Results of Bococizumab, A Monoclonal Antibody Against Proprotein Convertase Subtilisin/Kexin Type 9, from a Randomized, Placebo-Controlled, Dose-Ranging Study in Statin-Treated Subjects With Hypercholesterolemia

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Bococizumab is a humanized monoclonal antibody binding proprotein convertase subtilisin/kexin type 9, which may be a potential therapeutic option for reducing low-density lipoprotein cholesterol (LDL-C) levels in patients with hypercholesterolemia. In this 24-week, multicenter, double-blind, placebo-controlled, dose-ranging study (NCT01592240), subjects with LDL-C levels ≥80 mg/dl on stable statin therapy were randomized to Q14 days subcutaneous placebo or bococizumab 50, 100, or 150 mg or Q28 days subcutaneous placebo or bococizumab 200 or 300 mg. Doses of bococizumab were reduced if LDL-C levels persistently decreased to ≤25 mg/dl. The primary end point was the absolute change in LDL-C levels from baseline to week 12 after placebo or bococizumab administration. Continuation of bococizumab administration through to week 24 enabled the collection of safety data over an extended period. Of the 354 subjects randomized, 351 received treatment (placebo [n = 100] or bococizumab [n = 251]). The most efficacious bococizumab doses were 150 mg Q14 days and 300 mg Q28 days. Compared with placebo, bococizumab 150 mg Q14 days reduced LDL-C at week 12 by 53.4 mg/dl and bococizumab 300 mg Q28 days reduced LDL-C by 44.9 mg/dl; this was despite dose reductions in 32.5% and 34.2% of subjects at week 10 or 8, respectively. Pharmacokinetic/pharmacodynamic model-based simulation assuming no dose reductions predicted that bococizumab would lower LDL-C levels by 72.2 and 55.4 mg/dl, respectively. Adverse events were similar across placebo and bococizumab groups. Few subjects (n = 7; 2%) discontinued treatment because of treatment-related adverse events. In conclusion, bococizumab significantly reduced LDL-C across all doses despite dose reductions in many subjects. Model-based simulations predicted greater LDL-C reduction in the absence of bococizumab dose reduction. The Q14 days regimen is being evaluated in phase 3 clinical trials. © 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). **(Am J Cardiol 2015;115:1212−1221)**

The serine protease proprotein convertase subtilisin/kexin type 9 (PCSK9) regulates cholesterol homeostasis by binding to the epidermal growth factor−like A domain of the low-density lipoprotein receptor (LDLR), inducing its degradation.[1] LDL uptake is reduced, resulting in higher circulating levels of low-density lipoprotein cholesterol (LDL-C).[1] Gain-of-function mutations in PCSK9 increase LDL-C levels and the risk of cardiovascular events.[2] Conversely, loss-of-function PCSK9 mutations decrease plasma LDL-C and cardiovascular risk.[3] Bococizumab (RN316/PF-04950615) is a humanized IgG2Δa monoclonal antibody (mAb) that recognizes and binds to the LDLR-binding domain of PCSK9, thus preventing PCSK9-mediated degradation of LDLR, leading to improved LDL clearance and reduction of serum LDL-C levels.[4] In phase 1 and 2a clinical trials in hypercholesterolemic subjects, bococizumab reduced LDL-C levels by up to ∼75% and was generally well tolerated with few subjects discontinuing treatment because of adverse events (AEs).[5] This dose-ranging phase 2b study evaluated the LDL-C−lowering effect of subcutaneous doses of bococizumab administered every 2 weeks (Q14 days) or monthly (Q28 days). A unique aspect of the trial design was the incorporation of bococizumab dose reductions if persistent LDL-C values ≤25 mg/dl were achieved. To our knowledge, this is the first study of a PCSK9 inhibitor to report the impact

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See page 1220 for disclosure information.

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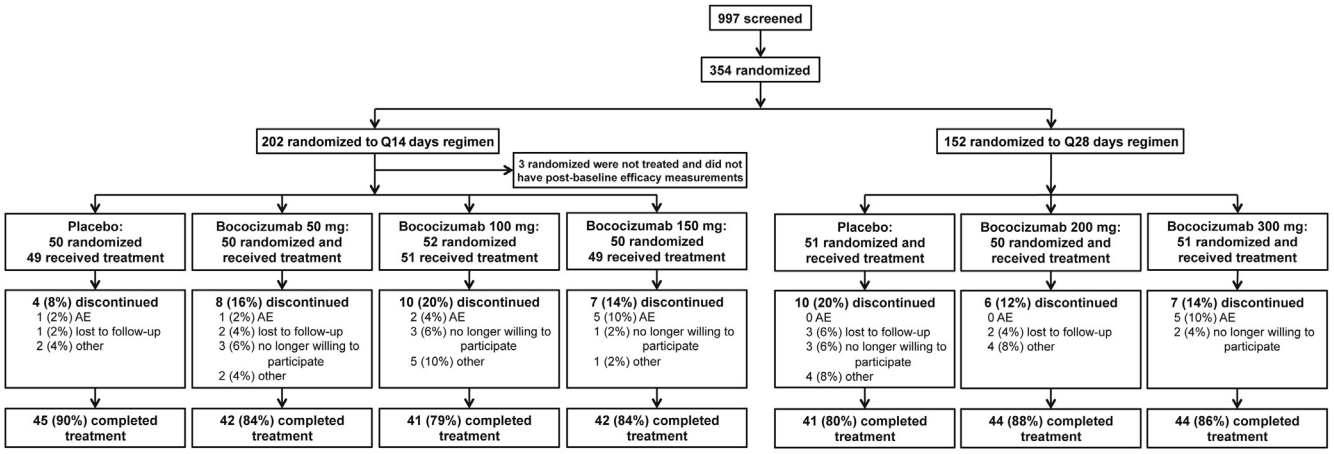


Figure 1. Patient flowchart. Subject allocation and disposition are shown for each of the bococizumab regimens, Q14 days and Q28 days.

Table 1
Baseline characteristics of all subjects randomized to placebo or bococizumab dosed every 14 days or every 28 days

Variable	Q14 days				Q28 days		
	Placebo* (n = 50)	Bococizumab (mg)			Placebo (n = 51)	Bococizumab (mg)	
		50 (n = 50)	100* (n = 52)	150* (n = 50)		200 (n = 50)	300 (n = 51)
Age (years)	61 ± 10	59 ± 11	62 ± 10	61 ± 10	58 ± 12	60 ± 10	60 ± 8
Male	25 (50%)	24 (48%)	26 (50%)	21 (42%)	29 (57%)	19 (38%)	25 (49%)
White	39 (78%)	33 (66%)	37 (71%)	36 (72%)	35 (69%)	39 (78%)	41 (80%)
Black	9 (18%)	15 (30%)	12 (23%)	10 (20%)	14 (27%)	9 (18%)	8 (16%)
Asian and other	2 (4%)	2 (4%)	3 (6%)	4 (8%)	2 (4%)	2 (4%)	2 (4%)
Weight (kg)	91 ± 22	91 ± 24	90 ± 21	90 ± 17	91 ± 21	88 ± 24	89 ± 19
BMI (kg/m^2)	32 ± 7	32 ± 8	32 ± 7	32 ± 6	31 ± 6	31 ± 7	31 ± 6
LDL-C (mg/dl)	109 ± 32†	108 ± 20	113 ± 26†	106 ± 18†	119 ± 45	106 ± 23	105 ± 22
TC (mg/dl)	189 ± 35†	186 ± 35	195 ± 34†	189 ± 25†	198 ± 45	185 ± 30	179 ± 30
TG (mg/dl)	124 (82, 180)†	109 (71, 155)	135 (96, 176)†	138 (100, 172)†	109 (80, 169)	123 (89, 158)	113 (79, 149)
Non−HDL-C (mg/dl)	137 ± 34†	134 ± 36	143 ± 31†	136 ± 23†	146 ± 45	133 ± 27	130 ± 29
HDL-C (mg/dl)	52 ± 14†	52 ± 16	51 ± 14†	53 ± 14†	53 ± 12	52 ± 14	49 ± 13
ApoB (mg/dl)	92 ± 20†	89 ± 22	97 ± 17†	90 ± 16†	97 ± 28	91 ± 16	87 ± 16
ApoA-I (mg/dl)	151 ± 25†	148 ± 24	149 ± 25†	151 ± 30†	150 ± 22	151 ± 26	145 ± 25
Lp(a) (mg/dl)	17 (5, 62)†	25 (8, 64)	18 (6, 66)†	22 (7, 86)†	18 (8, 48)	15 (5, 60)	31 (7, 60)
PCSK9 (ng/ml)	305 (260, 344)†	298 (257, 329)‡	314 (238, 363)‡	339 (308, 400)‡	301 (233, 349)†	317 (271, 378)†	299 (252, 364)
Baseline statin dose§							
Low	26 (52%)	25 (50%)	22 (42%)	20 (40%)	28 (55%)	23 (46%)†	22 (43%)†
High	24 (48%)	25 (50%)	30 (58%)	30 (60%)	23 (45%)	26 (52%)†	28 (55%)†

Values are mean ± SD, median (Q1, Q3), or n (%).

ApoA-I = apolipoprotein A-I; apoB = apolipoprotein B; BMI = body mass index; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; Lp(a) = lipoprotein(a); n = number of subjects randomized; SD = standard deviation; TC = total cholesterol; TG = triglycerides.

* Three subjects (1 each from the placebo, 100-mg, and 150-mg Q14 days groups) were randomized but did not receive treatment.

† Data are missing for 1 subject in each of these groups.

‡ Data are missing for 2 subjects in each of these groups.

§ Low statin dose defined as atorvastatin ≤10 mg, rosuvastatin ≤5 mg, simvastatin ≤20 mg, pravastatin ≤40 mg, lovastatin ≤80 mg, and fluvastatin ≤40 mg daily; high statin dose defined as all higher doses of these statins.

on LDL-C reduction when a dose reduction strategy is used in an effort to prevent extremely low levels of LDL-C. This report is the first full publication of bococizumab data from the phase 2 clinical trial program.

Methods

A more detailed description on the inclusion and exclusion criteria, study design, dose selection, drug administration, and laboratory evaluations can be found in the Supplementary Methods.

Enrolled subjects included men and women ≥18 years with hypercholesterolemia, on stable statin therapy (>6 weeks before screening), with a fasting LDL-C ≥80 mg/dl, and triglycerides ≤400 mg/dl. Subjects were excluded if they had a cardiovascular event during the previous 6 months, received treatment with systemic corticosteroids or an mAb during the previous 6 months, had

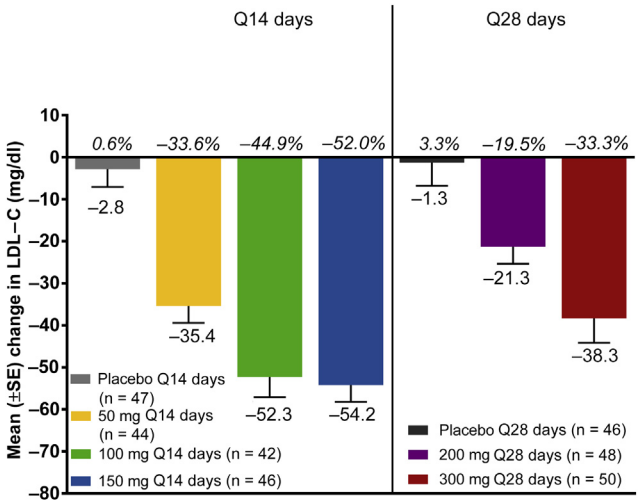


Figure 2. Mean absolute change from baseline in LDL-C at week 12. The placebo and bococizumab Q14 days and Q28 days dose groups are shown, with the corresponding mean percent changes from baseline in italics.

congestive heart failure (New York Heart Association class III or IV), poorly controlled diabetes mellitus or hypertension, or diagnosis of cancer, human immunodeficiency virus, or other serious diseases.

This 24-week, multicenter, randomized, double-blind, placebo-controlled, parallel-group, dose-ranging phase 2b study (NCT01592240) was conducted from July 2012 to May 2013. Subjects were randomized using an Interactive Voice Response System in a 1:1:1:1:1:1:1 ratio to Q14 days subcutaneous placebo or bococizumab 50, 100, or 150 mg or Q28 days subcutaneous placebo or bococizumab 200 or 300 mg. Follow-up was for 6 to 8 weeks after the last dose of study drug. For both the Q14 days and Q28 days regimens, the bococizumab dose was reduced if LDL-C levels decreased to ≤25 mg/dl: the first dose reduction required 2 consecutive LDL-C levels ≤25 mg/dl; all further dose reductions required 1 LDL-C level ≤25 mg/dl. LDL-C data collected at 14 days after the dose provided information on whether dose reduction of bococizumab was required. For the Q14 days regimen, day 43 was the first opportunity for dose reduction. The bococizumab dose reduction sequence for this cohort was 150 mg → 100 mg → 50 mg → 25 mg → placebo or 100 mg → 50 mg → 25 mg → placebo or 50 mg → 25 mg → placebo. For the Q28 days regimen, day 57 was the first opportunity for dose reduction. The bococizumab dose reduction sequence for this cohort was 300 mg → 200 mg → 100 mg → 50 mg → placebo or 200 mg → 100 mg → 50 mg → placebo (Supplementary Figures 1 and 2). The study was conducted under the guidelines of the Declaration of Helsinki and the Good Clinical Practice requirements of the International Conference on Harmonization. Local institutional review boards approved the protocol, and all subjects provided written informed consent. An independent data monitoring committee reviewed all study data.

The primary end point was the absolute change in LDL-C from baseline to week 12 after treatment with placebo or bococizumab, with the primary statistical analysis reporting this change as the placebo-adjusted treatment difference. Secondary end points included percentage change in LDL-C and absolute change and percentage change in non-HDL-C, HDL-C, total cholesterol, triglycerides, apolipoprotein (apo) A-I, apoB, and lipoprotein (a) from baseline at 12 and 24 weeks. The average placebo-adjusted change in LDL-C from weeks 10 to 12 in the Q28 days bococizumab dose groups was assessed as a tertiary end point. Safety end points included the incidence of AEs, serious AEs, laboratory abnormalities, incidence of antidrug antibodies (ADAs), and injection site reactions through to week 24 and up to 8 weeks of post-treatment follow-up. Bococizumab dose groups were compared with their respective placebo group using a mixed-model repeated measures analysis with the dependent variable being change from baseline (in LDL-C for the primary analysis) and including the fixed-effect terms treatment group, study visit time point, baseline value, treatment × study visit time point interaction, and baseline value × study visit time point interaction. An unstructured covariance matrix was used for the within-subject errors. The full analysis set population included all randomized subjects. However, the mixed-model repeated measures used for the statistical analyses of efficacy data only incorporated subjects who had a baseline and at least 1 postbaseline efficacy measurement. The analyses of safety data included all subjects who had received at least 1 dose of study medication. For all efficacy analyses, subjects remained in the dose group to which they were randomized, regardless of any subsequent dose reductions.

Demographic and baseline data were summarized as mean ± SD for continuous variables with the exception of triglyceride, lipoprotein (a), and PCSK9 values, which were not normally distributed and, therefore, presented as median (Q1 and Q3), and n (%) for categorical variables. Total cholesterol and triglyceride levels were assayed using standard enzymatic methods. LDL-C was measured by Friedewald and reflex ultracentrifugation for LDL-C levels ≤25 mg/dl.

Because of protocol-stipulated dose reductions of bococizumab in this study, a population pharmacokinetic/pharmacodynamic (PK/PD) model[6] was developed using data from 7 phase 1 and phase 2 clinical studies to construct a predictor of bococizumab LDL-C lowering in the absence of dose reductions. The population PK/PD data set consisted of 7,574 bococizumab PK observations and 10,177 LDL-C measurements from 674 subjects. The model accounted for dose interruptions or dose reductions implemented in the phase 2 trials and any missed doses that may have occurred during the trials. A 2-compartment PK model with parallel first-order and nonlinear (Michaelis-Menten) elimination was linked to an indirect PD response model describing LDL-C response. Performance of the final PK/PD model was verified with model diagnostics and visual predictive checks and was found to accurately capture the observed pharmacokinetics of bococizumab and LDL-C response in all studies and in this phase 2b study, which included dose reductions triggered by LDL-C levels ≤25 mg/dl. Clinical trial simulations using subject demography data from this study were performed with the final PK/PD model to estimate the expected LDL-C response in this phase 2b study assuming no missed doses or dose reductions.

Results

A total of 354 subjects were randomized and 351 received treatment with either placebo (n = 100) or bococizumab (n = 251) (Figure 1). The 3 subjects who were

Table 2
Lipid efficacy outcomes from baseline at week 12 in subjects randomized to placebo or bococizumab dosed every 14 days or every 28 days*

Variable	Q14 days				Q28 days		
	Placebo (n = 47)	Bococizumab (mg)			Placebo (n = 46–47)	Bococizumab (mg)	
		50 (n = 43–44)	100 (n = 42–44)	150 (n = 46)		200 (n = 48)	300 (n = 50)
LDL-C							
Mean (SD) change, mg/dl	−2.8 (29.2)	−35.4 (26.6)	−52.3 (31.3)	−54.2 (27.0)	−1.3 (37.2)	−21.3 (28.0)	−38.3 (41.3)
Mean (95% CI) placebo-adjusted change, mg/dl	—	−34.3 (−45.1, −23.5)	−45.1 (−55.9, −34.2)	−53.4 (−64.1, −42.7)	—	−27.6 (−40.5, −14.7)	−44.9 (−57.7, −32.1)
P-value	—	<0.001	<0.001	<0.001	—	<0.001	<0.001
Mean (SD) percent change, %	0.6 (25.5)	−33.6 (23.3)	−44.9 (23.4)	−52.0 (24.7)	3.3 (25.0)	−19.5 (26.6)	−33.3 (35.2)
Mean (95% CI) placebo-adjusted percent change, %	—	−35.0 (−44.9, −25.1)	−42.3 (−52.3, −32.3)	−53.1 (−63.0, −43.3)	—	−27.0 (−38.3, −15.7)	−41.1 (−52.3, −29.9)
P-value	—	<0.001	<0.001	<0.001	—	<0.001	<0.001
Total cholesterol							
Mean (SD) change, mg/dl	−5.6 (29.6)	−35.8 (24.7)	−52.7 (32.7)	−58.6 (32.9)	−0.4 (40.0)	−19.5 (27.4)	−37.6 (43.6)
Mean (95% CI) placebo-adjusted change, mg/dl	—	−29.7 (−41.8, −17.6)	−42.5 (−54.5, −30.4)	−55.6 (−67.6, −43.6)	—	−24.8 (−38.9, −10.7)	−45.6 (−59.8, −31.4)
P-value	—	<0.001	<0.001	<0.001	—	<0.001	<0.001
Mean (SD) percent change, %	−2.4 (14.4)	−19.0 (12.3)	−26.5 (14.9)	−31.6 (18.0)	1.2 (16.6)	−10.5 (15.3)	−19.4 (22.1)
Mean (95% CI) placebo-adjusted percent change, %	—	−15.7 (−22.1, −9.3)	−22.2 (−28.5, −15.8)	−30.1 (−36.5, −23.8)	—	−13.8 (−20.9, −6.7)	−23.8 (−30.9, −16.7)
P-value	—	<0.001	<0.001	<0.001	—	<0.001	<0.001
HDL-C							
Mean (SD) change, mg/dl	0.1 (7.0)	1.7 (7.1)	2.1 (7.5)	1.0 (7.5)	−1.0 (7.4)	3.4 (9.2)	3.1 (7.7)
Mean (95% CI) placebo-adjusted change, mg/dl	—	1.6 (−1.4, 4.6)	1.7 (−1.2, 4.7)	0.5 (−2.4, 3.5)	—	4.5 (1.2, 7.7)	3.9 (0.6, 7.1)
P-value	—	0.281	0.251	0.724	—	0.007	0.019
Mean (SD) percent change, %	0.8 (14.6)	3.9 (15.0)	3.9 (13.2)	2.7 (12.7)	−0.4 (14.0)	7.1 (17.1)	6.9 (16.9)
Mean (95% CI) placebo-adjusted percent change, %	—	3.4 (−2.4, 9.2)	2.6 (−3.1, 8.4)	1.4 (−4.4, 7.1)	—	7.7 (1.3, 14.0)	6.5 (0.2, 12.8)
P-value	—	0.246	0.371	0.643	—	0.018	0.043
Triglycerides†							
Median (Q1, Q3) change, mg/dl	−18.0 (−52.0, 13.0)	−12.0 (−40.0, 9.0)	−13.0 (−43.5, 10.0)	−21.0 (−43.0, 9.0)	5.0 (−23.0, 32.0)	−9.0 (−35.0, 11.5)	−14.5 (−51.0, 13.0)
Median (Q1, Q3) percent change, %	−14.5 (−32.5, 11.8)	−14.1 (−28.5, 7.8)	−14.8 (−35.6, 10.7)	−18.6 (−34.5, 5.0)	3.7 (−11.3, 31.1)	−7.6 (−24.2, 14.7)	−13.8 (−32.7, 11.3)

(*continued*)

Table 2
(*continued*)

Variable	Q14 days				Q28 days		
	Placebo (n = 47)	Bococizumab (mg)			Placebo (n = 46–47)	Bococizumab (mg)	
		50 (n = 43–44)	100 (n = 42–44)	150 (n = 46)		200 (n = 48)	300 (n = 50)
Non–HDL-C							
Mean (SD) change, mg/dl	−5.7 (26.9)	−37.5 (22.4)	−55.0 (32.8)	−59.6 (33.2)	0.6 (38.0)	−22.9 (29.1)	−40.7 (44.9)
Mean (95% CI) placebo-adjusted change, mg/dl	—	−31.1 (−42.6, −19.6)	−44.1 (−55.6, −32.6)	−55.6 (−67.0, −44.2)	—	−29.3 (−43.5, −15.1)	−48.6 (−62.7, −34.4)
P-value	—	<0.001	<0.001	<0.001	—	<0.001	<0.001
Mean (SD) percent change, %	−2.3 (20.2)	−28.3 (15.8)	−38.5 (21.4)	−44.9 (24.3)	2.8 (20.3)	−17.3 (22.3)	−28.7 (30.9)
Mean (95% CI) placebo-adjusted percent change, %	—	−24.6 (−33.3, −15.9)	−33.2 (−41.9, −24.6)	−42.8 (−51.4, −34.2)	—	−22.6 (−32.4, −12.8)	−34.7 (−44.5, −24.9)
P-value	—	<0.001	<0.001	<0.001	—	<0.001	<0.001

Values are mean (SD), mean (95% CI), or median (Q1, Q3).

CI = confidence interval; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; n = number of subjects treated; SD = standard deviation; TC = total cholesterol; TG = triglycerides.

* These values include subjects who had their bococizumab dose reduced in the weeks prior to week 12.

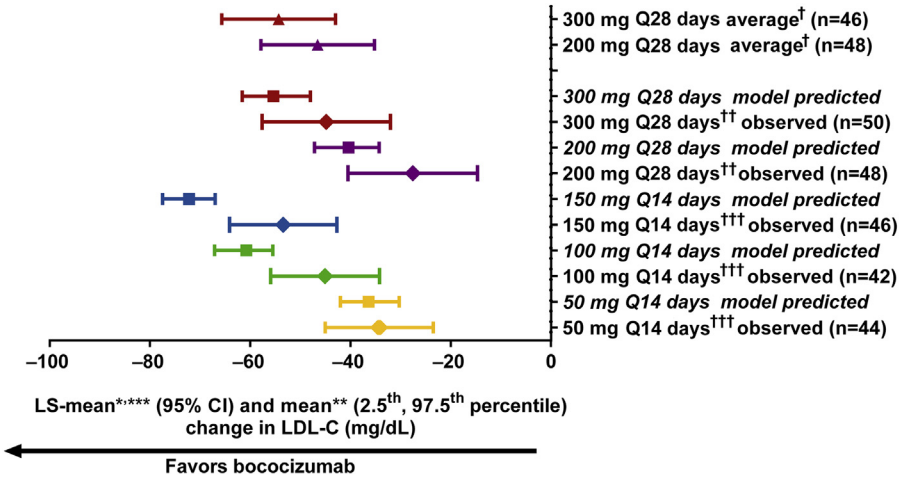
† The mixed model repeated measures analysis was not undertaken for triglycerides.

randomized but not treated did not have postbaseline efficacy measurements and so were excluded from further analysis (1 each from the placebo, 100-mg and 150-mg Q14 days groups). A total of 299 subjects (85% of those treated) completed treatment. The baseline demographics and clinical characteristics of subjects randomized to the various treatment groups were well balanced, including baseline LDL-C levels (Table 1).

Overall, 16%, 35%, 44%, and 39% of subjects randomized to bococizumab 100 mg Q14 days, 150 mg Q14 days, 200 mg Q28 days, and 300 mg Q28 days, respectively, had their dose reduced at any time during the study. No dose reductions occurred in the bococizumab 50-mg Q14 days group. At the week 10 visit (the Q14 days dosing time point before LDL-C measurement for the primary end point), 16% of available subjects in the bococizumab 100 mg Q14 days and 33% of subjects in the 150-mg Q14 days dose groups had their dose reduced (Supplementary Figure 3). At the week 8 visit (the Q28-days dosing time point before LDL-C measurement for the primary end point), 30% of available subjects in the bococizumab 200 mg Q28 days and 34% of subjects in the 300-mg Q28 days group had their dose reduced (Supplementary Figure 3).

Starting at week 2, Q14 days and Q28 days bococizumab dose regimens significantly reduced LDL-C levels, and this effect was sustained for the duration of the study. At week 12, the primary end point of mean absolute change from baseline in LDL-C was greatest in subjects receiving bococizumab 150 mg Q14 days (Figure 2, Table 2). The placebo-adjusted mean change from baseline in LDL-C in all subjects, including those with dose reductions, at week 12 ranged from −34.3 to −53.4 mg/dl for those receiving bococizumab Q14 days and from −27.6 to −44.9 mg/dl for those receiving bococizumab Q28 days (Figure 3, Table 2). This corresponded to placebo-adjusted mean percent changes from baseline of −35.0% to −53.1% for those receiving bococizumab Q14 days and −27.0% to −41.1% for those receiving bococizumab Q28 days (Table 2). The PK/PD model-predicted, placebo-adjusted mean change from baseline in LDL-C at week 12 ranged from −36.4 mg/dl for bococizumab 50 mg Q14 days to −72.2 mg/dl for bococizumab 150 mg Q14 days (Figure 3).

Before bococizumab dose reductions (up to week 6 for Q14 days and week 8 for Q28 days), placebo-adjusted mean reductions in LDL-C were greater than those observed at week 12 (Supplementary Figure 4). No dose reductions occurred in the 50-mg Q14 days group, and reductions in LDL-C were similar at weeks 8, 10, and 12 (−34.8, −33.3, and −34.3 mg/dl, respectively; Supplementary Figure 4). For the 100- and 150-mg Q14 days groups, reductions in LDL-C were maximal at week 6 (−59.9 mg/dl) and 8 (−66.9 mg/dl), respectively (Supplementary Figure 4). For the Q28 days regimen, changes were greatest at week 4 for both the 200-mg (−30.9 mg/dl) and the 300-mg groups (−54.9 mg/dl) (Supplementary Figure 4). Reductions in LDL-C were maintained between doses in subjects who received bococizumab Q14 days but not in those receiving bococizumab Q28 days (Figure 4). The individual subject LDL-C responses for subjects receiving bococizumab 150 mg Q14 days who had their dose reduced but did not miss a dose are depicted in Supplementary Figure 5, together with the percentage of these subjects on each bococizumab dose at each dosing visit, highlighting the effect of bococizumab dose reduction on LDL-C levels.



*LS-mean (95% CI) change calculated using a repeated measures model for longitudinal data. **Population pharmacokinetic/pharmacodynamic model predictions of mean (2.5th, 97.5th percentile) change for 100 trials (50 subjects/arm) using baseline demographics from this study. ***LS-mean (95% CI) average placebo-adjusted change from days 71 to 85 in the Q28 days bococizumab dose groups. †Placebo Q28 days average: n=44. ††Placebo Q28 days: n=46. †††Placebo Q14 days: n=47.

Figure 3. Placebo-adjusted mean change from baseline in LDL-C at week 12. A comparison of observed* (with dose reduction), model-predicted** (assuming no dose reduction), and Q28 days average*** (with dose reduction) data are shown. LS = least squares.

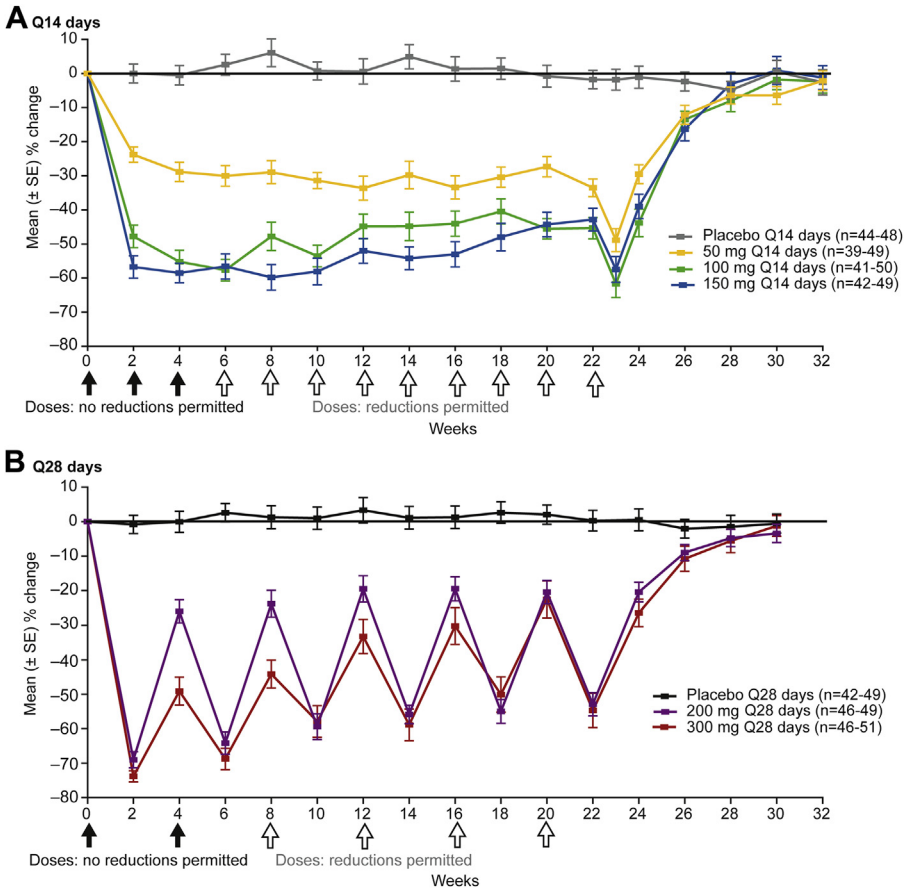


Figure 4. Mean percentage change from baseline in LDL-C. Change over time is shown for the *(A)* Q14 days and *(B)* Q28 days placebo and bococizumab dose groups.

Starting at week 2, bococizumab therapy produced dose-related reductions in non−HDL-C, which were maintained in the Q14 days 50-mg group throughout the 24-week treatment period. For the Q14 days 100- and 150-mg groups, change from baseline in non−HDL-C diminished after weeks 8 to 10, which may have been because of dose

Table 3
Percentage of subjects with all-causality and treatment-related* adverse events following randomization to placebo or bococizumab dosed every 14 days, or placebo or bococizumab dosed every 28 days

Variable	Q14 days				Q28 days		
	Placebo (n = 49)	Bococizumab (mg)			Placebo (n = 51)	Bococizumab (mg)	
		50 (n = 50)	100 (n = 51)	150 (n = 49)		200 (n = 50)	300 (n = 51)
AEs	84% [29%]	74% [24%]	84% [31%]	82% [37%]	80% [16%]	90% [28%]	82% [33%]
Serious AEs	14% [0%]	8% [0%]	4% [0%]	8% [2%]	4% [0%]	10% [0%]	8% [0%]
Discontinuation of treatment due to AE	2% [0%]	2% [2%]	4% [0%]	10% [8%]	0%	0%	10% [4%]
Most frequent AEs (≥∼10%)							
Nasopharyngitis	12% [0%]	16% [0%]	14% [0%]	10% [0%]	14% [0%]	12% [0%]	12% [0%]
Upper respiratory tract infection	14% [0%]	8% [0%]	10% [0%]	6% [2%†]	18% [0%]	10% [2%]	6% [0%]
Diarrhea	8% [0%]	6% [0%]	8% [4%]	10% [2%]	4% [0%]	4% [0%]	8% [0%]
Urinary tract infection	6% [0%]	2% [0%]	10% [0%]	8% [2%]	2% [0%]	4% [0%]	4% [0%]
Bronchitis	8% [0%]	4% [0%]	6% [0%]	10% [0%]	6% [0%]	6% [0%]	4% [0%]
Arthralgia	4% [0%]	0%	12% [2%]	8% [0%]	6% [0%]	0%	4% [0%]
Injection site erythema	4% [4%]	4% [2%]	8% [6%]	10% [10%]	0%	8% [8%]	10% [8%]
Injection site reaction	2% [2%]	6% [6%]	2% [0%]	12% [8%]	2% [2%]	2% [2%]	10% [8%]
Gastroesophageal reflux disease	2% [0%]	0%	10% [0%]	4% [0%]	2% [0%]	2% [0%]	0%
Cough	10% [0%]	4% [0%]	2% [0%]	4% [0%]	2% [0%]	2% [0%]	2% [0%]
Anemia	2% [2%]	0%	10% [0%]	4% [0%]	0%	4% [0%]	0%

Values are % of subjects with all-causality [treatment-related] AEs.
n = number of subjects treated.
* Investigator-determined treatment relatedness.
† Specified as viral upper respiratory tract infection.

reductions. The mean percent changes from baseline in non−HDL-C at week 12 are provided in Table 2. Bococizumab increased HDL-C at weeks 12 (Table 2) and 24. For both bococizumab dosing intervals, statistically significant dose-dependent decreases in mean serum levels of total cholesterol (Table 2) and apoB (Supplementary Table 1), but not apoA-I (Supplementary Table 1), relative to placebo were observed. There was a trendwise reduction in serum triglyceride levels with respect to bococizumab dose level across both dosing intervals tested (Table 2).

The percentage of subjects reporting AEs or serious adverse events (SAEs) was similar across placebo and bococizumab treatment groups (Table 3). Irrespective of investigator-determined causality, nasopharyngitis and upper respiratory tract infections were the most frequently reported all-causality AEs and showed a similar incidence in the placebo and bococizumab groups (Table 3). The most frequently reported treatment-related AEs were injection site events, of which the most common was injection site erythema (Table 3). One bococizumab 150 mg Q14 days subject experienced 2 concurrent SAEs that were considered treatment related: a viral upper respiratory tract infection and severe dyspnea. One subject—a 74-year-old man in the bococizumab 50-mg Q14 days treatment group—died from an accidental head injury after a fall during the follow-up period (57 days after last dose). This death was not considered related to study treatment.

Overall, 14 subjects (4%) discontinued treatment because of all-causality AEs, with more subjects in the higher than the lower dose bococizumab groups stopping treatment (Table 3); there was no pattern or trend in the types of AEs that led to discontinuation of treatment. Seven subjects (2%) discontinued treatment because of treatment-related AEs (Table 3). These were from the 50 mg Q14 days group, urticaria (n = 1); from the 150-mg Q14 days group, asthenia and fatigue, viral upper respiratory tract infection, renal impairment, and lip swelling (n = 1 for each AE); and from the 300-mg Q28 days group, abdominal pain and injection site reaction (n = 1 for each AE).

The incidence of AEs was similar among bococizumab-treated subjects who had an LDL-C ≤25 mg/dl during the study compared with those who did not (∼80% to 90% across most groups), and no trend was seen in the type of AEs reported between the 2 subgroups (Supplementary Table 2). Positive ADA titers were found in 18 of 251 bococizumab-treated subjects (7%), with one 150-mg Q14 days bococizumab-treated subject exhibiting reduced LDL-C lowering (0.4% of bococizumab-treated subjects). In this subject, ADAs were detected on day 113, and subsequently, LDL-C levels increased toward baseline (Supplementary Results). Otherwise, little variation was found in LDL-C response between subjects with and without ADAs (data not shown). The AEs reported in subjects with ADAs were similar to those observed in subjects without ADAs, with no signs or symptoms of hypersensitivity associated with positive ADA titers. Two subjects receiving bococizumab (0.8%) experienced nonserious AEs of memory loss (Supplementary Results). The incidences of other AEs of special interest are listed in Supplementary Table 3. Myalgia incidence across Q14 days dose groups was placebo, 2%; bococizumab 50 mg, 2%; bococizumab 100 mg, 4%; and bococizumab 150 mg, 0%. For Q28 days doses, the incidence of myalgia was placebo, 2%; bococizumab 200 mg, 0%; and bococizumab 300 mg, 0%. The incidence of creatine kinase abnormalities (>2× the upper limit of normal) was similar across placebo (12%

to 15%) and bococizumab groups (6% to 14%). No subjects had elevated alanine aminotransferase (ALT) or aspartate aminotransferase levels >3× the upper limit of normal; there were no cases of Hy's law.

Discussion

In this phase 2b dose-ranging study of subjects with hypercholesterolemia on stable doses of statin, compared with placebo, bococizumab significantly reduced LDL-C levels across all doses. This is despite protocol-stipulated dose reductions in a large proportion of subjects. Bococizumab Q14 days and Q28 days dosing regimens produced placebo-adjusted reductions in LDL-C at week 12 of up to 53.4 mg/dl (53.1%) for the 150-mg Q14 days dose and 44.9 mg/dl (41.1%) for the 300-mg Q28 days dose.

Bococizumab doses of up to 150 mg Q14 days and 300 mg Q28 days were generally well tolerated. Other than mild injection site reactions with bococizumab, AEs or SAEs were similar across placebo and bococizumab treatment groups, and few subjects (n = 7; 2%) discontinued treatment because of treatment-related AEs. Importantly, the incidence and type of AEs were similar among subjects in whom LDL-C levels decreased to ≤25 mg/dl and those in whom LDL-C remained >25 mg/dl, in line with a similar analysis of evolocumab from the Open-Label Study of Long-Term Evaluation Against LDL-C (OSLER) study.[7] Positive ADA titers were detected in ∼7% of bococizumab-treated subjects, with LDL-C lowering reduced in one of these subjects (0.4% of bococizumab-treated subjects). Recent clinical trials of other PCSK9 inhibitors have found ADA incidences of up to 11.5% in both phase 2 and phase 3 studies.[8,9] However, the absence of standardized methodologies for the analysis of ADAs makes a meaningful comparison of ADA rates across disparate studies or antibodies problematic.[10] A more comprehensive assessment of the proportion of patients who develop ADAs and the impact these may have on the LDL-C–lowering efficacy and safety of bococizumab, and the wider PCSK9 inhibitor class, will be evaluated in larger longer term phase 3 trials.

Although the lower levels of LDL-C achievable with currently available lipid-lowering therapies do not appear to be harmful,[11] the safety of the very low levels of LDL-C achievable with PCSK9 inhibitors, particularly when sustained over a prolonged period, is unclear,[5,11] although recently published safety data from longer term trials of evolocumab are encouraging.[7,12] Previous epidemiologic studies and data from earlier statin trials suggested an association between lower levels of LDL-C and increased risk of cancer, hemorrhagic stroke, and noncardiac death[5,11]: an association that has not been supported by subsequent outcome studies and large meta-analyses.[11,13] Concerns have also been raised regarding possible cognitive symptoms associated with very low LDL-C levels.[5,14] In this study, 2 subjects receiving bococizumab experienced a nonserious AE of memory loss; however, these subjects were both receiving concomitant medications (a statin and/or zolpidem tartrate) previously associated with memory loss (Supplementary Results). The phase 3 clinical trials of PCSK9 inhibitors will demonstrate whether prolonged very low LDL-C values (or PCSK9 inhibition) have any impact on cognition.

The advent of PCSK9 inhibitors has meant that LDL-C levels of ≤25 mg/dl, and even <10 mg/dl, can be achieved within a few weeks of the first dose. Owing to the limited data available on the physiological effects of very low levels of LDL-C, a unique aspect of this study was the protocol-stipulated bococizumab dose reductions in subjects with persistent LDL-C levels ≤25 mg/dl. Up to 44% of subjects receiving higher doses of bococizumab had dose reductions because of LDL-C levels ≤25 mg/dl, indicating that some subjects who showed the greatest response to bococizumab had their dose reduced (Supplementary Figure 6), thus tempering individual LDL-C responses (Supplementary Figure 5) and the overall group mean LDL-C reductions observed at week 12. Reductions in LDL-C were greater before dose reduction: for the 100- and 150-mg Q14 days dose groups, LDL-C reductions at weeks 6 and 8 were ∼13 to 15 mg/dl greater than those at week 12; for the 200- and 300-mg Q28 days dose groups, the respective LDL-C reductions at week 4 were ∼3 and ∼10 mg/dl greater than those at week 12. However, factors other than dose reduction, such as missed doses of study medication, may have had an effect on the outcomes of this study (Supplementary Figure 6). A population PK/PD model was used to predict the expected LDL-C response assuming no dose reductions or missed doses. This analysis suggested that LDL-C would have been lowered at week 12 by an additional ∼2 to 19 mg/dl with bococizumab Q14 days and by an additional ∼11 to 13 mg/dl with bococizumab Q28 days, in the absence of dose reductions or missed doses.

Recent studies have highlighted the importance of stable, sustained reductions in cardiovascular risk factors such as blood pressure[15] and LDL-C[16] for optimizing cardiovascular event reduction. For LDL-C, an analysis of the Treating to New Targets trial demonstrated that visit-to-visit variability in LDL-C was an independent predictor of cardiovascular risk.[16] Each 1-SD increase in LDL-C variability, measured as the average absolute difference between successive values, increased the risk of any coronary event by 16% and any cardiovascular event by 11%, independent of statin dose and achieved LDL-C.[16] This is a consideration with the dosing of PCSK9 mAbs, where high-dose monthly regimens can produce substantial fluctuations in LDL-C levels between doses, leading to large visit-to-visit LDL-C variability,[17–20] as confirmed in this study. The magnitude of LDL-C reductions from baseline achieved with bococizumab—up to ∼60% at week 8 with 150 mg Q14 days and up to ∼74% at the week 2 nadir with 300 mg Q28 days—was comparable with that reported for other PCSK9 inhibitors currently in clinical development.[9,17–22] Although greater maximal reductions in LDL-C were seen with higher doses of bococizumab administered Q28 days, these reductions were not as well maintained between doses. After the decrease in LDL-C levels 2 weeks after administration of each Q28 days bococizumab dose, LDL-C levels gradually increased in the 2 weeks post-nadir, resulting in a "saw-tooth" pattern (Figure 4) that has also been observed with monthly dosing of evolocumab[17,18] and alirocumab.[19,20] Lower doses of bococizumab administered Q14 days eliminated the cycle of LDL-C fluctuation seen with monthly dosing while still resulting in significant LDL-C reductions (Figure 4). The

clinical significance of LDL fluctuations because of different dosing regimens is unknown. In the present study, LDL-C levels at week 2 were reduced by ~70% from baseline with high-dose Q28 days bococizumab therapy, with LDL-C values falling to ≤25 mg/dl in ~40% of subjects in the 2 weeks after the first dose. Together, these observations support a Q14 days rather than a Q28 days bococizumab dosing regimen for phase 3 studies.

The achievement of substantial reductions in LDL-C levels after PCSK9 inhibition is likely to be of clinical benefit, particularly in patients at high cardiovascular risk who continue to have elevated LDL-C on maximally tolerated statin therapy. However, there are challenges associated with using a new therapy, such as PCSK9 inhibitors, that can lead to very low levels of LDL-C. First is a reliable method for measuring and reporting very low LDL-C values by clinical laboratories. Although the Friedewald equation has been routinely used to calculate LDL-C for >40 years, it can underestimate low levels of LDL-C compared with direct measurement after ultracentrifugation (used in this study for LDL-C levels ≤25 mg/dl).[23,24] Newer, more accurate methods for calculation of LDL-C from a standard lipid profile have been proposed, most recently by Martin et al.[25] External validation of this new method may provide clinicians with a reliable and inexpensive method to assess LDL-C at the very low levels likely to be achieved with PCSK9 inhibition.

Significant rapid reductions in LDL-C levels with PCSK9 inhibitors will also present physicians with a choice between starting with a low dose and up-titration, if necessary, or with high doses and subsequent dose reduction if required or ignoring LDL-C levels after initiation of therapy. Intensive initial therapy is often preferred to reduce cardiovascular outcomes, particularly in high-risk patients, and provides an argument for high initial doses. The LDL-C level at which dose reduction is triggered is crucial to attain maximal clinical benefit. This study used a threshold LDL-C of 25 mg/dl for dose reduction; however, LDL-C values ≤25 mg/dl were frequently achieved, and dose reduction occurred frequently and limited efficacy. LDL-C levels of ≤25 mg/dl were not associated with harm in this study and do not appear to be associated with harm in trials of other PCSK9 inhibitors conducted to date.[5,7,14] Hence, future studies may need to consider lowering the LDL-C threshold at which dose reduction is initiated (e.g., ≤10 mg/dl).

The clinical efficacy and safety of PCSK9 inhibitors look promising based on the results of phase 3 lipid-lowering trials.[9,12,21,22] However, the results of cardiovascular outcome trials are needed to confirm the long-term safety of PCSK9 inhibitors and the efficacy for reducing cardiovascular events. Although this phase 2 dose-ranging study excluded subjects with recent cardiovascular events and did not require a high cardiovascular risk status for entry (and, thus, may not represent a patient population at highest cardiovascular risk), the bococizumab phase 3 Studies on PCSK9 Inhibition and the Reduction of vascular Events (SPIRE) program consists of additional trials assessing the lipid-lowering efficacy of bococizumab and 2 cardiovascular outcome studies that include high-risk patients. SPIRE-1 (NCT01975376) will assess whether lowering LDL-C to levels well below previously recommended targets will lead to further reduction in cardiovascular events. This study includes high-risk patients with baseline LDL-C levels ≥70 to <100 mg/dl. SPIRE-2 (NCT01975389) will evaluate the efficacy and safety of bococizumab in a range of high-risk patients who have not achieved LDL-C levels <100 mg/dl despite high-dose statin therapy or who are partially or completely statin intolerant.

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Supplementary Data

Supplementary data related with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.amjcard.2015.02.006.

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