual maximum post-dose changes after first and last dose by treatment groups. Median and interquartile range values are provided in Tables 5 and 6. *Dose day, samples collected pre-dose. CRP indicates C-reactive protein; IL, interleukin; MIPO, mipomersen; PBO, placebo; QD indicates once daily; QW, once weekly; SC, subcutaneous; TIW, 3 times a week.

Table 5. C-Reactive Protein Concentrations Over Time, Median (Interquartile Range)

CRP, mg/L	30 mg QD*		70 mg TIW [†]		200 mg QW [‡]	
	Placebo	Mipomersen	Placebo	Mipomersen	Placebo	Mipomersen
Baseline	0.7 (0.3, 1.1)	1.7 (1.0, 3.2)	1.2 (0.4, 1.7)	0.5 (0.3, 1.0)	0.4 (0.4, 0.9)	0.7 (0.4, 1.8)
Day 2	0.6 (0.2, 0.8)	1.4 (0.8, 2.8)	0.8 (0.4, 1.1)	0.6 (0.3, 1.1)	0.3 (0.2, 0.9)	1.3 (0.4, 2.3)
Day 3	0.6 (0.3, 0.9)	2.0 (1.2, 4.2)	0.7 (0.5, 1.9)	1.1 (0.7, 2.1)	0.4 (0.3, 0.8)	4.3 (1.9, 11.6)
Day 4	0.65 (0.3, 0.8)	2.1 (1.2, 3.5)	0.7 (0.5, 1.7)	1.0 (0.6, 2.25)	0.4 (0.3, 0.8)	3.1 (1.5, 7.65)
Day 8	0.7 (0.6, 0.7)	2.2 (0.9, 4.2)	1.65 (1.2, 2.6)	0.9 (0.6, 2.8)	0.8 (0.3, 0.9)	1.1 (0.4, 2.1)
Day 9	0.7 (0.6, 0.8)	2.6 (1.1, 3.5)	1.25 (1.0, 2.4)	0.9 (0.4, 2.5)	0.6 (0.3, 1.6)	1.5 (0.8, 3.2)
Day 10	0.65 (0.6, 0.9)	2.1 (1.2, 3.7)	1.55 (1.0, 1.6)	1.3 (0.6, 4.0)	0.5 (0.3, 0.9)	5.7 (1.6, 15.1)
Day 11	0.6 (0.4, 0.8)	2.0 (1.4, 4.1)	1.5 (1.1, 2.1)	1.2 (0.45, 2.9)	0.6 (0.3, 0.6)	2.0 (1.2, 11.0)
Day 15	0.9 (0.3, 1.7)	2.1 (1.1, 3.1)	1.05 (0.5, 1.6)	1.1 (0.5, 2.8)	0.6 (0.3, 0.9)	1.5 (1.0, 2.4)
Day 16	0.8 (0.2, 1.3)	2.0 (1.5, 3.4)	0.85 (0.3, 1.4)	0.9 (0.4, 2.6)	0.8 (0.7, 1.6)	2.25 (1.0, 3.15)
Day 17	0.9 (0.3, 1.5)	1.9 (1.1, 2.4)	0.8 (0.5, 0.8)	1.0 (0.5, 2.5)	0.7 (0.6, 1.2)	4.2 (1.8, 6.7)
Day 18	0.8 (0.2, 1.0)	2.4 (1.0, 2.9)	1.1 (0.5, 1.3)	0.9 (0.5, 2.2)	0.8 (0.7, 1.0)	3.0 (2.0, 4.2)
Day 19	—	—	1.05 (0.7, 1.3)	1.0 (0.6, 2.2)	—	—
Day 20	—	—	0.95 (0.9, 1.2)	0.9 (0.4, 2.0)	—	—
Day 21	0.7 (0.3, 0.9)	1.9 (1.0, 2.3)	1.05 (0.8, 1.1)	0.9 (0.5, 1.8)	—	—
Day 22	0.6 (0.2, 0.8)	1.6 (1.0, 2.3)	1.2 (0.7, 2.2)	0.7 (0.4, 1.7)	1.1 (0.4, 1.4)	1.3 (0.5, 2.0)
Day 23	0.65 (0.5, 0.8)	1.6 (1.1, 2.2)	—	—	—	—
Day 24	0.7 (0.7, 0.8)	1.7 (1.0, 2.3)	—	—	—	—
Day 26	—	—	0.9 (0.8, 1.4)	0.6 (0.4, 2.0)	—	—
Day 28	0.95 (0.3, 1.2)	1.6 (0.6, 3.1)	—	—	—	—

QD indicates once daily; QW, once weekly; TIW, 3 times a week. Last dose, *Day 21; [†]Day 19; [‡]Day 15.

response, as well as the type or profile of the respective immune response. Mipomersen is engineered for use in humans based in part on this premise. For instance, the drug is void of known immune recognition motifs such as sequence elements which are prone to form higher-order structures,³⁴ and contains 5-methyl cytosine instead of cytosine residues as the latter may be recognized by the innate immune system as bacterial or viral DNA in origin.^{35,36} Mipomersen also contains five 2'-O-(2-methoxyethyl) (2'-MOE) sugar modifications at each end, which in addition to increasing drug stability and potency, dampen recognition by cellular nucleic acid sensors, eg, TLRs 7, 8, and 9, and possibly other, yet to be defined activation pathways. Further to these inherent characteristics, the route of administration and dose are 2 other factors, which may define the immuno-stimulatory profile of an oligonucleotide. These effects are exemplified by oligodeoxynucleotides specifically designed to elicit an immune response, where a systemic dose-dependent T_H1-like innate immune response is evident by SC injection of unmethylated CpG oligodeoxynucleotides in healthy men, but not by intravenous infusion.²¹ By design, mipomersen appears to avoid this type of response.

Beyond the transient post-dose increase in CRP levels observed in the mipomersen 200 mg QW group, there was no evidence of a systemic inflammatory signal, eg, innate immune response, in subjects dosed with mipomersen compared with placebo. Furthermore, there were no consistent clinical signs of inflammation evident in this study, such as flu-like symptoms. The basis of the dose-dependent transient increase in CRP expression is unknown. C-reactive protein expression is predominantly upregulated by IL-6 signal transduction.^{20,27,37} Notably though, a transient post-dose increase in IL-6 occurred in all dosing regimens and was independent of treatment assignment, with transient increases observed in both mipomersen and placebo-treated groups. Further to this nonspecific effect, the large majority of the post-dose IL-6 increases were not accompanied by increases in CRP levels. Decoupling of these 2 markers of systemic inflammation was particularly evident in the TIW dosing regimen where both treatment groups displayed transient increases in IL-6 levels without any subsequent change in CRP levels.

The nonspecific transient increases in IL-6 levels most likely reflect a local response to tissue injury which resulted

Table 6. Pre- and Post-Dose Interleukin-6 Concentrations, Median (Interquartile Range)

IL-6, pg/mL	30 mg QD*		70 mg TIW [†]		200 mg QW [‡]	
	Placebo	Mipomersen	Placebo	Mipomersen	Placebo	Mipomersen
Baseline	1.9 (1.1, 2.6)	4.3 (2.6, 5.9)	3.1 (2.7, 4.9)	4.1 (1.7, 7.7)	3.4 (2.5, 7.0)	3.4 (2.4, 5.30)
1st dose						
+1 h	2.6 (2.2, 5.5)	3.9 (2.5, 7.2)	3.4 (2.6, 7.7)	4.9 (3.1, 11.7)	3.7 (3.2, 6.0)	4.9 (2.5, 8.6)
+2 h	3.3 (2.2, 7.1)	4.1 (2.7, 6.7)	9.2 (3.4, 11.2)	7.0 (5.05, 13.45)	5.2 (2.8, 8.1)	3.6 (2.5, 13.3)
+3 h	7.7 (2.9, 11.2)	6.1 (3.0, 11.8)	7.9 (4.0, 9.6)	8.4 (6.5, 12.2)	5.8 (3.5, 9.1)	4.9 (3.0, 13.0)
+4 h	4.9 (3.4, 7.7)	6.7 (4.3, 8.5)	5.6 (3.9, 11.5)	9.4 (6.0, 14.7)	5.2 (3.9, 20.6)	6.5 (4.2, 15.2)
+6 h	5.9 (3.7, 7.7)	7.9 (4.1, 10.7)	11.1 (4.9, 23.2)	11.2 (6.2, 36.9)	5.9 (5.3, 11.7)	17.1 (5.2, 24.0)
+8 h	4.6 (3.4, 12.3)	10.0 (6.9, 16.5)	14.1 (6.1, 36.4)	11.5 (6.0, 33.6)	7.5 (5.2, 11.3)	11.5 (6.9, 21.7)
+12 h	3.8 (2.5, 10.8)	5.1 (3.2, 10.1)	7.2 (2.1, 19.0)	8.9 (4.0, 17.9)	7.9 (5.7, 8.4)	8.7 (4.6, 21.6)
+1 day	2.1 (1.3, 4.6)	4.2 (2.9, 8.2)	2.6 (1.7, 6.0)	5.9 (4.4, 10.4)	3.4 (1.8, 4.0)	10.9 (8.8, 14.5)
Last dose						
Pre	1.65 (1.4, 3.6)	3.9 (2.8, 8.7)	3.5 (2.4, 5.2)	3.6 (1.5, 8.0)	3.0 (2.4, 4.2)	3.7 (3.0, 5.0)
+1 h	2.35 (1.8, 2.9)	3.8 (2.0, 12.0)	4.35 (3.4, 5.1)	4.3 (2.4, 8.6)	3.3 (2.9, 6.0)	4.4 (3.1, 5.2)
+2 h	3.45 (3.4, 6.2)	4.8 (2.5, 9.3)	6.5 (6.1, 8.9)	6.1 (3.6, 9.7)	4.0 (2.9, 5.9)	4.1 (3.1, 5.6)
+3 h	3.95 (2.7, 4.7)	4.3 (2.9, 9.6)	10.25 (7.9, 11.9)	8.7 (4.6, 11.3)	4.4 (4.1, 5.7)	5.0 (4.0, 7.0)
+4 h	9.8 (3.0, 10.8)	5.3 (3.0, 8.8)	15.1 (13.5, 17.3)	9.8 (5.5, 12.8)	6.4 (4.7, 13.1)	6.5 (5.0, 8.1)
+6 h	5.85 (4.1, 8.2)	7.3 (4.2, 17.0)	11.05 (8.0, 17.8)	13.9 (8.0, 20.0)	8.2 (5.3, 14.8)	6.8 (4.7, 16.8)
+8 h	10.0 (6.0, 13.7)	8.5 (3.0, 18.1)	17.9 (13.3, 20.2)	13.7 (6.9, 20.6)	6.8 (5.6, 9.7)	11.0 (5.8, 17.8)
+12 h	5.4 (2.9, 23.0)	4.3 (2.8, 14.2)	7.7 (4.6, 16.2)	6.7 (3.0, 12.0)	9.9 (8.1, 19.5)	8.2 (5.3, 13.2)
+1 day	3.15 (1.3, 4.4)	4.3 (2.2, 11.8)	2.7 (1.6, 4.3)	3.6 (2.1, 8.5)	2.9 (2.3, 7.4)	6.9 (5.3, 11.45)
+2 days	2.8 (1.6, 4.1)	3.6 (2.2, 5.1)	2.5 (2.1, 4.4)	3.8 (1.5, 7.7)	2.9 (2.4, 3.6)	3.6 (2.2, 4.6)

QD indicates once daily; QW, once weekly; TIW, 3 times a week.
Last dose, *Day 21; [†]Day 19; [‡]Day 15.

from the mechanical action of the needle or bolus of fluid at the SC injection site. Other potential factors which may have affected IL-6 levels include diurnal variation in the immune system, or in the case of the placebo group the small amount of riboflavin added for color matching to the active solution. In regard to diurnal variation, dosing times had similar distribution profiles with respect to the time of day across cohorts and active to placebo within a cohort as a result of randomization. Furthermore, the majority of subjects were dosed within an hour of the same time of day and predominantly in the morning hours for the first and last doses, ie, the times of intensive sampling. Notably, there was no apparent association between time of day and IL-6 baseline or change from baseline levels. In regard to riboflavin, we found no indication of a “dose-dependent” effect in the IL-6 signal as a result of the different amounts of riboflavin injected by volume for each dose regime.

Whether the transient elevation in CRP levels observed in the 200 mg mipomersen is clinically significant is unknown. Nevertheless, the level of these increases are substantially

lower than those which may occur as a result of bacterial or viral infection,³⁸ surgery or dental procedures,³⁹ and other potential sources of tissue trauma and injury, such as exercise⁴⁰ and sunburn.⁴¹ Acute changes in CRP associated with such events can be quite robust, rising up to 1000-fold and peaking ≈24 to 48 hours after the stimulus with return to baseline levels ≈14 days later. These acute responses are considered a normal function of the immune system.²⁰

A chronic elevation in basal CRP level on the other hand is considered predictive of cardiovascular risk.^{13,42} When using CRP as a marker for cardiovascular risk, the AHA/CDC recommends taking the average of 2 measures, 2 weeks apart.⁴³ Furthermore, it is recommended to exclude results >10 mg/L that may reflect an acute condition and consequently falsely identify or overtly mask any coronary risk. CRP levels measured in this manner have proven to remain stable in patients treated for 26 weeks with 200 mg weekly SC doses of mipomersen compared to placebo in phase 3 trials.^{2,4,5} This latter result further establishes the absence of a systemic inflammatory response to mipomersen treatment.

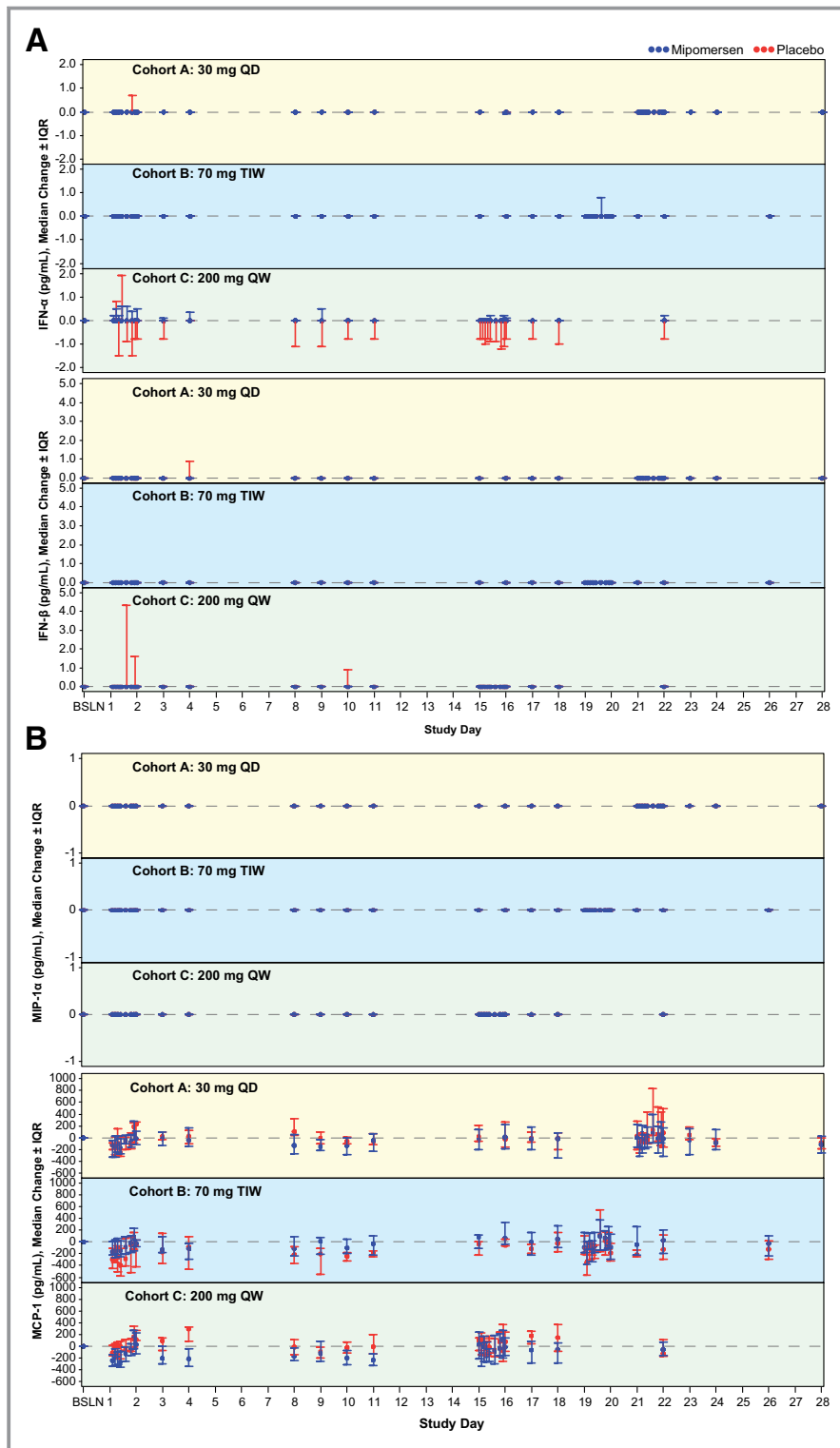


Figure 4. Effect of SC dosing regimen on inflammation biomarkers. Type I interferons, IFN- α and - β (A); chemokines, MIP-1 α , and MCP-1 (B); immune cell signaling and activation, IL-1 β , and IL-13 (C); complement split products, Bb and C5a (D); and IL-6 and CRP (E). Data presented are the median absolute changes from baseline (BSLN), \pm the interquartile range (IQR). Bb and C5a indicates complement split products; CRP, C-reactive protein; IFN, interferon; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; MIP-1 α , macrophage inflammatory protein 1 alpha; QD indicates once daily; QW, once weekly; SC, subcutaneous; TIW, 3 times a week.

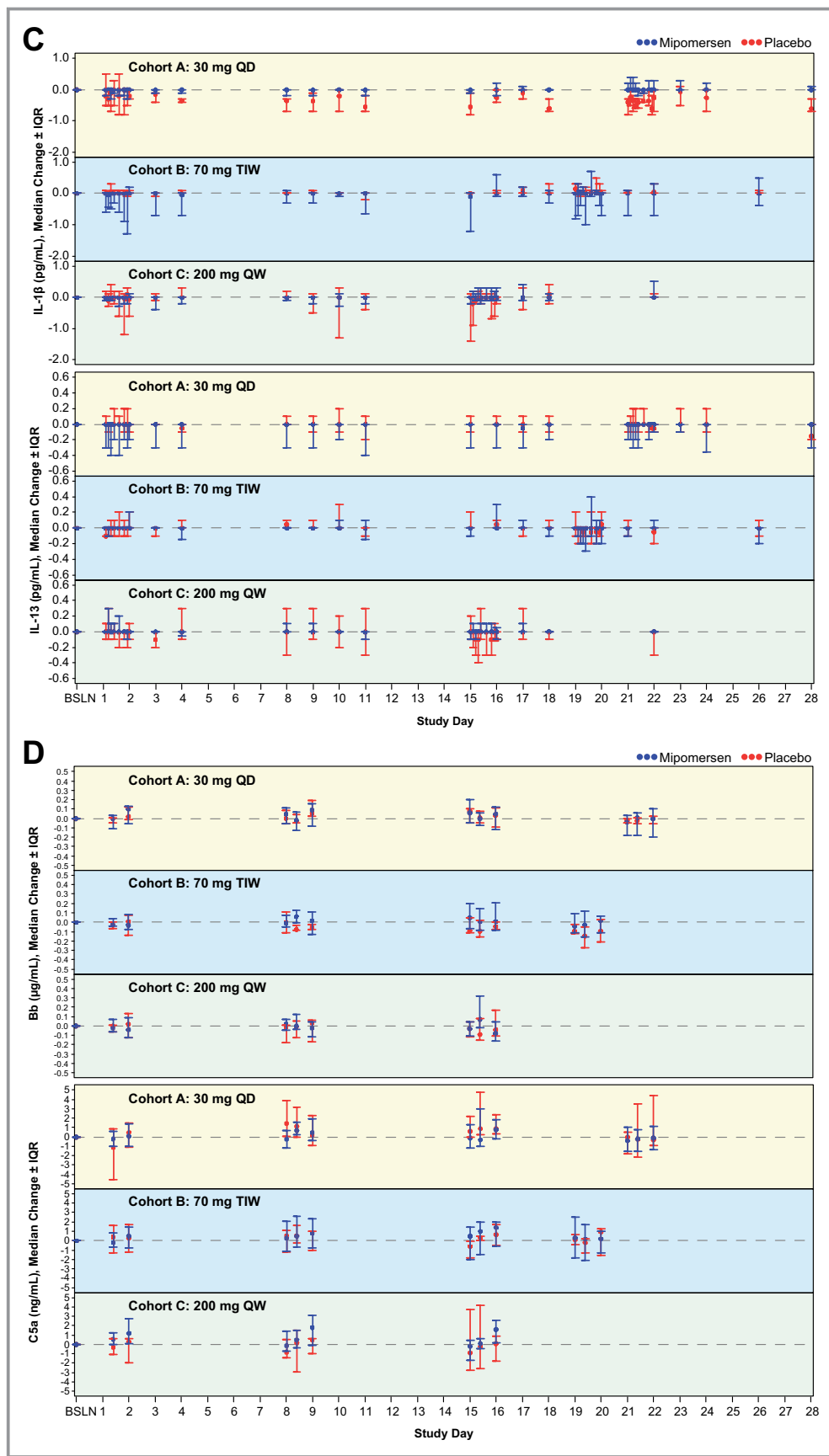


Figure 4. Continued.

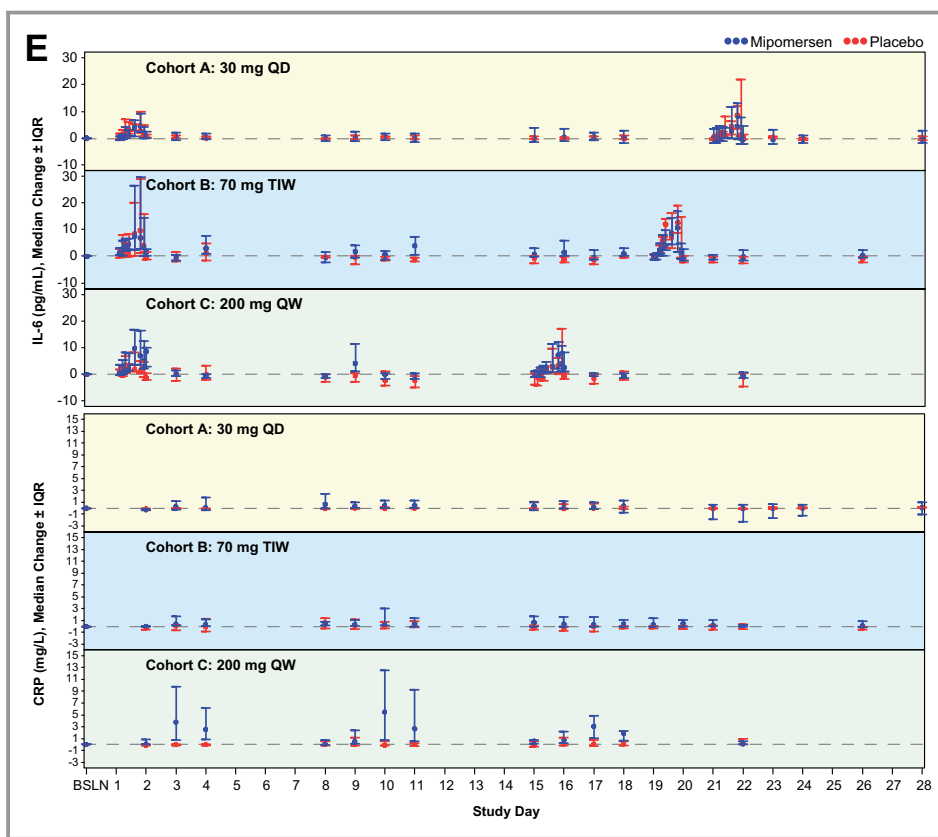


Figure 4. Continued.

Table 7. Exploratory Analysis of Lipid-lowering Response to Short-Term Mipomersen Treatment

	Placebo	30 mg QD	70 mg TIW	200 mg QW
LDL cholesterol				
Baseline, n	21	21	21	21
Mean, mmol/L	2.80 (0.68)	3.18 (0.62)	3.16 (0.90)	3.20 (0.85)
Day 28/ET, n	19	16	19	19
Mean, mmol/L	2.79 (0.60)	2.78 (0.62)	2.44 (0.64)	2.57 (0.86)
Change, n	19	16	19	19
Mean, %Δ	-1.2 (9.3)	-9.5 (18)	-21 (14)	-18 (17)
P-value		0.095	<0.001	<0.001
Apolipoprotein B				
Baseline, n	21	21	21	21
Mean, g/L	0.88 (0.20)	0.98 (0.19)	0.95 (0.24)	0.95 (0.22)
Day 28/ET, n	19	16	19	19
Mean, g/L	0.88 (0.18)	0.83 (0.16)	0.78 (0.21)	0.77 (0.23)
Change, n	19	16	19	19
Mean, %Δ	-0.03 (11.9)	-10.9 (11.1)	-16.5 (12.9)	-17.0 (15.5)
P-value		0.016	<0.001	<0.001

Values in parentheses are the standard deviations. Analysis excluded data from non-fasted samples. P-values were determined by ANOVA, compared to pooled placebo. To convert SI units to conventional units (mg/dL), divide absolute values for LDL cholesterol by 0.0259, and for apolipoprotein B by 0.01. ANOVA indicates analysis of variance; ET, early termination; LDL, low-density lipoprotein; QD, once daily; QW, once weekly; TIW, 3 times a week.

The current study was limited by sample size, the short-term dosing period, differences in visit schedules across cohorts and participation of healthy volunteers. Consequently the results from this study do not necessarily reflect the responses that may be observed with longer-term dosing or experienced by patients. Notably, the study was not powered to detect differences in safety and tolerability. For this reason, analyses of the relationships of injection site reaction incidence and injection dose and frequency were exploratory in nature. Future studies will be needed to systematically determine if these dosing parameters exert effects on the tolerability to longer-term treatment in patients.

In conclusion, this study demonstrated a similar drug exposure and overall safety profile between the 3 dosing regimens. Taken together, results from this short-term phase 1 study and the phase 3 studies in patients, indicates a lack of systemic inflammation associated with mipomersen. These findings collectively support further evaluation of alternative dosing regimens for mipomersen in longer-term studies involving patients at high-risk for coronary heart disease.

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Disclosures

Dr Kastelein is a consultant to Isis Pharmaceuticals, Aegerion Pharmaceuticals, Genzyme, and Alnylam Pharmaceuticals. Drs Flaim, Grundy, and Baker are employees of Isis Pharmaceuticals, Inc. Dr McGowan is an employee of Genzyme, a Sanofi company. Authors may own stock or stock options in Isis Pharmaceuticals and/or Genzyme, a Sanofi company.

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