ORIGINAL ARTICLE

Effect of a Monoclonal Antibody to PCSK9 on LDL Cholesterol

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ABSTRACT

BACKGROUND

Proprotein convertase subtilisin/kexin 9 (PCSK9), one of the serine proteases, binds to low-density lipoprotein (LDL) receptors, leading to their accelerated degradation and to increased LDL cholesterol levels. We report three phase 1 studies of a monoclonal antibody to PCSK9 designated as REGN727/SAR236553 (REGN727).

METHODS

In healthy volunteers, we performed two randomized, single ascending-dose studies of REGN727 administered either intravenously (40 subjects) or subcutaneously (32 subjects), as compared with placebo. These studies were followed by a randomized, placebo-controlled, multiple-dose trial in adults with heterozygous familial hypercholesterolemia who were receiving atorvastatin (21 subjects) and those with nonfamilial hypercholesterolemia who were receiving treatment with atorvastatin (30 subjects) (baseline LDL cholesterol, >100 mg per deciliter [2.6 mmol per liter]) or a modified diet alone (10 subjects) (baseline LDL cholesterol, >130 mg per deciliter [3.4 mmol per liter]). REGN727 doses of 50, 100, or 150 mg were administered subcutaneously on days 1, 29, and 43. The primary outcome for all studies was the occurrence of adverse events. The principal secondary outcome was the effect of REGN727 on the lipid profile.

RESULTS

Among subjects receiving REGN727, there were no discontinuations because of adverse events. REGN727 significantly lowered LDL cholesterol levels in all the studies. In the multiple-dose study, REGN727 doses of 50, 100, and 150 mg reduced measured LDL cholesterol levels in the combined atorvastatin-treated populations to 77.5 mg per deciliter (2.00 mmol per liter), 61.3 mg per deciliter (1.59 mmol per liter), and 53.8 mg per deciliter (1.39 mmol per liter), for a difference in the change from baseline of −39.2, −53.7, and −61.0 percentage points, respectively, as compared with placebo ($P<0.001$ for all comparisons).

CONCLUSIONS

In three phase 1 trials, a monoclonal antibody to PCSK9 significantly reduced LDL cholesterol levels in healthy volunteers and in subjects with familial or nonfamilial hypercholesterolemia. (Funded by Regeneron Pharmaceuticals and Sanofi; ClinicalTrials.gov numbers, NCT01026597, NCT01074372, and NCT01161082.)

In 2003, Abifadel and colleagues[1] described two families with autosomal dominant hypercholesterolemia that was associated with gain-of-function mutations in proprotein convertase subtilisin/kexin 9 (PCSK9), one of the serine proteases. These patients had high plasma levels of low-density lipoprotein (LDL) cholesterol, which was associated with an increased incidence of coronary heart disease. Shortly thereafter, studies of animal models identified a role for PCSK9 in the post-translational regulation of LDL-receptor activity.[2,3] PCSK9, which is synthesized primarily in the liver, enters the circulation, where it binds to hepatic LDL receptors and targets them for degradation. This process reduces the capacity of the liver to bind and remove LDL cholesterol and results in increased LDL cholesterol levels. Subsequent studies revealed that some patients with low levels of LDL cholesterol had PCSK9 loss-of-function mutations[4-7] and showed that these patients had a reduced incidence of coronary heart disease.[8] These studies raised the possibility that pharmacologic inhibition of PCSK9 might lower LDL cholesterol levels in patients with hypercholesterolemia.

REGN727/SAR236553 (REGN727) is an investigational, fully human monoclonal antibody that is highly specific for human PCSK9 and blocks its interaction with the LDL receptor. We report here the results from the initial studies of REGN727 in humans.

Methods

Study Designs

We performed three separate clinical studies of REGN727. Two were studies of single doses of REGN727, administered either intravenously or subcutaneously, in healthy volunteers. The third study was a multiple-dose study of subcutaneously administered REGN727 in subjects with either familial or nonfamilial hypercholesterolemia.

These studies were sponsored by Regeneron Pharmaceuticals and Sanofi. The single-dose studies were designed by Regeneron Pharmaceuticals, and the multiple-dose study was jointly designed by the first author and Regeneron Pharmaceuticals. The study protocols were approved by the investigational review board at each study center, and all subjects provided written informed consent. Data were collected at the study sites by several of the coauthors and were analyzed by representatives of Regeneron Pharmaceuticals. The first draft of the manuscript was jointly written by the first author and a representative of Regeneron Pharmaceuticals, with review and revision by the other authors. Editorial assistance was provided by an employee of the PharMed Group who was paid by Regeneron Pharmaceuticals. The first author and Regeneron representatives made the decision to submit the manuscript for publication. The academic authors vouch for the accuracy and completeness of the data and analyses as presented and for the fidelity of this report to the trial protocols, which are available with the full text of this article at NEJM.org.

Single-Dose Studies

For the single-dose studies, 40 subjects were enrolled in the group receiving intravenous REGN727 and 32 were enrolled in the group receiving subcutaneous REGN727. All subjects were healthy men and women between the ages of 18 and 65 years with a body weight ranging from 50 to 95 kg and a body-mass index (the weight in kilograms divided by the square of the height in meters) ranging from 18 to 30. All subjects had a serum LDL cholesterol level of more than 100 mg per deciliter (2.59 mmol per liter). The use of nonstudy agents to alter lipid levels was prohibited. Detailed enrollment criteria are provided in the Supplementary Appendix, available at NEJM.org.

In the single-dose study involving intravenous administration, an initial group of eight subjects was randomly assigned in a 3:1 ratio to receive either REGN727 at the lowest tested dose (0.3 mg per kilogram of body weight in six subjects) or placebo (in two subjects). After safety assessment, the dose of REGN727 was increased sequentially (to 1.0, 3.0, 6.0, and 12.0 mg per kilogram) in four further 3:1 randomized comparisons with placebo, with eight subjects in each group (Fig. S1A in the Supplementary Appendix). The single-dose study of subcutaneous administration had a similar design but included four sequential-dose groups receiving 50, 100, 150, and 250 mg (Fig. S1B in the Supplementary Appendix). These studies were conducted at two contract research organizations, Quintiles in Overland Park, Kansas (for intravenous and subcutaneous administration), and Comprehensive Phase One in Miramar, Florida (for subcutaneous administration).

All subjects in the single-dose studies were required to remain at the research facility for ob-

servation for 3 days after receiving a study drug. Blood was drawn for evaluation of serum lipid levels at baseline and at days 1, 2, 4, 8, 11, 15, 22, 29, 43, 64, 85 (intravenous administration only), and 106. Safety assessments that were performed on the same days included an evaluation of vital signs, a physical examination, blood tests, and electrocardiography. Details of the laboratory measurements are provided in the Supplementary Appendix.

MULTIPLE-DOSE STUDY

The multiple-dose study included three separate cohorts of subjects. The first cohort consisted of 21 subjects with heterozygous familial hypercholesterolemia (Fig. S1C in the Supplementary Appendix), and the second cohort consisted of 30 subjects with nonfamilial hypercholesterolemia (Fig. S1D in the Supplementary Appendix). All subjects in these two cohorts were receiving atorvastatin therapy and had an LDL cholesterol level of more than 100 mg per deciliter. The third cohort consisted of 10 subjects with nonfamilial hypercholesterolemia who were being treated with a modified diet only and who had an LDL cholesterol level of more than 130 mg per deciliter (3.36 mmol per liter) (Fig. S1E in the Supplementary Appendix). All subjects in the multiple-dose study were between the ages of 18 and 65 years, had a body-mass index of 18 to 35, and did not have diabetes or a known atherosclerotic vascular disease. Additional enrollment criteria are provided in the Supplementary Appendix.

Subjects in the multiple-dose study were randomly assigned to receive subcutaneous REGN727 (50, 100, or 150 mg) or placebo administered on days 1, 29, and 43. All three regimens were designed to provide a 4-week safety observation period between the first and second doses of REGN727 and to assess the pharmacodynamic effects of REGN727 at time points 2 weeks after administration (on study days 15, 43, and 57) and 4 weeks after administration (on study days 29 and 71). The multiple-dose trial was carried out at the Metabolic and Atherosclerosis Research Center in Cincinnati and the New Orleans Center for Clinical Research in Knoxville, Tennessee.

All subjects in the multiple-dose study were required to remain at the research facility for observation for 2 days after receiving the first dose of a study drug and for 2 hours after receiving the second and third doses. Serum lipid levels were evaluated at screening and 2 days before the administration of a study drug (for subjects receiving atorvastatin only), 1 day before administration, and on days 1, 2, 3, 8, 15, 29, 43, 57, 71, 85, 99, 120, and 148 after administration. Safety assessments that were performed on the same days included an evaluation of vital signs, a physical examination, blood tests, and electrocardiography. Details of the laboratory measurements are provided in the Supplementary Appendix.

STATISTICAL ANALYSIS

The primary end point for all three studies was the incidence and severity of treatment-emergent adverse events. The safety population for this purpose included all subjects in each study who had received at least one dose of a study drug. Secondary end points included the relative and absolute change in measured and calculated serum LDL cholesterol, total cholesterol, and other serum lipid and lipoprotein levels from baseline to each visit.

Results for all subjects in the placebo groups were pooled within each of the single-dose studies. In the multiple-dose study, results for all subjects in the placebo groups were pooled for each study cohort (subjects with heterozygous familial hypercholesterolemia or nonfamilial hypercholesterolemia receiving statin therapy and subjects with nonfamilial hypercholesterolemia receiving a modified diet only). Missing values were imputed with the last observation carried forward (in five subjects in the single-dose study of intravenous administration, six subjects in the single-dose study of subcutaneous administration, and no subjects in the multiple-dose study). In the multiple-dose study, the results on study day 57, the point at which the effects of administration at a biweekly interval could be observed, were chosen to compare the lipid and lipoprotein effects.

In the single-dose studies, the enrollment of six subjects per dose group was estimated to provide a power of at least 80% to detect a mean (±SD) difference of 30±15% between REGN727 and placebo in the mean percent change in LDL cholesterol from baseline, with a two-sided test at a significance level of 0.05. In the multiple-dose study, comparing the pooled group of six placebo-treated subjects with familial hypercholesterolemia with the five who received REGN727

in each dose group was also estimated to provide a power of at least 80% to detect a treatment difference of 30±15% on the basis of similar assumptions.

Effects of treatments on efficacy variables were assessed with the use of analysis-of-covariance models, with the study group as the fixed effect and the relevant baseline value as a covariate. Least-squares means of differences between the treatment group and the placebo group, 95% confidence intervals, and P values for comparison between the treatment group and the placebo group according to visit were obtained within the framework of analysis of covariance. For values for triglycerides and lipoprotein(a), a rank-based analysis of covariance was used. Categorical variables were analyzed with the use of Fisher's exact test. A P value of 0.05 was considered to indicate statistical significance. No adjustments were made for multiple comparisons.

RESULTS

STUDY POPULATIONS

Enrollment and outcomes for subjects in all three studies are summarized in Figure S1 in the Supplementary Appendix. For the single-dose studies, 40 and 32 subjects were enrolled in the intravenous and subcutaneous studies, respectively. For the multiple-dose study, 21 subjects with heterozygous familial hypercholesterolemia and 41 subjects with nonfamilial hypercholesterolemia were enrolled. One subject with nonfamilial hypercholesterolemia who was randomly assigned to receive REGN727 did not receive the drug owing to poor venous access for the required pharmacokinetic sampling. Baseline characteristics of the subjects in the three studies are provided in Table 1.

SAFETY

Two subjects in the single-dose studies had serious adverse events: a 33-year-old man receiving intravenous placebo, who had abdominal pain and rectal bleeding on study day 83, and a 19-year-old man with a history of appendectomy receiving 50 mg of subcutaneous REGN727, who had a small-bowel obstruction that was diagnosed on study day 75.

In the single-dose intravenous study, a 54-year-old man who received 12 mg per kilogram of REGN727 had a slightly elevated total bilirubin level at screening and throughout most of the trial (highest value, 2.5 mg per deciliter [43 μmol per liter] on day 15). Also, a 26-year-old man who received 0.3 mg of REGN727 per kilogram had a transient elevation of serum creatine kinase to more than 10 times the upper limit of the normal range (highest value, 5382 U per liter on day 22, with a return to less than 3 times the upper limit of the normal range on study day 43) in temporal proximity to strenuous physical exercise; there was no associated muscle pain or serum creatinine elevation. No subject in the single-dose trials discontinued participation early because of an adverse event.

In the multiple-dose study, no subject had a serious adverse event, and all subjects completed all visits. No subject receiving REGN727 in any of the three studies had an elevation of aspartate aminotransferase or alanine aminotransferase to more than 3 times the upper limit of the normal range or an increase in creatinine to more than 1.7 mg per deciliter (150 μmol per liter). Also in this study, among subjects who received REGN727, 5 of 39 subjects (13%) who were also receiving atorvastatin had creatine kinase levels that were more than 3 times the upper limit of the normal range, but none had levels that were more than 10 times the upper limit of the normal range; of the 8 subjects who were not receiving atorvastatin, none had such increased levels. Among subjects who received placebo, creatine kinase levels of more than 3 times the upper limit of the normal range occurred in 0 of 12 subjects who were receiving atorvastatin and in 2 of 2 subjects who were not receiving atorvastatin. There were a few injection-site reactions, which were mild.

The proportions of subjects who had at least one treatment-emergent adverse event were similar among subjects who received intravenous REGN727 and those who received placebo. As compared with subjects who received placebo, a higher proportion of subjects who received subcutaneous REGN727 had an adverse event in the single-dose and the multiple-dose studies. Headache was the most common adverse event. Additional safety data (treatment-emergent adverse events, clinical chemical analyses, and hematologic measurements) are provided in Tables S1 through S7 in the Supplementary Appendix.

LIPID AND LIPOPROTEIN RESPONSE

Single administration of intravenous or subcutaneous REGN727 in healthy volunteers resulted in a least-squares mean difference in the change from

Table 1. Baseline Characteristics of Healthy Volunteers and Subjects with Familial Hypercholesterolemia (FH) or Non-FH.*

Characteristic	Single-Dose Studies				Multiple-Dose Study					
	Intravenous (N=40)		Subcutaneous (N=32)		FH, with Atorvastatin (N=21)		Non-FH, with Atorvastatin (N=30)		Non-FH, with Modified Diet Alone (N=10)	
	Placebo (N=10)	*REGN727 (N=30)*	*Placebo (N=8)*	*REGN727 (N=24)*	*Placebo (N=6)*	*REGN727 (N=15)*	*Placebo (N=6)*	*REGN727 (N=24)*	*Placebo (N=2)*	*REGN727 (N=8)*
Mean age (yr)	30	38	35	34	39	41	50	53	45	54
Male sex (%)	80	63	100	67	83	80	67	54	100	38
Race (%)†										
White	40	60	50	42	100	80	100	92	100	100
Black	60	30	50	50		20		8		
American Indian or Alaskan Native		10		8						
Use of atorvastatin (%)										
10 mg	0	0	0	0	0	20	67	67	0	0
20 mg	0	0	0	0	50	27	33	29	0	0
40 mg	0	0	0	0	50	53	0	4	0	0
Body-mass index‡	25.0±2.2	26.4±2.6	25.1±3.2	25.3±2.9	25.8±3.4	27.8±5.2	29.4±3.2	27.4±2.8	23.9±4.0	29.5±3.9
Cholesterol (mg/dl)										
Low-density lipoprotein§	137.0±38.9	135.2±24.7	133.0±29.8	128.9±25.6	133.2±20.7	133.7±27.6	117.7±13.7	110.7±18.7	151.5±16.3	178.6±49.0
High-density lipoprotein	51.1±14.3	55.5±12.7	48.0±11.1	57.7±13.9	42.8±7.7	44.3±8.8	44.0±13.1	52.3±13.6	54.0±14.1	49.6±8.5
Apolipoprotein B (mg/dl)	117.7±30.8	109.9±23.1	106.0±22.6	99.1±20.9	109.7±13.8	112.9±18.9	103.5±11.0	97.2±17.4	117.5±23.3	138.5±36.7
Triglycerides (mg/dl)										
Median	125	87	88	98	104	105	127	129	116	127
Minimum:maximum	82:179	67:119	60:166	67:124	93:134	66:143	83:171	102:160	86:146	92:216

* Plus–minus values are means ±SD. To convert values for cholesterol to millimoles per liter, multiply by 0.02586. To convert values for triglycerides to millimoles per liter, multiply by 0.01129.
† Race was self-reported.
‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.
§ Low-density lipoprotein cholesterol was measured by means of preparative ultracentrifugation in the multiple-dose study and by homogeneous direct measurement in the single-dose studies.[9]

Table 2. Baseline and Lowest Values for Low-Density Lipoprotein (LDL) Cholesterol in Single-Dose Studies, According to Route of Administration.*

Variable	Placebo (N=10)	REGN727				
		0.3-mg/kg Dose (N=6)	1.0-mg/kg Dose (N=6)	3.0-mg/kg Dose (N=6)	6.0-mg/kg Dose (N=6)	12.0-mg/kg Dose (N=6)
Single-dose, intravenous						
LDL cholesterol						
Baseline (mg/dl)	137.0±38.9	132.0±14.3	126.3±16.9	151.8±40.7	127.2±8.3	138.7±28.3
Study day with lowest value	8	11	11	29	22	43
Lowest value (mg/dl)	128.6±30.6	88.2±17.4	66.7±24.6	56.7±24.4	55.2± 5.1	46.8±15.0
Difference in percent change from baseline vs. placebo (percentage points)†		−28.1±6.3	−42.2± 6.3	−57.4±7.6	−56.5±5.4	−65.4±8.4
P value vs. placebo†		<0.001	<0.001	<0.001	<0.001	<0.001
	Placebo (N=8)	50-mg Dose (N=6)	100-mg Dose (N=6)	150-mg Dose (N=6)	250-mg Dose (N=6)	
Single-dose, subcutaneous						
LDL cholesterol						
Baseline (mg/dl)	133.0±29.8	129.8±28.9	126.5±29.9	142.2±25.7	117.2±15.20	NA
Study day with lowest value	22	15	11	15	11	NA
Lowest value (mg/dl)	115.3±15.6	76.5±23.9	58.6±9.2	62.0±21.9	54.5±15.6	NA
Difference in percent change from baseline vs. placebo (percentage points)†		−32.5±8.5	−39.9±7.1	−38.5±8.5	−45.7±7.2	NA
P value vs. placebo†		<0.001	<0.001	<0.001	<0.001	NA

* Plus–minus values are means ±SD. LDL cholesterol was measured by means of homogeneous direct measurement.[9] To convert values for cholesterol to millimoles per liter, multiply by 0.02586. NA denotes not applicable.

† Least-squares mean differences and P values were calculated from an analysis-of-covariance model with use of values in the placebo and REGN727 groups from the same treatment day.

baseline in LDL cholesterol of up to 65 percentage points, as compared with placebo (Table 2 and Fig. 1). The degree and duration of LDL cholesterol lowering were dose-dependent, with higher doses producing prolonged reductions that were sustained up to day 64 (Fig. 1).

In the multiple-dose study, REGN727 doses of 50, 100, and 150 mg reduced measured LDL cholesterol in the combined atorvastatin-treated populations to 77.5 mg per deciliter (2.00 mmol per liter), 61.3 mg per deciliter (1.59 mmol per liter), and 53.8 mg per deciliter (1.39 mmol per liter), for a difference in the change from baseline of −39.2, −53.7, and −61.0 percentage points, respectively, as compared with placebo (P<0.001 for all comparisons) (Table S8 in the Supplementary Appendix). The degree and duration of LDL cholesterol reduction in the different dose groups corresponded to the reduction of free PCSK9 in plasma (Fig. S2 in the Supplementary Appendix). The mean difference in the change from baseline in LDL cholesterol, as compared with placebo, exceeded −46 percentage points 2 weeks after first administration of 150 mg in all three cohorts studied (Table 3 and Fig. 2). All but one patient (who had heterozygous familial hypercholesterolemia and was receiving atorvastatin) who received 150 mg in the multiple-dose study had a difference in the change from baseline in LDL cholesterol of at least −40 percentage points, as compared with placebo (Fig. S3C in the Supplementary Appendix).

The LDL cholesterol response was similar in all subjects, regardless of whether they had familial or nonfamilial hypercholesterolemia or whether they were treated with atorvastatin or with a modified diet alone. Corresponding changes were observed in levels of total and non–high-density lipoprotein (HDL) cholesterol and apolipoprotein B, and reductions were also observed in lipoprotein(a) (Tables S8 through S10 and Fig. S4 through

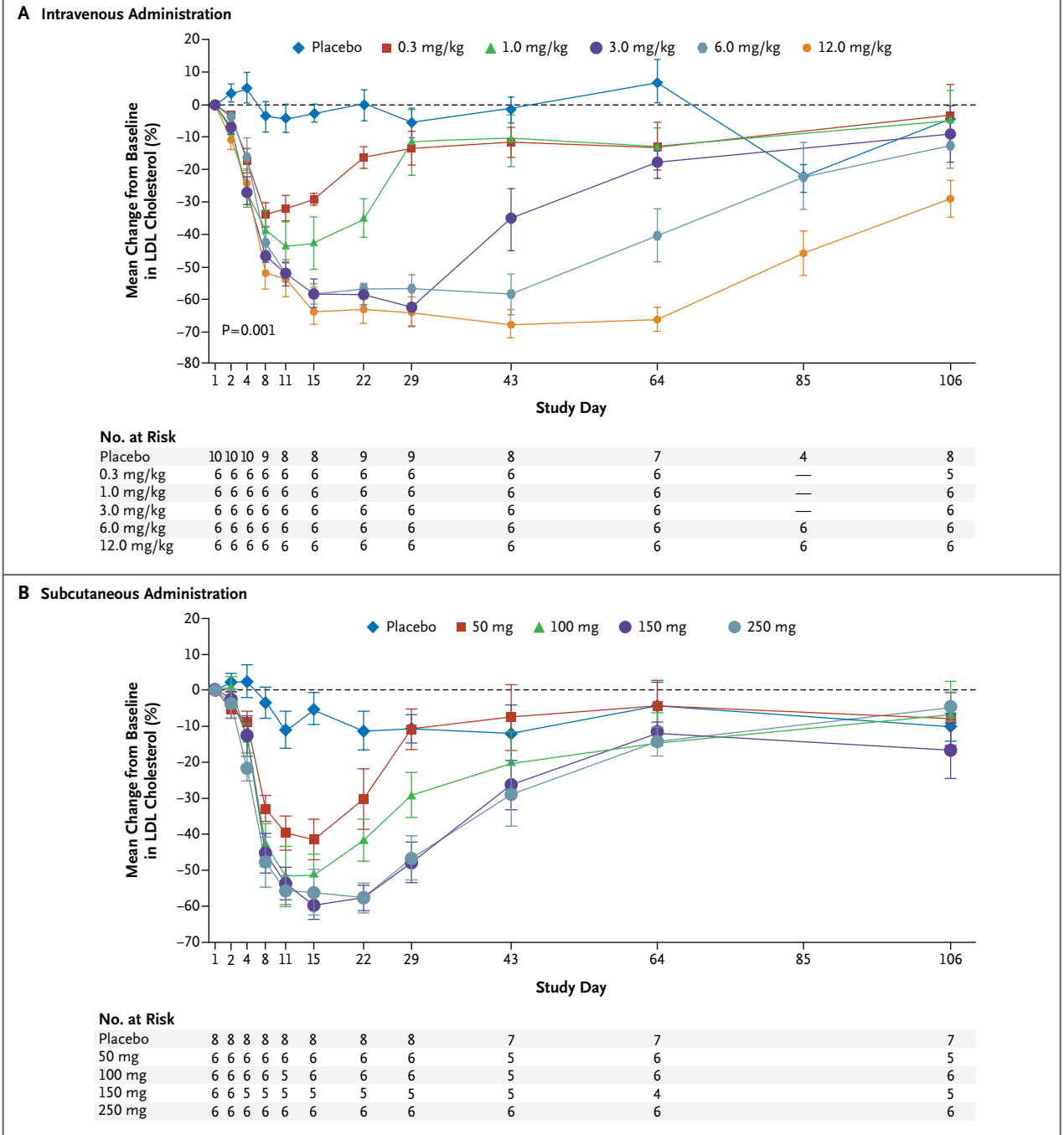


Figure 1. Mean Percent Change from Baseline in LDL Cholesterol Values among Healthy Volunteers in Single-Dose Studies.

Among subjects receiving increasing single doses of REGN727, values are shown for intravenous administration (Panel A) and subcutaneous administration (Panel B). LDL cholesterol values were calculated with the use of the Friedewald formula. The I bars indicate standard errors.

S8 in the Supplementary Appendix). In addition, as compared with placebo, increases were seen in both HDL cholesterol (up to 18 percentage points) and apolipoprotein A1 (up to 13 percentage points) in subjects taking atorvastatin.

DISCUSSION

In three early-phase randomized trials, we evaluated the effects of REGN727, a monoclonal antibody that blocks the interaction of PCSK9 with

Table 3. Baseline and Day 57 Values for LDL Cholesterol among Subjects with Familial Hypercholesterolemia (FH) or Non-FH in the Multiple-Dose Study, According to Atorvastatin Use.*

Variable	Placebo	REGN727		
		50-mg Dose	100-mg Dose	150-mg Dose
Subjects with FH taking atorvastatin				
No. of subjects	6	5	5	5
LDL cholesterol				
At baseline (mg/dl)	133.2±20.7	125.0±12.1	135.8±41.1	140.2±26.2
On day 57 (mg/dl)	137.2±12.5	80.6±21.9	60.0±15.7	65.4±21.2
Difference in percent change from baseline vs. placebo (percentage points)†		−41.4	−57.6	−55.7
P value vs. placebo†		<0.001	<0.001	<0.001
Subjects with non-FH taking atorvastatin				
No. of subjects	6	8	8	8
LDL cholesterol				
At baseline (mg/dl)	117.7±13.7	108.0±14.1	112.1±19.9	111.9±23.3
On day 57 (mg/dl)	123.2±13.6	75.5±13.7	62.1±12.7	46.5±19.9
Difference in percent change from baseline vs. placebo (percentage points)†		−38.2	−51.5	−64.7
P value vs. placebo†		<0.001	<0.001	<0.001
Subjects with non-FH not taking atorvastatin				
No. of subjects	2	NA	NA	8
LDL cholesterol				
At baseline (mg/dl)	151.5±16.3	NA	NA	178.6±49.0
On day 57 (mg/dl)	156.5±23.3	NA	NA	81.4±25.7
Difference in percent change from baseline vs. placebo (percentage points)†		NA	NA	−57.0
P value vs. placebo†		NA	NA	0.002

* Plus–minus values are means ±SD. LDL cholesterol was measured by means of preparative ultracentrifugation.[9] To convert values for cholesterol to millimoles per liter, multiply by 0.02586. NA denotes not applicable.
† Least-squares mean differences and P values were calculated from an analysis-of-covariance model.

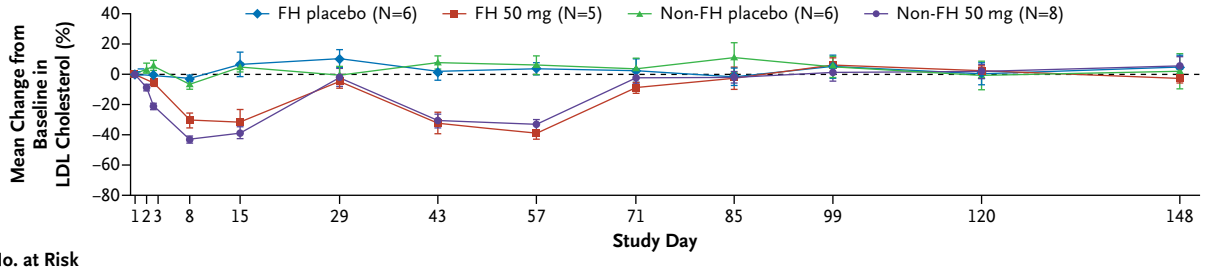
LDL receptors. In all three trials, REGN727 significantly reduced LDL cholesterol levels, as compared with placebo. This effect was significant both in healthy volunteers and in subjects with familial or nonfamilial forms of hypercholesterolemia. The effect was also significant in subjects who were concomitantly taking atorvastatin.

Our results confirm a role for PCSK9 in the regulation of LDL cholesterol levels. In addition, the demonstration of a good correlation between a reduction in free PCSK9 levels and a reduction in LDL cholesterol levels after the administration of REGN727 in humans supports previous reports from studies involving rodents and nonhuman primates that PCSK9 in the circulation, not intracellular PCSK9, is primarily responsible for regulating hepatic LDL receptors.[10,11]

In our studies, the effects of REGN727 and atorvastatin in lowering LDL cholesterol appeared to be additive, not synergistic, since mean percent reductions were similar when REGN727 was administered alone or in subjects already receiving atorvastatin. Although REGN727 and atorvastatin both lower LDL cholesterol by increasing hepatic LDL-receptor activity, atorvastatin does so primarily by enhancing the production of receptors, whereas REGN727 decreases the degradation of receptors. REGN727 induced a maximum lowering of LDL cholesterol within 2 weeks, whereas statins typically take longer.[12] The different modes of action of these agents may account for the more rapid effect seen with REGN727.

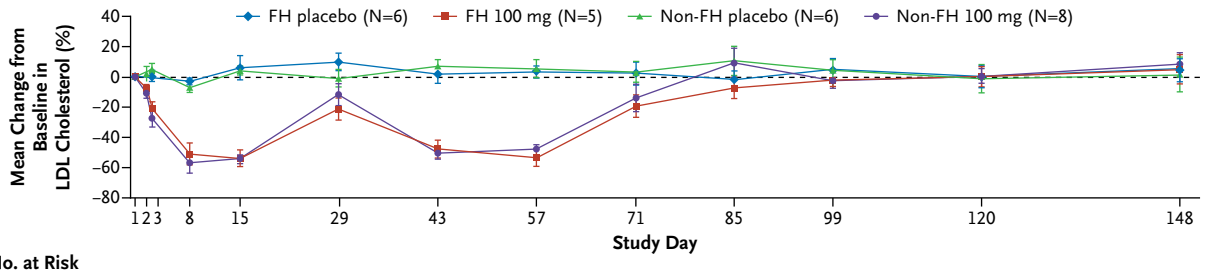
Statins increase the expression of sterol regulatory element–binding protein 2, a transcription

A Subcutaneous Dose of 50 mg with Atorvastatin



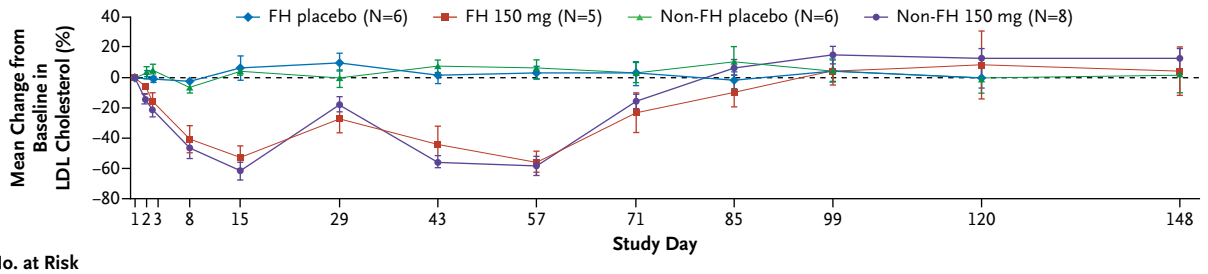
No. at Risk											
FH placebo	6	6	6	6	6	6	6	6	6	6	6
FH 50 mg	5	5	5	5	5	5	5	5	5	5	5
Non-FH placebo	6	6	6	6	6	6	6	6	6	6	6
Non-FH 50 mg	8	8	8	8	8	8	8	8	8	8	8

B Subcutaneous Dose of 100 mg with Atorvastatin



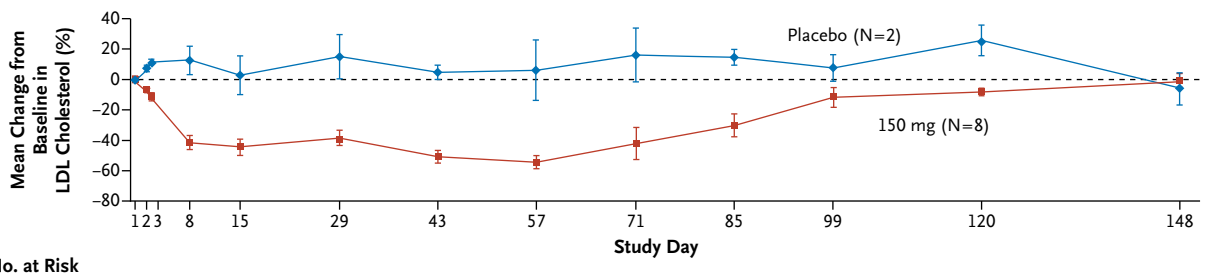
No. at Risk											
FH placebo	6	6	6	6	6	6	6	6	6	6	6
FH 100 mg	5	5	5	5	5	5	5	5	5	5	5
Non-FH placebo	6	6	6	6	6	6	6	6	6	6	6
Non-FH 100 mg	8	8	8	8	8	8	8	8	8	8	8

C Subcutaneous Dose of 150 mg with Atorvastatin



No. at Risk											
FH placebo	6	6	6	6	6	6	6	6	6	6	6
FH 150 mg	5	5	5	5	5	5	5	5	5	5	5
Non-FH placebo	6	6	6	6	6	6	6	6	6	6	6
Non-FH 150 mg	8	8	8	8	8	8	8	8	8	8	8

D Subcutaneous Dose of 150 mg without Atorvastatin



No. at Risk											
Placebo	2	2	2	2	2	2	2	2	2	2	2
150 mg	8	8	8	8	8	8	8	8	8	8	8

Figure 2 (facing page). Mean Percent Change from Baseline in LDL Cholesterol Values among Subjects with Familial Hypercholesterolemia (FH) or Non-FH.

Among subjects receiving multiple doses of REGN727, values are shown for subcutaneous doses of 50 mg (Panel A), 100 mg (Panel B), and 150 mg (Panel C) in subjects who were also receiving atorvastatin and for a subcutaneous dose of 150 mg in subjects who were not receiving atorvastatin (Panel D). All LDL cholesterol values, which were calculated with the use of the Friedewald formula, were available for all subjects at all visits in this study. The I bars indicate standard errors.

factor that in turn enhances the expression of both the low-density lipoprotein receptor gene (*LDLR*) and *PCSK9*. This apparent counter-regulation may limit the effectiveness of statins by PCSK9-mediated destruction of LDL receptors.[13] Subjects with no detectable circulating PCSK9 have LDL cholesterol levels of approximately 15 mg per deciliter (0.39 mmol per liter), a level that is not achieved by even the most efficacious statins.[14,15] Thus, the rate of destruction of LDL receptors appears to be an important determinant of LDL cholesterol levels. Statin-stimulated production of PCSK9, while not apparently altering the maximum LDL-lowering effect, might affect the duration of action of therapeutic antibodies because higher rates of PCSK9 production may result in greater clearance of free antibody. Thus, the duration of action of REGN727 may be longer in subjects who are treated with diet modification alone than in those treated with atorvastatin (Fig. 2C and 2D).

As might be anticipated with an agent that significantly reduces LDL cholesterol, REGN727 also significantly reduced apolipoprotein B levels. In subjects receiving both REGN727 and atorvastatin, there was a significant increase in HDL cholesterol levels. A reduction in lipoprotein(a) was also seen, although this effect was not significant at all doses. Other agents that up-regulate LDL receptors, such as statins and bile acid sequestrants, also raise HDL cholesterol levels, possibly by reducing the transfer of cholesterol from HDL to LDL particles. However, none of these drugs reduce levels of lipoprotein(a), which is not thought to be cleared through LDL receptors.[16] The explanation for a possible effect on lipoprotein(a) is thus uncertain, and additional work is required to confirm and explain this observation.

In our trials, we saw no clear evidence of drug-related adverse events. Five subjects in the multiple-dose trial who were receiving REGN727 with concomitant atorvastatin had brief elevations in creatine kinase to more than three times the upper limit of the normal range. Given the small number of subjects and the short duration of exposure, our ability to evaluate the safety profile of REGN727 in these trials was limited. Additional studies will be required to make a clearer assessment of this agent for potential adverse effects.

In summary, we evaluated the effects of REGN727, a fully human monoclonal antibody that blocks the interaction of PCSK9 with LDL receptors. In three small early-phase trials, REGN727 significantly reduced LDL cholesterol levels. This effect was significant both in healthy volunteers and in subjects with familial or nonfamilial forms of hypercholesterolemia. The effect was also significant in subjects who were concomitantly taking atorvastatin.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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REFERENCES

1. Abifadel M, Varret M, Rabeè JD, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet 2003;34:154-6.
2. Park SW, Moon YA, Horton JD. Post-transcriptional regulation of low density lipoprotein receptor protein by proprotein convertase subtilisin/kexin type 9a in mouse liver. J Biol Chem 2004;279:50630-8.
3. Maxwell KN, Breslow JL. Adenoviral-mediated expression of Pcsk9 in mice results in a low-density lipoprotein receptor knockout phenotype. Proc Natl Acad Sci U S A 2004;101:7100-5.
4. Cohen J, Pertsemlidis A, Kotowski I, Graham R, Garcia CK, Hobbs HH. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. Nat Genet 2005;37:161-5. [Erratum, Nat Genet 2005;37:328.]
5. Berge KE, Ose L, Leren TP. Missense mutations in the PCSK9 gene are associated with hypocholesterolemia and possibly increased response to statin therapy. Arterioscler Thromb Vasc Biol 2006;26:1094-100.
6. Kotowski IK, Pertsemlidis A, Luke A, et al. A spectrum of PCSK9 alleles contributes to plasma levels of low-density lipoprotein cholesterol. Am J Hum Genet 2006;78:410-22.
7. Yue P, Averna M, Lin X, Schonfeld G. The c.43_44insCTG variation in PCSK9 is associated with low plasma LDL-cholesterol in a Caucasian population. Hum Mutat 2006;27:460-6.
8. Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. N Engl J Med 2006;354:1264-7.
9. Department of Health and Human Services. Lipid Research Clinics manual of laboratory operations: lipid and lipoprotein analysis (revised). Washington, DC: Government Printing Office, 1982. (Publication no. (NIH) 75-67815.)
10. Lagace TA, Curtis DE, Garuti R, et al.

Secreted PCSK9 decreases the number of LDL receptors in hepatocytes and in livers of parabiotic mice. J Clin Invest 2006; 116:2995-3005.
11. Chan JCY, Piper DE, Cao Q, et al. A proprotein convertase subtilisin/kexin type 9 neutralizing antibody reduces serum cholesterol in mice and nonhuman primates. Proc Natl Acad Sci U S A 2009;106:9820-5.
12. Olsson AG. A new statin: a new standard. Clin Cardiol 2001;24:Suppl:III-18–III-23.
13. Rashid S, Curtis DE, Garuti R, et al. Decreased plasma cholesterol and hypersensitivity to statins in mice lacking Pcsk9. Proc Natl Acad Sci U S A 2005;102: 5374-9.
14. Zhao Z, Tuakli-Wosornu Y, Lagace TA, et al. Molecular characterization of loss-of-function mutations in PCSK9 and identification of a compound heterozygote. Am J Hum Genet 2006;79:514-23.
15. Hooper AJ, Marais AD, Tanyanyiwa DM, Burnett JR. The C679X mutation in PCSK9 is present and lowers blood cholesterol in a Southern African population. Atherosclerosis 2007;193:445-8.
16. Demant T, Seeberg K, Bedynek A, Seidel D. The metabolism of lipoprotein(a) and other apolipoprotein B-containing lipoproteins: a kinetic study in humans. Atherosclerosis 2001;157:325-39.