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Efficacy, safety and effect on biomarkers related to cholesterol and lipoprotein metabolism of rosuvastatin 10 or 20 mg plus ezetimibe 10 mg vs. simvastatin 40 or 80 mg plus ezetimibe 10 mg in high-risk patients: Results of the GRAVITY randomized study

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ABSTRACT

Objectives: Combination therapy may help high-risk patients achieve low-density lipoprotein cholesterol (LDL-C) goals. Impact of rosuvastatin 10 or 20 mg plus ezetimibe 10 mg (RSV10/EZE10 and RSV20/EZE10) has not been fully characterized previously. GRAVITY (NCT00525824) compared efficacy, safety and effect on biomarkers of RSV10/EZE10 and RSV20/EZE10 vs. simvastatin 40 mg and 80 mg plus EZE10 (SIM40/EZE10 and SIM80/EZE10) in patients with coronary heart disease (CHD) or CHD risk equivalent.
Methods: Adult patients ($n = 833$) were randomized to RSV10/EZE10, RSV20/EZE10, SIM40/EZE10 or SIM80/EZE10. Following a 6-week dietary lead-in, patients received 6 weeks' statin monotherapy followed by same statin dose plus ezetimibe for 6 more weeks. Primary endpoint was LDL-C change from baseline to 12 weeks.
Results: Significantly greater ($p < 0.05$) reductions in LDL-C and other atherogenic lipids were observed with RSV20/EZE10 vs. SIM40/EZE10 and SIM80/EZE10 and with RSV10/EZE10 vs. SIM40/EZE10. A significantly greater proportion of patients achieved LDL-C goals of <100 mg/dl and <70 mg/dl with RSV20/EZE10 vs. SIM40/EZE10 and SIM80/EZE10 and with RSV10/EZE10 vs. SIM40/EZE10. LDL-C was reduced ~10–14% further with combination therapy vs. monotherapy. Statin monotherapy reduced cholesterol and bile acid synthesis biomarkers, ezetimibe reduced β-sitosterol (sterol absorption marker), and combination therapy achieved additive reductions in lipoprotein-associated phospholipase A_2 mass and activity, free cholesterol and 7-ketocholesterol. Safety profiles of rosuvastatin/ezetimibe and simvastatin/ezetimibe combinations were comparable.
Conclusion: Co-administration of rosuvastatin 10 or 20 mg plus ezetimibe achieved significant improvements in lipid profiles in high-risk patients vs. simvastatin 40 or 80 mg plus ezetimibe.

1. Introduction

Patients at high risk of cardiovascular disease may require statin therapy in combination with another treatment such as a bile acid sequestrant, nicotinic acid or the cholesterol absorption inhibitor ezetimibe for effective reduction of low-density lipoprotein cholesterol (LDL-C) [14,25]. In the EXamination of Potential Lipid-modifying effects Of Rosuvastatin in combination with Ezetimibe versus Rosuvastatin alone (EXPLORER) study in high-risk patients, rosuvastatin at its highest prescribed dose (40 mg) co-administered with ezetimibe 10 mg lowered LDL-C levels to a significantly greater extent and allowed a greater proportion of patients to achieve their LDL-C goals compared with rosuvastatin alone [4]. The effects of rosuvastatin 10 mg or 20 mg plus ezetimibe have not previously been fully characterized.

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The aim of the current 12-week study (GRAVITY: **G**auging the lipid effects of **R**osuv**A**statin plus ezetimibe **V**ersus s**I**mvastatin plus ezetimibe **T**herap**Y**; NCT00525824) was to compare efficacy and safety of rosuvastatin 10 mg and 20 mg plus ezetimibe 10 mg with that of the commercially available fixed-dose combinations of simvastatin 40 mg and 80 mg plus ezetimibe 10 mg, in patients at high cardiovascular risk. As an exploratory objective, changes in several biomarkers were assessed, including those relating to cholesterol synthesis (lanosterol), metabolism (free cholesterol) and absorption (β-sitosterol), bile acid synthesis (7-α-hydroxy-4-cholesten-3-one [C4]), and atherosclerosis (7-ketocholesterol and lipoprotein-associated phospholipase A_2 [Lp-PLA_2] concentration and activity). There is currently little information available on the relationship between these biomarkers and either the atherogenic lipid profile or the response to lipid-lowering statin monotherapy or combination therapies.

2. Methods

This was a 12-week open-label, randomized, parallel-group, multicenter, phase IIIb study conducted in 111 centers in the USA, South America and Europe, from August 2007 to September 2008. The study was performed in accordance with the ethical principles in the Declaration of Helsinki, the International Conference on Harmonization/Good Clinical Practice and appropriate regulatory requirements. The study protocol was approved by the appropriate local independent ethics committees. Written, informed consent was received from all patients before enrollment.

2.1. Patients

Male or female patients aged ≥18 years with hypercholesterolemia and a history of coronary heart disease (CHD) or a CHD risk equivalent, clinical evidence of atherosclerosis or a 10-year Framingham risk score >20% for CHD were eligible for enrollment into the initial dietary lead-in phase. For inclusion in the randomized treatment phase, patients were required to have fasting LDL-C 130 to <220 mg/dl (3.4 to <5.7 mmol/l) and fasting triglycerides (TG) < 400 mg/dl (4.5 mmol/l). Patients were excluded if they received lipid-lowering drugs after start of lead-in phase or other prohibited concomitant medications (medications considered necessary for a patient's safety were allowed); had history of statin-induced myopathy or serious hypersensitivity reaction to statins and/or a history of hypersensitivity to any ezetimibe components; were considered to be unstable after a myocardial infarction, recent episode of unstable angina, myocardial revascularization, transient ischemic attack or stroke; had severe congestive cardiac failure (New York Heart Association class IIIb or IV); were awaiting a planned myocardial revascularization; had history of malignancy apart from resected basal cell or squamous cell carcinoma of the skin; had homozygous familial hypercholesterolemia; had current active liver disease (alanine aminotransferase or serum glutamic-pyruvic transaminase ≥2 times the upper limit of normal [ULN]) or severe hepatic impairment, uncontrolled hypothyroidism, unexplained creatine kinase >1 × ULN <3 months before enrollment or serum creatinine >176 μmol/l (2.0 mg/dl). Women who were pregnant, breastfeeding, or of childbearing potential but not using contraception were also excluded, as were patients whose hormone replacement therapy or oral contraceptive therapy was initiated or changed within 3 months before enrollment in the dietary lead-in phase.

Patients could also participate in an optional analysis of plasma biomarkers, provided separate written, informed consent was received in addition to that required for the main study.

2.2. Study design

Patients were required to discontinue their current lipid-lowering therapy before entering a 6-week dietary lead-in period, during which they followed the Therapeutic Lifestyle Change diet. Eligible patients were then randomly assigned (1:1:1:1) to one of four treatments (rosuvastatin 10 mg or 20 mg or simvastatin 40 mg or 80 mg) for 6 weeks' monotherapy. Thereafter, patients continued with their assigned statin dose in combination with ezetimibe 10 mg for a further 6 weeks (Supplemental Fig. 1). The 6-week treatment periods were considered adequate time to attain a relatively stable estimate of the treatment effect based on previous studies [18]. All treatments were dosed once daily. Monotherapy with rosuvastatin (AstraZeneca Pharmaceuticals, Wilmington, DE) and simvastatin (Merck, Whitehouse Station, NJ) and the simvastatin/ezetimibe combination (Merck) were administered as single tablets. The combination of rosuvastatin with ezetimibe (Merck) was administered as two separate tablets.

Study treatments were not blinded to allow use of the fixed-dose simvastatin/ezetimibe combinations. However, investigators and sponsor staff were blinded to the randomization scheme and efficacy measurements (lipid levels).

Patients were randomized sequentially using a central Interactive Voice Response System, and according to a standard computerized randomization scheme blocked by site (generated by the study sponsor).

The primary efficacy variable was change in LDL-C from baseline to 12 weeks, i.e. after 6 weeks' statin monotherapy plus 6 weeks' combination therapy. Secondary variables included changes in other lipid markers of cardiovascular risk and proportion of patients achieving LDL-C goals of <100 mg/dl or <70 mg/dl [14,25].

2.3. Assessments

Fasting blood samples were collected at each visit (at weeks −6, −2, 0, 4, 6, 10 and 12) to determine total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C), TG, nonHDL-C, and ratios of LDL-C/HDL-C, TC/HDL-C, and nonHDL-C/HDL-C and high-sensitivity C-reactive protein (hsCRP). Apolipoprotein B (ApoB) and apolipoprotein A-1 (ApoA-I) and the ApoB/ApoA-I ratio were determined from week 0 onward. Analyses were performed by a central laboratory. LDL-C levels were determined using the Friedewald equation, unless TG level was >400 mg/dl (4.5 mmol/l), in which case a β-quantification measurement of LDL-C was used.

Blood samples for hematology and clinical chemistry (including creatine kinase, serum creatinine, alanine aminotransferase and aspartate aminotransferase) were collected at weeks −6, 0, 6 and 12.

Blood samples for analysis of biomarkers were drawn at weeks 0, 6 and 12 (Supplemental Fig. 1). At each timepoint, two 5-ml samples of blood were taken from a peripheral vein into ethylenediamine tetra-acetic acid tubes and centrifuged for 10 min at room temperature or lower at 2000 g. Samples were stored at −80 °C prior to analysis. Levels of β-sitosterol, lanosterol, C4, 7-ketocholesterol, free cholesterol and both concentration and activity of Lp-PLA_2 were analyzed by a specialized laboratory. Specific methods are detailed below.

2.3.1. Biomarker analyses

Gas-liquid chromatography:mass spectrometry techniques, with slight modifications of previously published protocols, were used to determine circulating levels of β-sitosterol [1], lanosterol [20], C4 [7] and 7-ketocholesterol [10]. Details are provided in the Supplemental Materials.

Lp-PLA_2 concentration was determined by a solid-phase sandwich ELISA (PLAC test, diaDexus Inc., South San Francisco, CA). Lp-PLA_2 activity was measured by an automated Colorimetric Activity Method assay (diaDexus Inc., South San Francisco, CA) using a Beckman Coulter AU400e autoanalyzer (Olympus, Center Valley, PA). Unesterified cholesterol (free cholesterol) was determined by an automated chemistry assay (Wako Chemicals USA, Inc., Richmond, VA) using a Beckman Coulter AU400e autoanalyzer.

2.3.2. Safety

Safety was assessed by adverse event (AE) and serious AE (SAE) reporting using the Medical Dictionary for Regulatory Activities (MedDRA) version 10.1 and laboratory findings. Myopathy was defined as symptoms of muscle pain and/or creatine kinase $>5 \times$ ULN. Compliance was assessed from visit 4 onward by counting the number of returned tablets; patients were considered non-compliant if they took <80% of any study medication.

2.4. Statistical analyses

Efficacy analyses were performed on the intention-to-treat population using the last observation carried forward method. The intention-to-treat population comprised patients with a baseline measurement and at least one post-baseline measurement during the randomized phase, and who had taken at least one dose of study medication.

The primary endpoint was the geometric mean percentage change in LDL-C from baseline (mean of values determined at weeks −2 and 0) to end of 6-week combination therapy period (calculated as the mean after 4 and 6 weeks of combination therapy [visits 6 and 7]) (Fig. 1), assessed using analysis of covariance (ANCOVA). The response variable was the logarithm of the ratio of the treatment value divided by the corresponding baseline value for LDL-C levels with a main effect for treatment, and baseline LDL-C level as covariate. Estimates for treatment effect and their 95% confidence intervals were exponentiated to produce estimates of percentage change. To obtain a statistical power of 95%, it was calculated that 177 intention-to-treat patients would be required per treatment arm to detect a 5% difference in the primary endpoint between the rosuvastatin and simvastatin combinations, with a standard deviation (for the mean percentage reduction) of 13%.

Statistical significance was assessed for rosuvastatin 10 mg/ezetimibe 10 mg vs. simvastatin 40 mg/ezetimibe 10 mg; rosuvastatin 20 mg/ezetimibe 10 mg vs. simvastatin 40 mg/ezetimibe 10 mg and rosuvastatin 20 mg/ezetimibe 10 mg vs. simvastatin 80 mg/ezetimibe 10 mg. The Hochberg procedure [6] was used to correct for multiple comparisons.

ANCOVA was also used to assess between-group differences for secondary lipid variables, including TC, HDL-C, TG, nonHDL-C, ApoB, ApoA-1 and ratios of LDL-C/HDL-C, TC/HDL-C, nonHDL-C/HDL-C, ApoB/ApoA-I and hsCRP. The proportion of patients achieving LDL-C goals was analyzed by logistic regression with terms for baseline LDL-C, center and treatment in the model and hypothesis testing was performed using likelihood ratio tests.

Changes in biomarker levels (exploratory analysis) from baseline to 6 weeks (end of monotherapy) and 12 weeks (end of combination therapy) per treatment group were summarized using descriptive statistics, and ANCOVA was used to assess within- and between-group differences.

Safety was assessed in the safety population, which comprised all randomized patients who received at least one dose of study medication. Frequencies of AEs, SAEs and abnormal laboratory values were summarized by treatment group.

3. Results

3.1. Study population

Of 833 randomized patients, 828 were included in the safety population and 814 were included in the intention-to-treat population. The most frequent reason for withdrawal from the randomized phase (between 3% and 5% of all treatment groups) was AE occurrence (Supplemental Fig. 2). There were no major differences between treatment groups in baseline characteristics (Table 1). Mean treatment compliance was ≥95% in all groups during monotherapy and combination therapy phases.

3.2. Efficacy

3.2.1. LDL-C reduction

Rosuvastatin 20 mg/ezetimibe 10 mg reduced LDL-C from baseline by 63.5% vs. reductions of 55.2–57.4% achieved with the simvastatin/ezetimibe doses ($p < 0.001$; Fig. 1A). LDL-C reductions with rosuvastatin 10 mg/ezetimibe 10 mg (59.7%) were significantly ($p = 0.002$) greater vs. simvastatin 40 mg/ezetimibe 10 mg (Fig. 1A). When compared with statin monotherapy, further LDL-C reductions of ~10%–14% were achieved with the addition of ezetimibe (Fig. 1A). Absolute LDL-C levels and reductions from

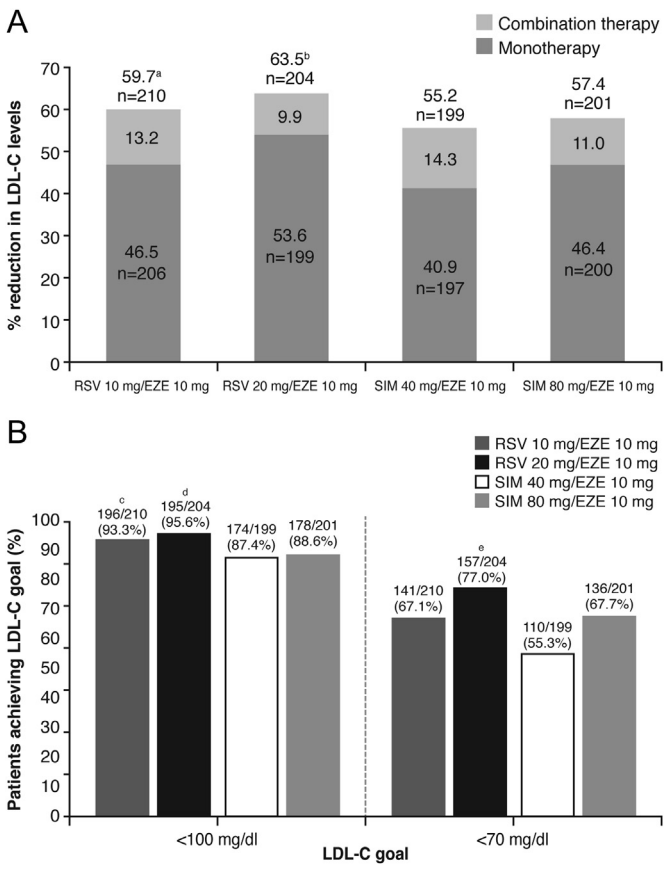

Fig. 1. (A) Mean percentage change in LDL-C from baseline to end of monotherapy (week 0 to week 6; grey shaded boxes) and from end of monotherapy to end of combination therapy (week 6 to week 12; white boxes). Values above bars show overall changes from baseline to end of combination therapy (week 0 to week 12). (B) Percentage of patients achieving LDL-C goals at week 12 (after 6 weeks combination therapy) (intention-to-treat population). [a]$p = 0.002$ vs. SIM 40 mg/EZE 10 mg, [b]$p < 0.001$ vs. SIM 40 mg/EZE 10 mg and SIM 80 mg/EZE 10 mg, [c]$p = 0.030$ vs. SIM 40 mg/EZE 10 mg, [d]$p = 0.007$ vs. SIM 80 mg/EZE 10 mg and $p = 0.001$ vs. SIM 40 mg/EZE 10 mg, [e]$p < 0.001$ vs. SIM 80 mg/EZE 10 mg and SIM 40 mg/EZE 10 mg.

Table 1
Patient demographics and baseline characteristics.

	RSV 10 mg/EZE 10 mg ($n = 214$)	RSV 20 mg/EZE 10 mg ($n = 214$)	SIM 40 mg/EZE 10 mg ($n = 202$)	SIM 80 mg/EZE 10 mg ($n = 203$)
Demographics[a]				
Age, years; mean (SD)	62.2 (10.1)	61.8 (9.9)	61.9 (9.4)	62.0 (9.2)
Male, *n* (%)	123 (57.5)	117 (54.7)	105 (52.0)	113 (55.7)
Race, *n* (%)				
Caucasian	127 (59.3)	136 (63.6)	115 (56.9)	127 (62.6)
Hispanic	45 (21.0)	45 (21.0)	45 (22.3)	36 (17.7)
Black	20 (9.3)	18 (8.4)	18 (8.9)	19 (9.4)
Asian	3 (1.4)	1 (0.5)	5 (2.5)	6 (3.0)
Other	19 (8.9)	14 (6.5)	19 (9.4)	15. (7.4)
Body mass index, kg/m^2; mean (SD)	30.3 (6.0)	31.0 (6.6)	30.2 (6.2)	31.0 (6.5)
Blood pressure, mmHg; mean (SD)				
Systolic	137.7 (17.7)	136.6 (16.0)	137.9 (18.5)	137.6 (18.2)
Diastolic	81.7 (10.2)	81.6 (9.9)	81.0 (10.7)	81.2 (10.6)
Baseline laboratory parameters, mg/dl; mean (SD)[b]	$n = 210$	$n = 204$	$n = 199$	$n = 201$
LDL-C	162.7 (22.7)	164.8 (24.7)	164.8 (23.6)	163.1 (24.1)
HDL-C	48.4 (12.7)	48.1 (13.2)	49.1 (13.5)	48.2 (12.2)
Total cholesterol	248.1 (28.3)	251.6 (30.3)	251.3 (28.2)	247.7 (24.9)
Triglycerides	184.8 (79.0)	192.5 (80.0)	187.2 (78.4)	181.9 (81.2)
nonHDL-C	199.8 (28.4)	203.5 (29.1)	202.3 (28.4)	199.5 (25.2)
ApoB	127.3 (20.7)	129.4 (21.7)	127.8 (20.0)	126.4 (18.3)
ApoA-I	144.0 (26.4)	143.4 (27.2)	145.6 (27.5)	143.2 (24.5)
hsCRP, mg/l; median (range)	2.8 (0.2–103.0)	2.6 (0.2–68.7)	2.6 (0.2–47.1)	2.5 (0.3–44.1)
Baseline biomarkers, median (IQR)[c]	$n = 130$	$n = 132$	$n = 124$	$n = 129$
C4 (ng/ml)	57.4 (82.4)	57.1(81.4)	55.0(82.8)	56.6(64.7)
7-ketocholesterol (ng/ml)	48.3 (25.5)	48.7 (31.3)	47.1 (30.5)	45.4 (24.1)
β-sitosterol (μg/ml)	2.2 (1.4)	2.1 (1.1)	2.3 (1.3)	2.1 (1.4)
Free cholesterol (mg/dl)	60.0 (17.7)	62.8 (15.1)	61.4 (14.5)	62.4 (12.6)
Lanosterol (μg/ml)	1.0 (0.8)	1.1 (0.8)	1.1 (1.0)	1.1 (0.9)
Lp-PLA$_2$ activity (nmol/min/ml)	217.5 (76.8)	224.5 (66.1)	211.1 (67.9)	208.3 (68.2)
Lp-PLA$_2$ concentration (ng/ml)	272.7 (120.5)	259.4 (116.2)	283.8 (150.7)	285.5 (124.1)

Apo = apolipoprotein; C4 = 7-α-hydroxy-4-cholestene-3-one; EZE = ezetimibe; HDL-C = high-density lipoprotein cholesterol; hsCRP = high-sensitivity C-reactive protein; IQR = interquartile range; LDL-C = low-density lipoprotein cholesterol; Lp-PLA$_2$ = lipoprotein-associated phosphatase A$_2$; RSV = rosuvastatin; SD = standard deviation; SIM = simvastatin.

[a] All randomized patients.

[b] Intention-to-treat population.

[c] Patients with evaluable biomarker data in the intention-to-treat population. Some patients (up to 4.8%) had missing values for particular biomarkers.

baseline with standard deviation are shown in Supplemental Table 1.

3.2.2. LDL-C goal achievement

A significantly ($p \leq 0.007$) greater proportion of patients achieved the LDL-C goal of <100 mg/dl and <70 mg/dl with rosuvastatin 20 mg/ezetimibe 10 mg (95.6% and 77.0%, respectively) than with either simvastatin/ezetimibe dose (87.4–88.6% and 55.3–67.7%, respectively; Fig. 1B). The proportion of patients achieving LDL-C goal <100 mg/dl was significantly greater with rosuvastatin 10 mg/ezetimibe 10 mg (93.3%) vs. simvastatin 40 mg/ezetimibe 10 mg ($p = 0.03$, Fig. 1B).

3.2.3. Changes in other parameters

With combination therapy, HDL-C levels increased from baseline by a mean of 3.9%–7.5% across the groups, with significant ($p < 0.05$) increases observed with rosuvastatin 20 mg/ezetimibe 10 mg vs. either of the simvastatin/ezetimibe doses (Table 2). Rosuvastatin 20 mg/ezetimibe 10 mg achieved significantly ($p < 0.001$) greater reductions in TC, TG, nonHDL-C and ApoB levels and TC/HDL-C, LDL-C/HDL/C, nonHDL-C/HDL-C and ApoB/ApoA-I ratios vs. either of the simvastatin/ezetimibe doses (Table 2). Reductions in these parameters were also significantly ($p < 0.05$) greater with rosuvastatin 10 mg/ezetimibe 10 mg vs. simvastatin 40 mg/ezetimibe 10 mg (Table 2). All treatments resulted in median hsCRP level reductions from baseline (Table 2). There were no statistically significant differences between groups in ApoA-1 or hsCRP changes.

3.3. Biomarkers

Of 814 patients in the intention-to-treat population, 542 agreed to participate in the optional biomarker analysis. Baseline biomarker levels were similar across the groups (Table 1). Other baseline characteristics for the sub-population participating in the biomarker analysis were similar across the groups, and generally consistent with those of the overall study population (Supplemental Table 2).

Significant ($p < 0.05$) reductions from baseline to end of monotherapy were observed in all groups for lanosterol, 7-ketocholesterol, free cholesterol and both concentration and activity of Lp-PLA2 and for C4 for both rosuvastatin groups; changes from baseline to end of monotherapy in β-sitosterol were not significant for any treatment group (Fig. 2).

Median reductions from baseline to end of combination therapy were numerically larger for most biomarkers compared with changes from baseline to end of monotherapy, except for C4 and lanosterol (Fig. 2). Reductions from end of monotherapy to end of combination therapy were significant ($p \leq 0.002$) for all treatment groups for 7-ketocholesterol (except for rosuvastatin 20 mg/ezetimibe 10 mg), β-sitosterol, free cholesterol and Lp-PLA$_2$ activity and concentration (Fig. 2). There were no significant reductions from end of monotherapy to end of combination therapy for C4 and lanosterol (Fig. 2).

Median changes in biomarker levels are shown in Supplemental Table 3.

Table 2
Mean (SD) percentage change from baseline in lipids, lipoproteins and hsCRP at week 12 after 6 weeks combination therapy (intention-to-treat population).

	RSV 10 mg/EZE 10 mg ($n = 210$)	RSV 20 mg/EZE 10 mg ($n = 204$)	SIM 40 mg/EZE 10 mg ($n = 199$)	SIM 80 mg/EZE 10 mg ($n = 201$)
LDL-C	−59.7 (14.2)[a]	−63.5 (16.7)[b]	−55.2 (15.8)	−57.4 (20.5)
HDL-C	6.4 (13.9)	7.5 (16.4)[c]	3.9 (12.7)	4.3 (12.6)
Total cholesterol	−43.0 (11.2)[a]	−46.6 (12.8)[b]	−39.6 (12.7)	−41.7 (15.2)
Triglycerides	−28.9 (23.7)[d]	−35.0 (24.0)[b]	−23.0 (28.1)	−25.8 (26.6)
nonHDL-C	−54.7 (13.7)[e]	−58.9 (14.9)[b]	−49.9 (14.7)	−52.4 (18.4)
ApoB[f]	−46.1 (12.6)[a]	−49.5 (13.8)[b]	−42.0 (14.8)	−44.2 (17.2)
ApoA-I[f]	3.8 (13.0)	2.7 (11.5)	1.5 (9.7)	2.1 (10.7)
Total cholesterol/HDL-C	−45.5 (13.2)[e]	−49.5 (13.6)[b]	−41.3 (13.0)	−43.5 (16.3)
LDL-C/HDL-C	−61.5 (15.5)[e]	−65.3 (16.7)[b]	−57.1 (14.8)	−58.7 (21.1)
nonHDL-C/HDL-C	−56.4 (15.4)[e]	−60.9 (15.5)[b]	−51.1 (15.4)	−53.5 (20.2)
ApoB/ApoA-I	−47.4 (13.9)[e]	−50.2 (14.3)[b]	−42.5 (14.2)	−44.8 (17.8)
hsCRP[g]	−25.2 (−99.0, 20295.3)	−34.1 (−97.9, 2368.3)	−28.5 (−91.5, 1611.4)	−30.6 (−94.9, 1270.4)

[a] $p < 0.01$ vs. SIM 40 mg/EZE 10 mg.
[b] $p < 0.001$ vs. SIM 40 mg/EZE 10 mg and SIM 80 mg/EZE 10 mg.
[c] $p < 0.05$ vs. SIM 80 mg/EZE 10 mg and SIM 40 mg/EZE 10 mg.
[d] $p < 0.05$ vs. SIM 40 mg/EZE 10 mg.
[e] $p < 0.001$ vs. SIM 40 mg/EZE 10 mg.
[f] $n = 206$, 199, 194, and 199 for RSV 10 mg/EZE 10 mg, RSV 20 mg/EZE 10 mg, SIM 40 mg/EZE 10 mg and SIM 80 mg/EZE 10 mg, respectively.
[g] Values are median (range) % change from baseline: $n = 207$, 202, 197, and 199 for RSV 10 mg/EZE 10 mg, RSV 20 mg/EZE 10 mg, SIM 40 mg/EZE 10 mg and SIM 80 mg/EZE 10 mg, respectively.

3.4. Safety

AEs were experienced by 32.7% and 31.4% of patients overall during monotherapy and combination therapy, respectively (Supplemental Table 4). Overall incidence of liver, muscle and renal AEs was low in all treatment groups during monotherapy and combination therapy: one case of myopathy was reported in the rosuvastatin 10 mg group and two cases of myopathy in the simvastatin 80 mg group during monotherapy; one case of myopathy was reported in the rosuvastatin 20 mg/ezetimibe 10 mg group during combination therapy. No cases of rhabdomyolysis were reported.

Nineteen patients discontinued due to an AE during monotherapy (Supplemental Table 4). The most common AE leading to withdrawal was myalgia, occurring in one patient each in the rosuvastatin 10 mg and simvastatin 40 mg groups and three patients in the rosuvastatin 20 mg group. Twelve patients discontinued due to an AE during combination therapy (Supplemental Table 4). The most frequent AEs leading to withdrawal were fatigue, muscle spasms and dizziness, with each occurring in no more than one patient in any group and no notable differences in the frequency of AEs between groups.

During monotherapy, 12 patients (1.4%), distributed across all groups, experienced SAEs (Supplemental Table 4). Two patients in the rosuvastatin 20 mg group experienced a cerebrovascular accident, and all other SAEs occurred in no more than one patient. Sixteen patients (2.1%) experienced an SAE during combination therapy (Supplemental Table 4). The most frequent SAE was unstable angina occurring in two patients receiving rosuvastatin 10 mg plus ezetimibe and three patients receiving simvastatin 40 mg plus ezetimibe. All other SAEs were isolated reports. No deaths occurred during the study.

4. Discussion

4.1. Efficacy

The primary findings in this study were that rosuvastatin 20 mg/ezetimibe 10 mg had a significantly greater LDL-C lowering effect than simvastatin 40–80 mg/ezetimibe 10 mg. Rosuvastatin 10 mg/ezetimibe 10 mg also achieved significantly greater LDL-C reductions vs. simvastatin 40 mg/ezetimibe 10 mg. In all treatment groups, mean LDL-C reductions from baseline were >50%, in keeping with the 2011 European guideline recommendations for very high-risk patients [25]. Furthermore, a significantly greater proportion of patients receiving rosuvastatin 20 mg/ezetimibe 10 mg achieved LDL-C goals of <100 mg/dl and <70 mg/dl vs. both simvastatin/ezetimibe combinations, and significantly more patients receiving rosuvastatin 10 mg/ezetimibe 10 mg achieved the LDL-C goal of <100 mg/dl vs. simvastatin 40 mg/ezetimibe 10 mg. Significant reductions in other atherogenic lipids were also observed with rosuvastatin/ezetimibe vs. simvastatin/ezetimibe combinations. Previously, significantly greater reductions in LDL-C, TC, nonHDL-C and ApoB were reported following co-administration of simvastatin 20 mg with ezetimibe 10 mg vs. monotherapy with rosuvastatin 10 mg in hypercholesterolemic patients [11]. To our knowledge there is no study directly comparing rosuvastatin–ezetimibe with atorvastatin–ezetimibe, but the LDL-C reductions observed with rosuvastatin–ezetimibe in this study are well in line with what has previously been reported for atorvastatin–ezetimibe combinations [3].

4.2. Biomarkers

Levels of various biomarkers relating to cholesterol metabolism were assessed in this study as an exploratory analysis. Cholesterol synthesis, as judged by levels of lanosterol, was reduced with statin monotherapy in all treatment groups in the present study, which was expected from the statin mode of action as a cholesterol synthesis inhibitor [29], as lanosterol precedes mevalonate in the cholesterol biosynthesis pathway [24]. Conversely, there were essentially no further changes in lanosterol levels (and therefore in cholesterol synthesis) when ezetimibe was added to statin. Instead, reductions in levels of the cholesterol absorption marker β-sitosterol (plant sterol) were observed in all treatment groups in the combination therapy phase but not during statin monotherapy. This is in line with previous results [17] and confirms ezetimibe's mode of action as a cholesterol absorption inhibitor [28].

Cholesterol is excreted in the form of bile acids. However, there have been suggestions that bile acid synthesis may be reduced in patients with coronary artery disease compared with those without coronary artery disease [9]. Previously, it was reported that atorvastatin reduced synthesis of the bile acid precursor C4 in familial hypercholesterolemia patients, but only in those with partial ileal

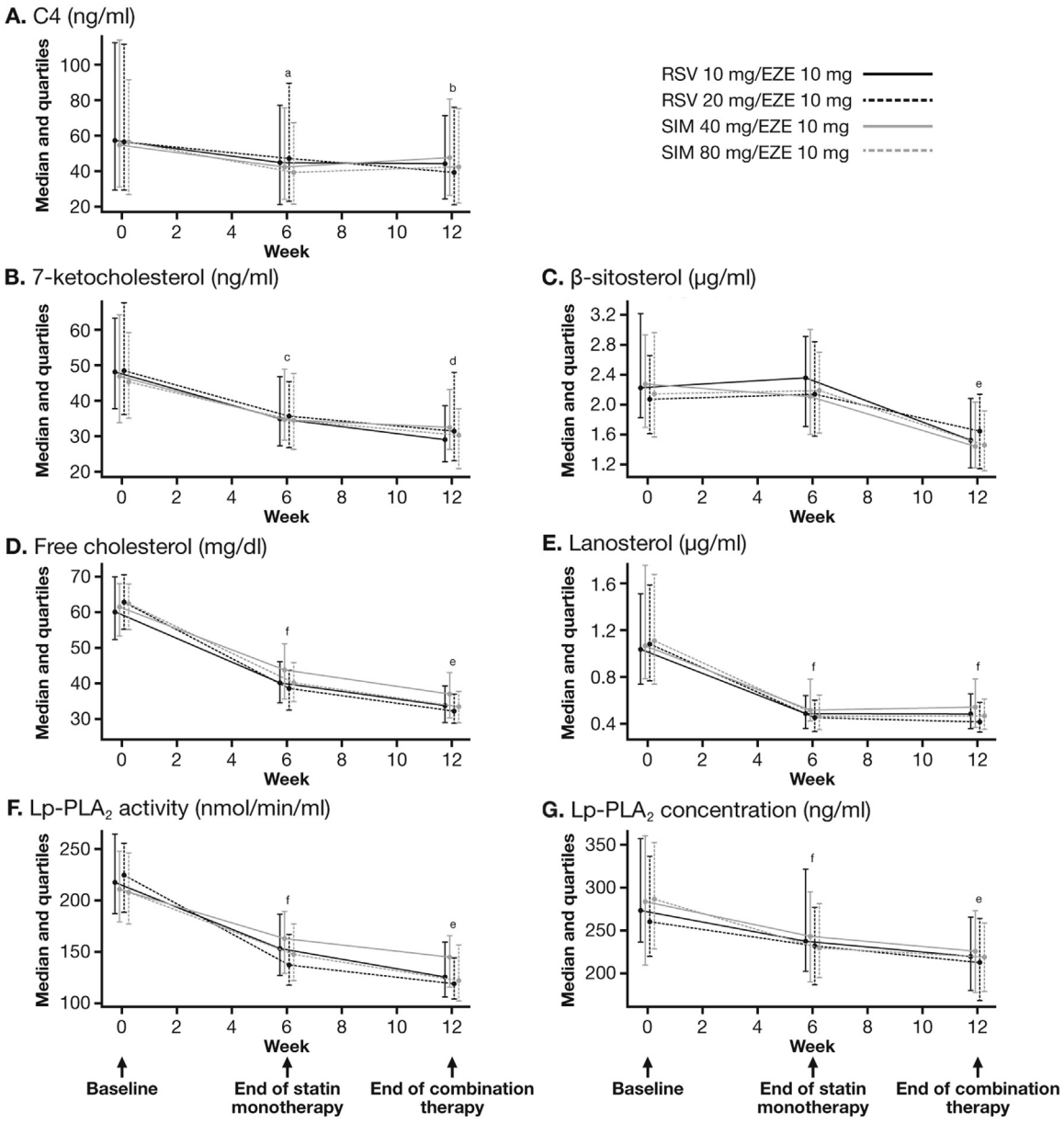

Fig. 2. Median (interquartile range) biomarker measurements by treatment and phase. Error bars show interquartile ranges (intention-to-treat population patients for whom biomarker measurements were available). [a]$p < 0.05$ vs. baseline for RSV 10 mg/EZE 10 mg and RSV 20 mg/EZE 10 mg, [b]$p < 0.05$ vs. baseline for all treatment groups except SIM 40 mg/EZE 10 mg, [c]$p < 0.001$ vs. baseline for RSV 10 mg/EZE 10 mg and RSV 20 mg/EZE 10 mg, and $p < 0.05$ vs. baseline for SIM 40 mg/EZE 10 mg and SIM 80 mg/EZE 10 mg, [d]$p \leq 0.002$ vs. monotherapy for all treatment groups except RSV 20 mg/EZE 10 mg, and $p < 0.001$ vs. baseline for all treatment groups, [e]$p \leq 0.001$ vs. baseline and $p < 0.001$ vs. monotherapy for all treatment groups, [f]$p < 0.001$ vs. baseline for all treatment groups. For numbers of patients in each treatment group see Table 1.

bypass or following administration of bile acid sequestrants, conditions that increase C4 levels [22]. The present study noted a trend for C4 reduction with rosuvastatin and simvastatin monotherapy, which reached statistical significance with rosuvastatin treatment; the clinical significance of these findings is unclear. Ezetimibe addition appeared to have no further effect on C4 levels.

7-ketocholesterol is a marker of atherosclerotic disease progression [15,16], the burden of atherosclerosis and lipid rich core [16], and reductions in plasma levels of 7-ketocholesterol have been noted following treatment with atorvastatin and fenofibrates [2]. This is in agreement with the results of the present study, where 7-ketocholesterol levels were significantly reduced in all treatment groups after 12 weeks' treatment, with the majority of the reductions occurring over the first 6 weeks of statin monotherapy.

Esterification of free cholesterol in plasma lipoproteins by lecithin:cholesterol acyltransferase (LCAT) has a key role in HDL metabolism; a lack of LCAT activity may contribute to atherogenesis although evidence for this is contradictory [27]. Levels of free (unesterified) cholesterol were significantly reduced with all treatments in the present study, with further reductions achieved with addition of ezetimibe to statin. The ratio of free cholesterol to TC can be used as an indirect measure of the cholesterol esterification rate (i.e. LCAT activity), and was roughly 0.25 at baseline and virtually unchanged after 12 weeks of treatment across the treatment groups, suggesting that the study treatments did not affect cholesterol esterification rates.

Significant reductions in the activity and concentrations of Lp-PLA_2, which have both been shown to be associated with

cardiovascular risk [30], were also observed in all treatment groups, with roughly 70%–80% of the overall reductions in Lp-PLA_2 activity or concentrations occurring during statin monotherapy. Statins have been previously reported to reduce Lp-PLA_2 activity and mass [21,26]; however, there are conflicting reports in the literature on whether ezetimibe reduces Lp-PLA_2 levels and mass [19,23,26] or has no effect [13,21].

4.3. Safety

Combination therapy with rosuvastatin 10–20 mg plus ezetimibe 10 mg was well tolerated overall, with a safety profile similar to that of simvastatin/ezetimibe combination therapy. Moreover, a similar proportion of patients experienced AEs across all treatment groups during combination therapy. SAEs were infrequent during the study. In EXPLORER study, 6 weeks' treatment with rosuvastatin 40 mg/ezetimibe 10 mg was generally well tolerated [4]. In another study, rosuvastatin 5–10 mg plus ezetimibe 10 mg had a similar safety profile to rosuvastatin 10–20 mg without ezetimibe after 6 weeks' treatment [5]. It should be noted that since 2011, the US Food and Drug Administration has recommended limiting prescription of the simvastatin 80 mg dose to only those individuals who have already been taking the drug for 1 year or more and the drug should not be started at this dose in new patients because of an increased risk of myopathy [12,31].

In summary, the addition of ezetimibe to rosuvastatin or simvastatin was associated with further reductions in atherogenic lipids and lipoproteins and some biomarkers. It is hoped that results of the large (>18,000 patients) IMProved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) (ClinicalTrials.gov identifier: NCT00202878), comparing simvastatin alone with fixed-dose simvastatin/ezetimibe combinations, will provide more definitive information of the clinical benefits and risks of ezetimibe added to a statin [8].

5. Conclusions

For difficult-to-treat patients with very high LDL-C levels who cannot achieve their LDL-C treatment goal, data from both the present study and the previous EXPLORER study indicate that combining rosuvastatin with ezetimibe could be an effective option to achieve further reductions in LDL-C and is more effective than combining simvastatin with ezetimibe in bringing patients to LDL-C goals. Measurement of biomarkers related to cholesterol and lipoprotein metabolism confirm that statins reduce lanosterol, a marker of cholesterol synthesis, and ezetimibe reduces sitosterol, a marker of cholesterol absorption.

The observation of additive reductions in Lp-PLA_2 mass and activity and 7-ketocholesterol along with ApoB, non-HDL-C and LDL-C suggests that combination therapy may provide additional benefit in patients who continue to have high levels of atherogenic lipoproteins or biomarkers after statin monotherapy. Unfortunately, this hypothesis is not being tested in the IMPROVE IT trial which enrolled only individuals with well controlled LDL-C on statin therapy and remains to be addressed in future studies of statins combined with other therapies that reduce atherogenic lipoproteins.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2013.10.022.

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