Cardiol Ther (2019) 8:91–102
https://doi.org/10.1007/s40119-019-0133-6

ORIGINAL RESEARCH

Effects of Evolocumab on the ApoA1 Remnant Ratio: A Pooled Analysis of Phase 3 Studies

Heidi T. May · Joseph B. Muhlestein · Yuhui Ma · J. Antonio G. López · Blai Coll · John Nelson

Received: January 11, 2019 / Published online: March 9, 2019

ABSTRACT

Introduction: The apolipoprotein A1 (apoA1) remnant ratio has been identified as an independent cardiovascular (CV) risk factor. Higher apoA1 remnant ratios may predict lower CV risk in some patients. This analysis aimed to evaluate the effects of evolocumab on the change from baseline in the apoA1 remnant ratio compared with placebo.

Methods: This pooled post hoc analysis included 2464 patients with mixed dyslipidemia treated with evolocumab 140 mg every 2 weeks (Q2W) or 420 mg once monthly (QM) in three phase 3 evolocumab trials. The apoA1 remnant ratio was calculated by dividing apoA1 by the difference between non-high-density lipoprotein cholesterol (non-HDL-C) and low-density lipoprotein cholesterol (LDL-C). ApoA1 remnant ratio strata were generated using previously published tertile (< 4.7, 4.7–6.8, and > 6.8) and partitioning categories (< 3.6, 3.6–6.0, and > 6.0).

Results: The baseline apoA1 remnant ratio in evolocumab and placebo treatment arms was 7.1 and 7.3, respectively. At week 12, evolocumab 140 mg Q2W and 420 mg QM increased the apoA1 remnant ratio by 25.0% and 33.6%, respectively, versus placebo ($p < 0.0001$ for both groups). When patients were categorized by week 12 apoA1 remnant ratio thresholds (< 3.6 vs. > 3.6, and < 4.7 vs. > 4.7), those with higher week 12 apoA1 remnant ratios were significantly more likely to have also achieved a target non-HDL-C level of < 100 mg/dl. In the subset of women > 50 years of age, the proportion of patients at apoA1 remnant ratio thresholds < 3.6, 3.6–6.0, and > 6.0 at baseline shifted toward or remained at higher thresholds at week 12.

Conclusions: This post hoc analysis suggests that evolocumab increases the apoA1 remnant ratio.

Funding: Amgen Inc.

Plain Language Summary: Plain language summary available for this article.

Enhanced digital features To view enhanced digital features for this article go to https://doi.org/10.6084/m9.figshare.7751324.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s40119-019-0133-6) contains supplementary material, which is available to authorized users.

H. T. May (✉) · J. B. Muhlestein
Intermountain Heart Institute, Intermountain Medical Center, Murray, UT, USA
e-mail: heidi.may@imail.org

J. B. Muhlestein
Department of Medicine, University of Utah, Salt Lake City, UT, USA

Y. Ma · J. A. G. López · B. Coll
Amgen Inc., Thousand Oaks, CA, USA

J. Nelson
California Cardiovascular Institute, Fresno, CA, USA

Keywords: Evolocumab; Mixed dyslipidemia; Non-HDL-C; PCSK9; Remnant lipoprotein; Threshold; VLDL

PLAIN LANGUAGE SUMMARY

Evolocumab is a medication that is used to lower the risk of heart attack and stroke in adults with cardiovascular disease that is caused by atherosclerosis, also known as atherosclerotic cardiovascular disease or ASCVD. Atherosclerosis is the narrowing of blood vessels known as arteries from a buildup of plaque, usually made up of cholesterol and other fatty substances.

Evolocumab reduces LDL cholesterol, often called "bad" cholesterol.

Levels of LDL cholesterol are commonly used to determine a patient's risk of ASCVD. Lowering LDL generally reduces the risk of ASCVD. Still, LDL levels may not completely explain a person's risk.

The apoA1 remnant ratio is a possible additional indicator of the risk of ASCVD. The apoA1 remnant ratio is calculated in this study by dividing apoA1, a protective factor, by the difference between non-HDL and LDL cholesterol, risk factors for ASCVD.

Higher apoA1 remnant ratios may be tied to a lower risk of ASCVD. Previous studies have shown that the apoA1 remnant ratio may predict risk better than traditional indicators in women over 50.

This study found that evolocumab increased the apoA1 remnant ratio after 12 weeks of treatment compared with placebo. Higher apoA1 remnant ratios were able to identify patients who were more likely to reach goal levels of non-HDL.

INTRODUCTION

The pharmacologic focus of the prevention of cardiovascular (CV) events in the management of dyslipidemia has long been the lowering of low-density lipoprotein cholesterol (LDL-C) [1–3]; however, in some patients, lowering LDL-C may not account for the entirety of the risk [3–5]. In some patients with dyslipidemia, other markers may enhance CV risk prediction and complement standard lipid parameters [6, 7].

Elevated levels of remnant lipoproteins (RLP) correlate with risk for CV events and are being investigated as potential biomarkers for assessing CV risk in a select population of patients [8–10]. RLP are partially catabolized chylomicrons and very-low-density lipoprotein (VLDL) from which some triglycerides have been removed by the action of lipoprotein lipase and hepatic lipase [11]. RLP are primarily found in the VLDL/chylomicrons—intermediate-density lipoprotein (IDL) density range [12]. Higher levels of both RLP and IDL predicted incident coronary heart disease in a primary prevention population [13]. Higher triglyceride-rich lipoprotein cholesterol (TRL-C) levels were similarly associated with increased risk of major adverse cardiovascular events (MACE) in an unadjusted analysis of patients on 10 mg/day atorvastatin in a secondary prevention setting (TNT trial) [14]. Remnant lipoproteins are removed by receptors including the LDL receptor, LDL-receptor-related protein-1, and the VLDL receptor [15–17].

Apolipoprotein A1 (ApoA1), the principle protein component of high-density lipoprotein (HDL), has a protective effect for CV risk and therefore an inverse relationship with CV risk [18]. Beyond individual particle concentrations, ratios take into account the relationship between multiple markers. The apoA1 remnant ratio (apoA1/[$VLDL_3$-C + IDL-C]) measures the relative amount of the protective factor apoA1 and the risk factor, RLP. An increase in the ratio predicts a decrease in risk. The apoA1 remnant ratio provided better prediction of risk than traditional lipid markers in women over 50 years of age and African Americans [6, 19]. High-risk apoA1 remnant ratios were found in the majority of a cohort of very high-risk women over 55 years of age who met the National Lipid Association recommended treatment goals on statin therapy [20]. The effect of lipid-lowering therapy on the apoA1 remnant ratio has never been previously described. The purpose of this post hoc analysis was to examine the effects of evolocumab on

this ratio, including in women over 50 years of age.

Evolocumab is a human monoclonal antibody that targets proprotein convertase subtilisin/kexin type 9 (PCSK9), a serine protease that inhibits LDL receptor (LDLR) recycling [21]. PCSK9 reduces the recycling of LDLR by binding to the receptor along with LDL and targeting the receptor for lysosomal degradation [22]. This action leads to decreased LDLR density on the surface of hepatocytes, which in turn increases circulating LDL-C [23]. PCSK9 has been shown to degrade not only the LDLR, but also the VLDL receptor and the LDL-receptor–related protein 1 [24, 25].

By reversing those effects, evolocumab reduces LDL-C levels across a diverse range of patient populations [26], and reduces CV events [27], including in higher-risk populations, such as patients with diabetes [28] or peripheral artery disease [29]. In addition to LDL-C, evolocumab lowers remnant lipoproteins [30]; however, the effect of evolocumab on the apoA1 remnant ratio is unknown. The objective of this post hoc subanalysis of three evolocumab studies was to evaluate the effects of evolocumab treatment on the change from baseline in the apoA1 remnant ratio compared with placebo.

METHODS

Patient Characteristics

Patients with mixed dyslipidemia who were treated with evolocumab 140 mg every 2 weeks (Q2W) or 420 mg once every month (QM) from three completed phase 3 evolocumab trials of 12-week duration were included in the analysis [31–33]. Evolocumab biweekly and monthly doses have shown clinical equivalence [34]. The MENDEL-2 trial evaluated the safety and efficacy of evolocumab monotherapy versus placebo or ezetimibe on LDL-C reduction in patients with a 10-year Framingham risk score of $\leq$ 10% (NCT01763827) [31]. The RUTHERFORD-2 trial examined the safety and efficacy of evolocumab on LDL-C in patients with heterozygous familial hypercholesterolemia (HeFH; NCT01763918) [32]. The LAPLACE-2 trial tested the efficacy and safety of evolocumab in combination with statin therapy versus statin therapy alone or statin therapy plus ezetimibe alone on LDL-C levels in patients with hypercholesterolemia and mixed dyslipidemia (NCT01763866) [33]. These three trials were selected and pooled because they were of the same duration and utilized similar LDL-C inclusion criteria.

Patients included in this post hoc subanalysis were $\geq$ 18 years of age with fasting LDL-C $\geq$ 150 mg/dl (not receiving any statin therapy, including all patients in the MENDEL study), $\geq$ 100 mg/dl (not receiving intensive statin therapy), $\geq$ 80 mg/dl (receiving intensive statin therapy), and fasting triglycerides $\leq$ 400 mg/dl by central laboratory. Intensive statin use was defined as atorvastatin (40 mg or greater), rosuvastatin (20 mg or greater), simvastatin (80 mg), or any statin with ezetimibe.

All procedures performed in the trials reported in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the mentioned studies. Approval of institutional review boards was received for these trials and has been previously reported [31–33]. This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

Lipid Measurements

Blood samples for determining lipid levels (total cholesterol, LDL-C, HDL-C, non-HDL-C, apolipoprotein B (apoB), apoA1, lipoprotein(a) (Lp(a)), triglycerides, VLDL-C) were collected at baseline and weeks 2, 8, 10, and 12. LDL-C concentration was calculated using the Friedewald formula unless the calculated LDL-C was $<$ 40 mg/dl or triglycerides were $>$ 400 mg/dl, in which case, ultracentrifugation LDL-C was used. VLDL-C was

Table 1 Baseline demographics and clinical characteristics

Characteristic	Placebo (n = 821)	Evolocumab (n = 1643)	Total (n = 2464)
Age (years), mean (SD)	57 (11)	57 (11)	57 (11)
Women, n (%)	411 (50)	787 (48)	1198 (49)
Race/ethnicity, n (%)			
White	758 (92)	1506 (92)	2264 (92)
Hispanic/Latino	46 (6)	87 (5)	133 (5)
Black or African American	27 (3)	69 (4)	96 (4)
Asian	26 (3)	50 (3)	76 (3)
American Indian or Alaska native	0 (0)	2 (< 1)	2 (< 1)
Native Hawaiian or other Pacific Islander	3 (< 1)	1 (< 1)	4 (< 1)
Mixed race	0 (0)	3 (< 1)	3 (< 1)
Other	7 (1)	12 (< 1)	19 (1)
Coronary artery disease, n (%)	150 (18)	343 (21)	493 (20)
Cardiovascular risk factors, n (%)			
Current cigarette use	114 (14)	238 (15)	352 (14)
Type 2 diabetes mellitus	84 (10)	190 (12)	274 (11)
Family history of coronary heart disease[a]	186 (23)	390 (24)	576 (23)
Metabolic syndrome[b]	248 (30)	513 (31)	761 (31)
NCEP risk category, n (%)			
High risk	257 (31)	543 (33)	800 (33)
Moderately high risk	75 (9)	157 (10)	232 (9)
Moderate risk	228 (28)	492 (30)	720 (29)
Lower risk	261 (32)	451 (27)	712 (29)
Statin intensity at baseline per ACC/AHA definition			
High intensity	301 (37)	612 (37)	913 (37)
Moderate intensity	360 (44)	716 (44)	1076 (44)
Low intensity	5 (0.6)	6 (0.4)	11 (0.4)
Unknown	0 (0.0)	1 (0.1)	1 (0.0)
Lipid parameters at baseline			
ApoA1 remnant ratio, mean (SD)	7.3 (3.9)	7.1 (3.8)	7.2 (3.8)
LDL-C (mmol/l), mean (SD)	3.1 (1.1)	3.2 (1.1)	3.1 (1.1)
HDL-C (mmol/l), mean (SD)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)
Triglycerides (mmol/l), mean (SD)	1.5 (0.8)	1.5 (0.9)	1.5 (0.8)

Table 1 continued

Characteristic	Placebo (n = 821)	Evolocumab (n = 1643)	Total (n = 2464)
Non-HDL-C (mmol/l), mean (SD)	3.8 (1.2)	3.9 (1.2)	3.8 (1.2)
VLDL-C (mmol/l), median (Q1, Q3)	0.6 (0.4, 0.8)	0.6 (0.4, 0.8)	0.6 (0.4, 0.8)
Lp(a) (nmol/l), median (Q1, Q3)	35 (12, 141)	36 (11, 152)	35 (11, 148)
ApoA1 (g/l), mean (SD)	1.5 (0.3)	1.5 (0.3)	1.5 (0.3)
ApoB (g/l), mean (SD)	0.9 (0.3)	1.0 (0.3)	1.0 (0.3)
RLP-C, mean (SD)	0.7 (0.3)	0.7 (0.4)	0.7 (0.4)

ApoA1 apolipoprotein A1, *ApoA1 remnant ratio* apoA1/(non-HDL-C–LDL-C), *ApoB* apolipoprotein B, *BMI* body mass index, *ACC* American College of Cardiology, *AHA* American Heart Association, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *Lp(a)* lipoprotein(a), *NCEP* National Cholesterol Education Program, *Q* quartile, *RLP* remnant lipoprotein, *SOC* standard of care, *SD* standard deviation, *VLDL-C* very-low-density lipoprotein cholesterol

[a] Based on the presence of coronary heart disease in a first-degree male relative 55 years of age or younger or female 65 years of age or younger

[b] Defined as having 3 or more of the following factors: elevated waist circumference, triglyceride level of 1.69 mmol/l (150 mg/dl) or greater, low HDL-C level (< 1.03 mmol/l [< 40 mg/dl] in men and < 1.29 mmol/l [< 50 mg/dl] in women), systolic blood pressure of 130 mmHg or greater or diastolic blood pressure of 85 mmHg or greater, or hyperglycemia (fasting blood glucose ≥ 5.55 mmol/l [≥ 100 mg/dl])

determined by ultracentrifugation. Total cholesterol, LDL-C, HDL-C, triglycerides, and VLDL-C were calculated by taking the mean of screening and day 1 values, if available.

Measurement of ApoA1 Remnant Ratio

A surrogate measurement for RLP was used to determine the apoA1 remnant ratio, since IDL data from these studies were unavailable, by dividing apoA1 with the difference between non-HDL-C and LDL-C in place of VLDL + IDL as reported by Varbo et al. [8–10]. Stratifications of the apoA1 remnant ratio were generated using tertile (< 4.7, 4.7–6.8, and > 6.8) and partitioning categories (< 3.6, 3.6–6.0, and > 6.0) that were previously identified [19].

Statistical Analyses

Baseline demographics, measurements of lipid parameters, and clinical characteristics were summarized descriptively by treatment groups. The mean percentage change in the apoA1 remnant ratio, RLP, or apoA1 from baseline to week 12 was compared between treatment groups using a repeated-measures model with study, treatment group, visit, and the interaction between treatment group and visit as covariates. Categorical variables were compared using the Chi-square test. All p values reported are two-sided and were not adjusted for multiple comparisons. All statistical analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

A total of 2464 patients, the full cohort receiving evolocumab or placebo from the three parent studies, were included in this analysis. Demographics and clinical characteristics, including CV risk factors, are reported in Table 1. Overall, 49% of patients were women; the mean (standard deviation [SD]) age was 57 (11) years, and 92% of patients were white. Approximately 20% of patients had coronary artery disease at baseline. Over 40% were at high or moderately-high risk based on National Cholesterol Education Program Adult

Table 2 Effects of evolocumab on percent change from baseline in apoA1, RLP, and the apoA1 remnant ratio

	Placebo Q2W (*n* = 411)	Evolocumab 140 mg Q2W (*n* = 818)	Placebo QM (*n* = 410)	Evolocumab 420 mg QM (*n* = 825)
ApoA1				
Mean (SE) at week 12	3.1 (0.6)	6.9 (0.5)	1.8 (0.7)	6.4 (0.5)
LS mean (SE) at week 12	1.8 (0.6)	5.7 (0.5)	0.7 (0.7)	5.9 (0.5)
Mean (SE) treatment	–	4.0 (0.7)[b]	–	5.2 (0.8)[b]
Difference vs. placebo[a]				
RLP				
Mean (SE) at week 12	5.8 (1.7)	− 9.0 (1.2)	11.4 (2.5)	− 8.8 (1.1)
LS mean (SE) at week 12	5.3 (1.5)	− 10.0 (1.2)	11.0 (1.8)	11.7 (1.4)
Mean (SE) treatment	–	− 15.3 (1.7)[b]	–	− 22.7 (2.0)[b]
Difference vs. placebo[a]				
ApoA1 remnant ratio				
Mean (SE) at week 12	6.7 (1.8)	31.2 (2.2)	1.1 (2.1)	29.0 (1.7)
LS mean (SE) at week 12	1.4 (2.3)	26.4 (1.8)	− 3.7 (2.3)	29.9 (1.8)
Mean (SE) treatment	–	25.0 (2.6)[b]	–	33.6 (2.6)[b]
Difference vs. placebo[a]				

ApoA1 apolipoprotein A1, *ApoA1 remnant ratio* apoA1/(non-HDL-C–LDL-C), *LS* least squares, *n* number of subjects in the full analysis set, *Q2W* every 2 weeks, *QM* monthly, *RLP* remnant lipoprotein

[a] Fixed-effects treatment differences are from the repeated measures model, which includes parent study, treatment group, visit, and the interaction between treatment group and visit

[b] $p < 0.0001$

Treatment Panel III criteria, and 81% of patients were receiving moderate- or high-intensity statin regimens. The mean (SD) baseline apoA1 remnant ratio was 7.2 (3.8) and the mean (SD) baseline LDL-C concentration was 3.1 (1.1) mmol/l. The baseline apoA1 remnant ratio was similar across the evolocumab and placebo

Table 3 Spearman correlation between change from baseline at week 12 in apoA1 remnant ratio and change from baseline at week 12 in other lipids

Lipid parameter	Evolocumab 140 mg Q2W (*n* = 818)	Evolocumab 420 mg QM (*n* = 825)
n	737	746
ApoA1	0.165[a]	0.184[a]
ApoB	− 0.160[a]	− 0.168[a]
Triglycerides	− 0.750[a]	− 0.751[a]
HDL-C	0.404[a]	0.378[a]
Non-HDL-C	− 0.214[a]	− 0.208[a]
VLDL-C	− 0.756[a]	− 0.752[a]
LDL-C	− 0.065	− 0.044
Lp(a)	0.012	0.012

ApoA1 apolipoprotein A1, *ApoA1 remnant ratio* apoA1/(non-HDL-C–LDL-C), *ApoB* apolipoprotein B, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *Lp(a)* lipoprotein(a), *VLDL-C* very-low-density lipoprotein cholesterol

[a] $p < 0.0001$

arms, as were baseline lipid levels, including apoA1, LDL-C, and non-HDL-C.

The effects of evolocumab treatment on the apoA1 remnant ratio and on its components, apoA1 and RLP, are shown in Table 2. Evolocumab increased apoA1 relative to placebo for a mean (standard error [SE]) treatment difference of 4.0 (0.7) for 140 mg Q2W and 5.2 (0.8) for 420 mg QM (both $p < 0.0001$). Evolocumab decreased RLP relative to placebo for a mean treatment difference of – 15.3 (1.7) and – 22.7 (2.0), respectively (both $p < 0.0001$). The mean (SE) apoA1 remnant ratio at week 12 was 8.8 (0.2) in patients receiving evolocumab 140 mg Q2W, 7.4 (0.2) in patients receiving placebo Q2W, 8.7 (0.2) in patients receiving evolocumab 420 mg QM, and 7.3 (0.2) in patients receiving placebo QM. The mean (SE) percent change from baseline to week 12 in the apoA1 remnant ratio was 26.4% (1.8%) in patients receiving evolocumab Q2W, 29.9% (1.8%) in patients receiving evolocumab QM, 1.4% (2.3%) in patients receiving placebo Q2W, and – 3.7% (2.3%) in patients receiving placebo QM. These percent changes from baseline corresponded to mean (SE) treatment differences for evolocumab versus placebo of 25.0% (2.6%) for 140 mg Q2W ($p < 0.0001$) and 33.6% (2.6%) for the 420 mg QM dose ($p < 0.0001$). Spearman correlations of the change in the apoA1 remnant ratio from baseline to week 12 and the change from baseline to week 12 in other lipids were statistically significant for apoB, HDL-C, non-HDL-C, VLDL-C, apoA1, and triglycerides (all $p < 0.0001$) (Table 3). Changes in LDL-C and Lp(a) did not correlate with change in the apoA1 remnant ratio.

Patients were grouped by week 12 apoA1 remnant ratio threshold and evolocumab dose frequency to examine reductions in lipid levels by achieved apoA1 remnant ratio (see Table S1 in the electronic supplementary material).

Patients were evaluated according to their evolocumab dose/interval and week 12 apoA1 remnant ratio level to examine the impact of these factors on LDL-C and non-HDL-C goal achievement. No trend in week 12 LDL-C goal achievement (< 70 mg/dl) was observed across various thresholds of achieved apoA1 remnant ratio. Patients with higher week 12 apoA1 remnant ratios ≥ 3.6 vs. < 3.6 and ≥ 4.7 vs. < 4.7 were significantly more likely to achieve a non-HDL-C treatment goal of < 100 mg/dl than were those with lower week 12 apoA1 remnant ratio values at both dose groups (< 3.6 or < 4.7; Table 4). We observed an increase in the numerical distribution of women over 50 years of age at higher thresholds of apoA1 remnant ratio at week 12 (Table 5) although the number of patients in some of the threshold categories was small with less precision.

DISCUSSION

The results of this post hoc analysis suggest that evolocumab, a PCSK9 antibody inhibitor, increases the apoA1 remnant ratio. This is the first study reporting the effects of a PCSK9 inhibitor on the apoA1 remnant ratio, and the first study examining this in women over 50 years of age. As demonstrated in prior

Table 4 Week 12 goal achievement by apoA1 remnant ratio threshold

Goal	Evolocumab dose/dose frequency							
	140 mg Q2W		420 mg QM		140 mg Q2W		420 mg QM	
	ApoA1 remnant ratio threshold							
	< 3.6 (*n* = 48)	≥ 3.6 (*n* = 693)	< 3.6 (*n* = 53)	≥ 3.6 (*n* = 695)	< 4.7 (*n* = 128)	≥ 4.7 (*n* = 613)	< 4.7 (*n* = 123)	≥ 4.7 (*n* = 625)
LDL-C < 70 mg/dl	94%	83%	76%	81%	87%	83%	75%	81%
p value[a]	0.0556		0.3541		0.3466		0.0901	
Non-HDL-C < 100 mg/dl	77%	89%	57%	86%	79%	90%	64%	87%
p value[a]	0.0129		< 0.0001		0.0003		< 0.0001	

ApoA1 apolipoprotein A1, *ApoA1 remnant ratio* apoA1/(non-HDL-C–LDL-C), *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *Q2W* every 2 weeks, *QM* monthly, *SE* standard error, *VLDL-C* very-low-density lipoprotein cholesterol

[a] For the difference between the apoA1 remnant ratio categories within each evolocumab dose/dose frequency

studies, the on-treatment apoA1 remnant ratio is an independent CV risk factor [7, 19]. Evolocumab's effect on the apoA1 remnant ratio is achieved not only by its effect on increasing the numerator (apoA1) (+ 4% evolocumab 140 mg Q2W compared with placebo), but also by an even greater decrease in the denominator (RLP) (– 15% evolocumab 140 mg Q2W compared with placebo). These non-LDL-C-related effects have potential significant clinical relevance. First, from a mechanistic standpoint, the reduction in RLP-C by evolocumab supports the biologic plausibility that by inhibiting PCSK9, which downregulates not only LDL receptors but also the VLDL receptor and LDL-receptor–related proteins, the clearance of RLP would increase. These data are also consistent with a recent study showing that evolocumab reduces VLDL-C and total, medium, and small particles of VLDL, in addition to IDL particles as measured by nuclear magnetic resonance [30]. The importance of reducing small VLDL particles was recently demonstrated in the JUPITER trial [35]. In 11,984 patients on rosuvastatin 20 mg/day with a median LDL-C of 51 mg/dl, VLDL particles were associated with a significant increase in the incidence of atherosclerotic CV disease risk, with a 68% increase in risk for every 1 standard deviation increase in VLDL particle (adjusted hazard ratio 1.68, 95% CI 1.28–2.22). In a post hoc analysis of the TNT trial, atorvastatin 80 mg compared to atorvastatin 10 mg significantly reduced risk, independent of LDL-C, in patients with higher baseline TRL-C levels (measured by non-HDL-C minus LDL-C; relative risk reduction: 29–41%; all $p < 0.0250$) [14]. This analysis suggests that the reduction in CV event rates by evolocumab may be attributable to a combination of reductions in not only LDL-C but also in RLP; future analyses could confirm this.

In this study, we also evaluated women over 50 years of age since the initial discovery paper involving the apoA1 remnant ratio was in women over 50 years of age referred for cardiac catheterization. In that study, death/myocardial infarction at 3 years occurred in 20.4, 17.1, and 8.9% of patients with apoA1 remnant ratios < 3.6, 3.6–6.0, and > 6.0, respectively. These stratifications have been shown in a previous study to partition those at low or moderate-to-high cardiovascular risk [19]. The same tertile and partitioning categories were used in this study to determine risk levels, as is done with other lipid parameters. Using those same partitioning categories here, 2.6, 16.0, and 73.2% of patients treated with evolocumab 140 mg Q2W had apoA1 remnant ratios of < 3.6, 3.6–6.0,

Table 5 ApoA1 remnant ratio threshold shift from baseline to week 12 in women and women > 50 years of age

Treatment	Baseline apoA1 threshold	Week 12 apoA1 remnant ratio threshold, *n* (%)				
		< 3.6	3.6–6	> 6	Missing	Total
Women						
Evolocumab 140 mg Q2W (*n* = 386)	< 3.6	11 (2.8)	24 (6.2)	4 (1.0)	7 (1.8)	46 (11.9)
	3.6–6	2 (0.5)	29 (7.5)	54 (14.0)	8 (2.1)	93 (24.1)
	> 6	1 (0.3)	11 (2.8)	216 (56.0)	17 (4.4)	245 (63.5)
	Missing	0 (0.0)	1 (0.3)	1 (0.3)	0 (0.0)	2 (0.5)
	Total	14 (3.6)	65 (16.8)	275 (71.2)	32 (8.3)	386 (100.0)
Evolocumab 420 mg QM (*n* = 401)	< 3.6	13 (3.2)	17 (4.2)	6 (1.5)	2 (0.5)	38 (9.5)
	3.6–6	3 (0.7)	49 (12.2)	48 (12.0)	10 (2.5)	110 (27.4)
	> 6	0 (0.0)	9 (2.2)	222 (55.4)	20 (5.0)	251 (62.6)
	Missing	0 (0.0)	1 (0.2)	1 (0.2)	0 (0.0)	2 (0.5)
	Total	16 (4.0)	76 (19.0)	277 (69.1)	32 (8.0)	401 (100.0)
Women > 50 years of age						
Evolocumab 140 mg Q2W (*n* = 313)	< 3.6	6 (1.9)	18 (5.8)	3 (1.0)	5 (1.6)	32 (10.2)
	3.6–6	2 (0.6)	23 (7.3)	48 (15.3)	8 (2.6)	81 (25.9)
	> 6	0 (0.0)	8 (2.6)	177 (56.5)	13 (4.2)	198 (63.3)
	Missing	0 (0.0)	1 (0.3)	1 (0.3)	0 (0.0)	2 (0.6)
	Total	8 (2.6)	50 (16.0)	229 (73.2)	26 (8.3)	313 (100.0)
Evolocumab 420 mg QM (*n* = 333)	< 3.6	9 (2.7)	15 (4.5)	5 (1.5)	2 (0.6)	31 (9.3)
	3.6–6	3 (0.9)	38 (11.4)	37 (11.1)	8 (2.4)	86 (25.8)
	> 6	0 (0.0)	7 (2.1)	191 (57.4)	16 (4.8)	214 (64.3)
	Missing	0 (0.0)	1 (0.3)	1 (0.3)	0 (0.0)	2 (0.6)
	Total	12 (3.6)	61 (18.3)	234 (70.3)	26 (7.8)	333 (100.0)

n number of subjects in the full analysis set, *ApoA1* apolipoprotein A1, *ApoA1 remnant ratio* apoA1/(non-HDL-C–LDL-C), *Q2W* every 2 weeks, *QM* monthly

and > 6.0, respectively at 12 weeks, compared with 10.2, 25.9, and 63.3% at baseline. The apoA1 remnant ratio may have utility, then, in this population, although further studies using this as a primary or secondary target are necessary. Furthermore, examination of the response of the apoA1 remnant ratio to statin therapy merits exploration. Limitations of this study include that the analysis was conducted post hoc and was not powered to detect cardiovascular events, was unadjusted for potential confounders, and included trials of limited duration (12 weeks); therefore, further prospective investigation of the effect of evolocumab on the apoA1 remnant ratio and CV risk is warranted. In addition, some of the apoA1 remnant ratio threshold subgroups had relative small numbers of patients (< 60) and thus lower precision.

CONCLUSIONS

This post hoc analysis is the first to suggest that evolocumab increases the apoA1 remnant ratio following 12 weeks of treatment. This effect is independent of standard lipids, non-HDL-C, and apoB. Evolocumab's effect on increasing this novel ratio, which is independent of LDL-C, is consistent with previously published data showing an increase in apoA1 and reductions in remnant lipoproteins. The on-treatment apoA1 remnant ratio was shown to identify patients who are less likely to attain non-HDL-C < 100 mg/dl treatment goals after 12 weeks of treatment. In further subgroup analyses, while limited by the number of patients in apoA1 remnant ratio thresholds, these analyses suggest that evolocumab increased the apoA1 remnant ratio in women > 50 years of age moving them into higher levels which have previously been shown to be associated with less CV risk.

ACKNOWLEDGEMENTS

We thank the participants of these studies.

Funding. This work was funded by Amgen Inc. Amgen Inc. funded the article processing charges. All authors had full access to the data in this study and take complete responsibility for the integrity of the data and accuracy of the analysis.

Medical Writing, Editorial, and Other Assistance. Medical writing assistance was funded by Amgen Inc., and provided by Wanda Krall, PhD, on behalf of Amgen Inc. and Annalise Nawrocki, PhD, of Amgen Inc.

Authorship. All authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole and have given their approval for this version to be published.

Disclosures. Heidi T. May and Joseph B. Muhlestein have nothing to disclose. Yuhui Ma: Employed on behalf of Amgen Inc. J. Antonio G. López: Employee and stockholder of Amgen Inc. Blai Coll: Employee of Amgen Inc. and owns Amgen stock/stock options. John Nelson: Amgen Speakers Bureau, Advisory Board, and Stock.

Compliance with Ethics Guidelines. All procedures performed in the trials reported in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the mentioned studies. Approval of institutional review boards was received for these trials and has been previously reported [31–33]. This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

Data Availability. Qualified researchers may request data from Amgen clinical studies. Complete details are available at the following: http://www.amgen.com/datasharing.

REFERENCES

1. No authors listed. The Lipid Research Clinics Coronary Primary Prevention Trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. JAMA. 1984;251:365–74.

2. No authors listed. The Lipid Research Clinics Coronary Primary Prevention Trial results. I. Reduction in incidence of coronary heart disease. JAMA. 1984;251:351–64.

3. Cholesterol Treatment Trialists Collaboration, Baigent C, Blackwell L, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet. 2010;376:1670–81.

4. Fruchart JC, Davignon J, Hermans MP, et al. Residual macrovascular risk in 2013: what have we learned? Cardiovasc Diabetol. 2014;13:26.

5. Wong ND. Residual risk after treatment of patients with atherosclerotic cardiovascular disease with proprotein convertase subtilisin-kexin type 9 monoclonal antibody therapy (from the Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk Trial). Am J Cardiol. 2017;120:1220–2.

6. May HT, Nelson JR, Lirette ST, et al. The utility of the apolipoprotein A1 remnant ratio in predicting incidence coronary heart disease in a primary prevention cohort: the Jackson Heart Study. Eur J Prev Cardiol. 2016;23:769–76.

7. Averna M, Stroes E, The lipid alterations beyond LDL expert working group. How to assess and manage cardiovascular risk associated with lipid alterations beyond LDL. Atheroscler Suppl. 2017;26:16–24.

8. Varbo A, Benn M, Nordestgaard BG. Remnant cholesterol as a cause of ischemic heart disease: evidence, definition, measurement, atherogenicity, high risk patients, and present and future treatment. Pharmacol Ther. 2014;141:358–67.

9. Varbo A, Benn M, Tybjaerg-Hansen A, Jorgensen AB, Frikke-Schmidt R, Nordestgaard BG. Remnant cholesterol as a causal risk factor for ischemic heart disease. J Am Coll Cardiol. 2013;61:427–36.

10. Varbo A, Benn M, Tybjaerg-Hansen A, Nordestgaard BG. Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation. Circulation. 2013;128:1298–309.

11. Toth PP, Grabner M, Punekar RS, Quimbo RA, Cziraky MJ, Jacobson TA. Cardiovascular risk in patients achieving low-density lipoprotein cholesterol and particle targets. Atherosclerosis. 2014;235:585–91.

12. Jialal I, Devaraj S. Remnant lipoproteins: measurement and clinical significance. Clin Chem. 2002;48:217–9.

13. Joshi PH, Khokhar AA, Massaro JM, et al. Remnant lipoprotein cholesterol and incident coronary heart disease: the Jackson Heart and Framingham Offspring Cohort Studies. J Am Heart Assoc. 2016;5:e002765.

14. Vallejo-Vaz AJ, Fayyad R, Boekholdt SM, et al. Triglyceride-rich lipoprotein cholesterol and risk of cardiovascular events among patients receiving statin therapy in the Treating to New Targets (TNT) trial. Circulation. 2018;138:770–81.

15. Cooper AD. Hepatic uptake of chylomicron remnants. J Lipid Res. 1997;38:2173–92.

16. Chappell DA, Medh JD. Receptor-mediated mechanisms of lipoprotein remnant catabolism. Prog Lipid Res. 1998;37:393–422.

17. Takahashi S, Suzuki J, Kohno M, et al. Enhancement of the binding of triglyceride-rich lipoproteins to the very low density lipoprotein receptor by apolipoprotein E and lipoprotein lipase. J Biol Chem. 1995;270:15747–54.

18. Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner E. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. Lancet. 2001;358:2026–33.

19. May HT, Nelson JR, Kulkarni KR, et al. A new ratio for better predicting future death/myocardial infarction than standard lipid measurements in women > 50 years undergoing coronary angiography: the apolipoprotein A1 remnant ratio (Apo A1/[VLDL(3) + IDL]). Lipids Health Dis. 2013;12:55.

20. Nelson J, He L, Dansinger M. ApoA1 remnant ratio in very high risk patients with LDL-c, 70 mg/dl and nonHDL-c, 100 mg/dl: ramifications for residual risk in women > 55 years of age [abstract 186]. J Clin Lipidol. 2018;12 515–6.

21. Langslet G, Emery M, Wasserman SM. Evolocumab (AMG 145) for primary hypercholesterolemia. Expert Rev Cardiovasc Ther. 2015;13:477–88.

22. Awan Z, Baass A, Genest J. Proprotein convertase subtilisin/kexin type 9 (PCSK9): lessons learned from patients with hypercholesterolemia. Clin Chem. 2014;60:1380–9.

23. Chan JC, 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 USA. 2009;106:9820–5.

24. Poirier S, Mayer G, Benjannet S, et al. The proprotein convertase PCSK9 induces the degradation of

low density lipoprotein receptor (LDLR) and its closest family members VLDLR and ApoER2. J Biol Chem. 2008;283:2363–72.

25. Canuel M, Sun X, Asselin MC, Paramithiotis E, Prat A, Seidah NG. Proprotein convertase subtilisin/kexin type 9 (PCSK9) can mediate degradation of the low density lipoprotein receptor-related protein 1 (LRP-1). PLoS One. 2013;8:e64145.

26. Blom DJ, Hala T, Bolognese M, et al. A 52-week placebo-controlled trial of evolocumab in hyperlipidemia. N Engl J Med. 2014;370:1809–19.

27. Sabatine MS, Giugliano RP, Keech AC, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. N Engl J Med. 2017;376:1713–22.

28. Sabatine MS, Leiter LA, Wiviott SD, et al. Cardiovascular safety and efficacy of the PCSK9 inhibitor evolocumab in patients with and without diabetes and the effect of evolocumab on glycaemia and risk of new-onset diabetes: a prespecified analysis of the FOURIER randomised controlled trial. Lancet Diabetes Endocrinol. 2017;5:941–50.

29. Bonaca MP, Nault P, Giugliano RP, et al. Low-density lipoprotein cholesterol lowering with evolocumab and outcomes in patients with peripheral artery disease: insights from the FOURIER trial (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk). Circulation. 2018;137:338–50.

30. Toth PP, Sattar N, Blom DJ, et al. Effect of evolocumab on lipoprotein particles. Am J Cardiol. 2018;121:308–14.

31. Koren MJ, Lundqvist P, Bolognese M, et al. Anti-PCSK9 monotherapy for hypercholesterolemia: the MENDEL-2 randomized, controlled phase III clinical trial of evolocumab. J Am Coll Cardiol. 2014;63:2531–40.

32. Raal FJ, Stein EA, Dufour R, et al. PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolaemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial. Lancet. 2015;385:331–40.

33. Robinson JG, Nedergaard BS, Rogers WJ, et al. Effect of evolocumab or ezetimibe added to moderate- or high-intensity statin therapy on LDL-C lowering in patients with hypercholesterolemia: the LAPLACE-2 randomized clinical trial. JAMA. 2014;311:1870–82.

34. Stroes E, Robinson JG, Raal FJ, et al. Clinical equivalence of evolocumab among patient subgroups in PROFICIO: a pooled analysis of 3146 patients from phase 3 studies. London: European Society of Cardiology; 2015.

35. Lawler PR, Akinkuolie AO, Harada P, et al. Residual risk of atherosclerotic cardiovascular events in relation to reductions in very-low-density lipoproteins. J Am Heart Assoc. 2017;6:e007402.