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ORIGINAL RESEARCH ARTICLE

Effect of the BET Protein Inhibitor, RVX-208, on Progression of Coronary Atherosclerosis: Results of the Phase 2b, Randomized, Double-Blind, Multicenter, ASSURE Trial

Stephen J. Nicholls[1] · Rishi Puri[2] · Kathy Wolski[2] · Christie M. Ballantyne[3] · Philip J. Barter[4] · H. Bryan Brewer[5] · John J. P. Kastelein[6] · Bo Hu[2] · Kiyoko Uno[2] · Yu Kataoka[1] · Jean-Paul R. Herrman[7] · Bela Merkely[8] · Marilyn Borgman[2] · Steven E. Nissen[2]

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Abstract

Background Bromodomain and extra-terminal (BET) proteins regulate transcription of lipoprotein and inflammatory factors implicated in atherosclerosis. The impact of BET inhibition on atherosclerosis progression is unknown.

Methods ASSURE was a double-blind, randomized, multicenter trial in which 323 patients with angiographic coronary disease and low high-density lipoprotein cholesterol (HDL-C) levels were randomized in a 3:1 fashion to treatment with the BET protein inhibitor RVX-208 200 mg or placebo for 26 weeks. Plaque progression was measured with serial intravascular ultrasound imaging. Lipid levels, safety, and tolerability were also assessed.

Results During treatment, apolipoprotein (apo)A-I increased by 10.6 % with placebo ($P < 0.001$ compared with baseline) and 12.8 % with RVX-208 ($P < 0.001$ compared with baseline), between groups $P = 0.18$. HDL-C increased by 9.1 % with placebo ($P < 0.001$ compared with baseline) and 11.1 % with RVX-208 ($P < 0.001$ compared with baseline), between groups $P = 0.24$. Low-density lipoprotein cholesterol (LDL-C) decreased by 17.9 % with placebo ($P < 0.001$ compared with baseline) and 15.8 % with RVX-208 ($P < 0.001$ compared with baseline), between groups $P = 0.55$. The primary endpoint, the change in percent atheroma volume, decreased 0.30 % in placebo-treated patients ($P = 0.23$ compared with baseline) and 0.40 % in the RVX-208 group ($P = 0.08$ compared with baseline), between groups $P = 0.81$. Total atheroma volume decreased 3.8 mm^3 in the placebo group ($P = 0.01$ compared with baseline) and 4.2 mm^3 in the RVX-208 group ($P < 0.001$ compared with baseline), $P = 0.86$ between groups. A greater incidence of elevated liver enzymes was observed in RVX-208-treated patients (7.1 vs. 0 %, $P = 0.009$).

Conclusion Administration of the BET protein inhibitor RVX-208 showed no greater increase in apoA-I or HDL-C or incremental regression of atherosclerosis than administration of placebo.

Trial Registration ClinicalTrials.gov identifier—NCT01067820.

Key Points

RVX-208 had no incremental effect on lipids compared with placebo.

RVX-208 had no incremental effect on plaque regression.

The impact of RVX-208 on cardiovascular outcomes remains to be determined.

✉ Stephen J. Nicholls
stephen.nicholls@sahmri.com

1 South Australian Health and Medical Research Institute, PO Box 11060, Adelaide, SA 5001, Australia

2 Department of Cardiovascular Medicine and Cleveland Clinic Coordinating Center for Clinical Research, Cleveland Clinic, Cleveland, OH, USA

3 Section of Cardiovascular Research, Baylor College of Medicine and the Methodist DeBakey Heart and Vascular Center, Houston, TX, USA

4 University of New South Wales, Sydney, NSW, Australia

5 Medstar Research Institute, Hyattsville, MD, USA

6 Department of Vascular Medicine, Academic Medical Center, University of Medicine, Amsterdam, The Netherlands

7 Department of Cardiology, Onze Lieve Vrouwe Gasthuis, Amsterdam, The Netherlands

8 Heart and Vascular Center, Semmelweis University, Budapest, Hungary

1 Introduction

Persistent cardiovascular risk despite widespread use of established medical therapies has stimulated interest in developing new strategies for secondary prevention in patients with coronary artery disease. Favorable findings from human [1] and animal [2] population studies have resulted in considerable interest in development of agents that promote greater levels or activity of high-density lipoproteins (HDLs). However, to date, the success of HDL-cholesterol (HDL-C)-raising therapies in randomized controlled trials has been disappointing [3–5], with the exception of infusing delipidated forms of HDL [6–8].

The induction of endogenous synthesis of apolipoprotein A-I (apoA-I), the major protein associated with HDL particles, represents a novel approach to lipid modification. Enhanced hepatic synthesis of apoA-I should theoretically generate new HDL particles, resulting in greater biological activity of HDL. Preliminary studies of the bromodomain and extra-terminal (BET) inhibitor RVX-208 demonstrated increased apoA-I and HDL-C levels [9] and enhanced systemic cholesterol efflux activity [10]. The BET family of proteins plays an important role in transcriptional regulation and has been implicated in a variety of disease processes. As a result, these effects of RVX-208 have the theoretical potential to favorably affect atherosclerotic plaque.

Intravascular ultrasonography (IVUS) has been successfully employed in clinical trials to evaluate the impact of medical therapies on progression of coronary atherosclerosis [6, 11–17]. These studies have demonstrated that infusion of HDL mimetics has a beneficial impact on plaque progression [6–8]. Accordingly, the objective of the ASSURE (ApoA-I Synthesis Stimulation and Intravascular Ultrasound for Coronary Atheroma Regression Evaluation) study was to determine the 26-week impact of the BET protein inhibitor RVX-208 on the burden of coronary atherosclerosis in patients with coronary disease and low HDL-C levels.

2 Methods

2.1 Study Design

ASSURE was a prospective, randomized, multicenter, double-blind clinical trial [18]. The trial was designed by the Cleveland Clinic Coordinating Center for Clinical Research (C5Research) in collaboration with the Executive Steering Committee and the sponsor. The study protocol was approved by the institutional review board at each site. Patients provided written informed consent prior to study entry.

Patients aged at least 18 years were eligible if they demonstrated at least one 20 % stenosis on clinically indicated coronary angiography and a target vessel for imaging with less than 50 % obstruction. Patients were required to have a low HDL-C level ($\leq$45 mg/dl in females, $\leq$40 mg/dl in males) within the 60 days prior to enrolment and receive treatment with either atorvastatin 10–40 mg daily or rosuvastatin 5–20 mg daily during the study. Patients treated with another statin agent were switched to rosuvastatin prior to randomization. Patients were excluded if they were receiving either a fibrate or nicotinic acid at a dose of at least 250 mg daily, had uncontrolled hypertension, or had heart failure, severe renal dysfunction, or liver disease.

Patients meeting the inclusion criteria underwent randomization in a 3:1 ratio via interactive voice response system to treatment with RVX-208 100 mg or placebo administered twice daily for 26 weeks. Patients were seen in the clinic every 2 weeks for the first 8 weeks and then every 3 weeks for the remainder of the study.

2.2 Acquisition and Analysis of Ultrasound Images

Following coronary angiography, baseline IVUS was performed. Previous reports have described the methods of image acquisition and analysis [6, 11–17]. Imaging was performed in a single artery using either the s5^{TM} (Volcano, Sacramento, CA, USA) or iLab^{TM} (Boston Scientific, Boston, MA, USA) systems and screened by the core laboratory at the C5Research. Patients meeting prespecified requirements for image quality were eligible for randomization. After 26 weeks of treatment, patients underwent a second ultrasonographic examination in the same artery with the same imaging system. Using digitized images, personnel, who were unaware of the treatment status and time sequence of paired imaging, performed measurements of the lumen and external elastic membrane in images within a matched artery segment. The accuracy and reproducibility of this method have been reported previously [19].

The primary efficacy measure, percent atheroma volume (PAV), was calculated as follows

$$\mathrm{PAV} = \frac{\sum (\mathrm{EEM}_{\mathrm{area}} - \mathrm{Lumen}_{\mathrm{area}})}{\sum \mathrm{EEM}_{\mathrm{area}}} \times 100$$

where $\mathrm{EEM}_{\mathrm{area}}$ is the cross-sectional area of the external elastic membrane and $\mathrm{Lumen}_{\mathrm{area}}$ is the cross-sectional area of the lumen. The change in PAV was calculated as the PAV at 26 weeks minus the PAV at baseline. A secondary measure of efficacy, normalized total atheroma volume (TAV), was calculated as follows:

$$\mathrm{TAV}_{\mathrm{Normalized}} = \frac{\sum (\mathrm{EEM}_{\mathrm{area}} - \mathrm{Lumen}_{\mathrm{area}})}{\text{number of images in pullback}} \times \text{median number of images in cohort}$$

where the average plaque area in each image was multiplied by the median number of images analyzed in the entire cohort to compensate for differences in segment length between subjects. The efficacy measure of change in normalized TAV was calculated as the TAV at 26 weeks minus the TAV at baseline. The efficacy measure of change in atheroma volume in the most diseased sub-segment was calculated in the 10-mm segment containing the greatest plaque burden at baseline. Regression was defined as a decrease in PAV or TAV from baseline.

2.3 Biochemical Measures

A central laboratory performed all biochemical determinations (ACM, Rochester, NY, USA; and York, UK). Lipid profiles were determined by enzymatic assay. Levels of apoA-I were determined by turbidimetric immunoassay (Boston Heart Diagnostics, Framingham, MA, USA). Lipoprotein particle number and size were measured with nuclear magnetic resonance (LipoScience, Raleigh, NC, USA) as previously described [20]. Particle concentrations of lipoprotein subclasses of different sizes were calculated from the measured amplitudes of their spectroscopically distinct lipid methyl signals. Lipoprotein levels and safety laboratory measurements were obtained and any adverse reactions recorded at each study visit. High-sensitivity C-reactive protein (CRP) was determined by nephelometry.

2.4 Statistical Analysis

Categorical variables are summarized using frequencies, while laboratory parameters are reported as median and interquartile range (IQR). The primary endpoint was the change in PAV from baseline to 26 weeks post-randomization within the RVX-208 treatment arm and was tested using the Wilcoxon signed-rank test. A similar test was performed separately on the placebo group. The median percent change in laboratory parameters from baseline to 26 weeks within and between treatment groups was tested using the Wilcoxon signed-rank test. Although the study was not sufficiently powered to detect differences between treatment groups, an analysis of covariance (ANCOVA) on ranked data was performed with treatment group as a factor and baseline value as a covariate. The safety analysis of laboratory abnormalities and cardiovascular events was tested using either Chi-squared or Fisher's exact test.

A sample size of 186 was selected to provide 85 % power to detect a decrease from baseline in PAV of 0.6 % with a standard deviation of 2.7 % in the active RVX-208-treated group. An additional 62 patients were planned to be randomized into the placebo group. With an anticipated non-completion rate of 20 %, a total of 310 patients were planned for enrollment into the trial.

All reported P values are two-sided.

The lead academic investigator (SJN) wrote the manuscript and vouches for the accuracy and completeness of the data and the analyses. While the Executive Steering Committee and C5Research had confidentiality agreements with the sponsor, the study contract specified that a copy of the study database be provided to C5Research for independent analysis and granted the academic authors the unrestricted rights to publish the results.

3 Results

3.1 Subject Characteristics

The disposition of patients in the study is summarized in Fig. 1. From 30 September 2011 to 25 September 2012, at 60 centers, 676 patients were screened, with 244 patients randomized to the RVX-208 group and 80 to the placebo group. After 26 weeks of treatment, 281 patients (87.0 %) remained in the study and had IVUS imaging that permitted measurement of atheroma burden at baseline and follow-up. Of these patients, 208 were in the RVX-208 group and 73 in the placebo group. No significant differences were observed between the treatment groups in demographic characteristics, medication use, and laboratory values at baseline (Table 1). No significant differences were observed in baseline characteristics and laboratory values between those patients who completed the study and those who did not. The dose of rosuvastatin (13.8 ± 5.4 vs. 13.2 ± 5.6 mg, $P = 0.54$) and atorvastatin (29.1 ± 11.4 vs. 27.7 ± 11.7 mg, $P = 0.60$) did not differ in placebo- or RVX-208-treated patients, respectively. The rate of statin discontinuation did not differ between placebo and RVX-208 groups during the study (14.1 vs. 20.0 %, $P = 0.27$).

3.2 Biochemical Measurements

Table 2 summarizes the percentage change of laboratory values for the 281 patients who completed the trial. During the 26 weeks of treatment, apoA-I increased 10.6 % ($P < 0.001$ compared with baseline) in the placebo group and 12. 8% ($P < 0.001$ compared with baseline) in the RVX-208 group, between groups $P = 0.18$. HDL-C increased by 9.1 % ($P < 0.001$ compared with baseline) in the placebo group and 11.1 % ($P < 0.001$ compared with baseline) in the RVX-208 group, between groups $P = 0.24$. Low-density lipoprotein cholesterol (LDL-C)

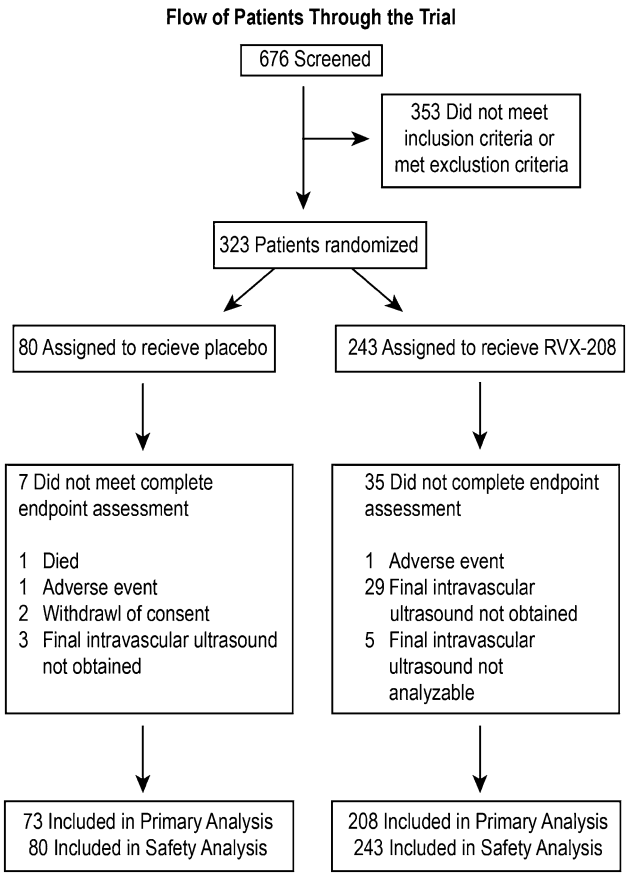

Fig. 1 Disposition of patients in study. The final disposition of patients in each group includes all patients assigned to study drug. Patients who withdrew from the study include those discontinued due to subject or physician decision. Adverse events include discontinuation due to adverse events or abnormal laboratory or electrocardiographic results

levels decreased by 17.9 % with placebo ($P < 0.001$ compared with baseline) and 15.8 % with RVX-208 ($P < 0.001$ compared with baseline), between groups $P = 0.55$. There were no incremental reductions in other atherogenic lipid parameters or high-sensitivity CRP with RVX-208 compared with placebo. There were no significant differences in nuclear magnetic resonance-derived measures of HDL particle concentrations between the treatment and placebo groups.

3.3 Intravascular Ultrasound Endpoints

Table 3 summarizes the change in IVUS efficacy measures. Greater measures of total atheroma volume [199.9 mm^3, 95 % confidence interval (CI) 154.4–258.3 vs. 154.8 mm^3, 95 % CI 118.1–209.7; $P < 0.001$] and atheroma volume in the most diseased 10-mm segment (61.6 mm^3, 95 % CI 41.9–82.9 vs. 50.7 mm^3, 95 % CI 37.1–70.4; $P = 0.05$), but not percent atheroma volume (38.1 %, 95 % CI 33.4–44.3 vs. 36.2 %, 95 % CI 30.2–44.8; $P = 0.11$) were observed in the RVX-208 group. The primary endpoint, change in PAV within the RVX-208 group, decreased by 0.40 % ($P = 0.08$ compared with baseline). PAV decreased by 0.30 % ($P = 0.23$ compared with baseline) in the placebo group, between groups $P = 0.81$. TAV decreased by 3.8 mm^3 ($P = 0.01$ compared with baseline) and 4.2 mm^3 ($P < 0.001$ compared with baseline) in the RVX-208 group, between groups $P = 0.86$. Atheroma volume in the 10-mm subsegment that contained the greatest disease burden at baseline decreased by 1.3 mm^3 ($P = 0.01$ compared with baseline) in the placebo group and 2.2 mm^3 ($P < 0.001$ compared with baseline) in the RVX-208 group, between groups $P = 0.79$. Changes in the primary efficacy parameter, PAV, stratified according to prespecified subgroups are summarized in Fig. 2. There was no evidence of statistical heterogeneity, suggesting a differential effect of RVX-208 in different subgroups.

3.4 Clinical and Laboratory Adverse Events

Reasons for study discontinuation, laboratory abnormalities, and investigator-reported clinical events are summarized in Table 4. A greater number of discontinuations due to adverse events were observed in the RVX-208 group (3.7 vs. 2.5 %). Elevations of hepatic transaminases greater than three times the upper limit of normal occurred more frequently in the RVX-208 group (7.0 vs. 0 %, $P = 0.009$). No episode of liver enzyme elevation was accompanied by an increase in bilirubin levels above the upper limit of normal. All episodes of liver enzyme elevation were observed between 4 and 8 weeks of treatment and resolved spontaneously when study drug administration continued. No significant differences in cardiovascular events were observed between the groups (13.8 % in the placebo treatment group vs. 7.4 % in the RVX-2008 group, $P = 0.09$).

4 Discussion

The ASSURE study evaluated the early impact of a BET protein inhibitor previously reported to increase endogenous synthesis of apoA-1. While modest increases in apoA-I and HDL-C and a decrease in LDL-C from baseline were observed with RVX-208, these changes did not differ from the placebo group. Furthermore, while reductions from baseline in plaque burden were observed in the RVX-208 group, these effects did not differ from changes observed in placebo-treated patients. These data demonstrate that RVX-208 treatment did not result in any measureable

Table 1 Patient demographics, concomitant medications, and baseline laboratory values in placebo- and RVX-208-treated patients

Parameter	Placebo ($n = 80$)	RVX-208 ($n = 243$)	P value
Age (years)	57.6 ± 9.6	58.3 ± 8.5	0.57
Male	57 (71.3)	189 (77.8)	0.24
Caucasian	80 (100)	238 (97.9)	0.76
Body mass index (kg/m^2)	30.5 ± 5.4	30.0 ± 4.7	0.73
Hypertension	69 (86.3)	193 (79.4)	0.18
Diabetes	23 (28.8)	76 (31.3)	0.67
Smoking history	52 (65.0)	170 (70.0)	0.41
Prior myocardial infarction	32 (40.0)	98 (40.3)	0.96
Prior PCI	35 (43.8)	92 (37.9)	0.35
Prior CABG	2 (2.5)	3 (1.2)	0.60
Concomitant medication use			
Prior statin use	63 (78.8)	203 (83.5)	0.33
Aspirin	67 (83.8)	208 (85.6)	0.69
Beta blocker	59 (73.8)	196 (80.7)	0.19
ACE inhibitor	37 (46.3)	102 (42.0)	0.50
Baseline biochemistry			
Total cholesterol (mmol/l)	4.0 (3.3–4.9)	4.0 (3.4–4.8)	0.71
LDL cholesterol (mmol/l)	2.5 ± 0.9	2.5 ± 0.9	0.67
HDL cholesterol (mmol/l)	1.0 (0.9–1.1)	1.0 (0.9–1.1)	0.97
Triglycerides (mmol/l)	1.5 (1.0–2.1)	1.5 (1.1–2.1)	0.97
Non-HDL cholesterol (mmol/l)	3.0 (2.4–3.8)	3.1 (2.5–3.8)	0.60
Apolipoprotein B (g/l)	0.8 (0.7–1.0)	0.9 (0.7–1.0)	0.84
Apolipoprotein A-I (g/l)	1.2 (1.0–1.3)	1.2 (1.0–1.3)	0.45
Total HDL particles (μmol/l)	25.4 (22.9–28.0)	26.2 (23.6–29.3)	0.12
Small HDL particles (μmol/l)	16.6 (14.5–18.7)	16.8 (14.5–19.5)	0.45
Large HDL particles (μmol/l)	2.1 (1.6–3.1)	2.3 (1.6–3.0)	0.93
hsCRP (mg/l)	3.1 (1.6–5.7)	2.3 (1.1–5.4)	0.24

Data are expressed as mean ± standard deviation, n (%) or median (interquartile range)

ACE angiotensin-converting enzyme, *CABG* coronary artery bypass grafting, *HDL* high-density lipoprotein, *hsCRP* high-sensitivity C-reactive protein, *LDL* low-density lipoprotein, *PCI* percutaneous coronary intervention

incremental benefit on plaque regression for patients with coronary artery disease and low HDL-C levels.

While there is considerable interest in the discovery of new anti-atherosclerotic agents for patients with coronary disease, their clinical development is challenging. Selection of appropriate efficacy biomarkers is critical in the early evaluation of new compounds, in order to focus on agents with the greatest likelihood of success in large and expensive outcome trials. However, to date, no biomarker has consistently predicted clinical benefit for HDL-raising strategies. While substantial interest has focused on measures of HDL functionality [21, 22], none of these techniques are validated to the point that they can reliably determine the potential efficacy of new agents.

Since the expected effects of HDL-modifying strategies involve stimulation of reverse cholesterol transport, the current study and other efforts have employed techniques that measure changes in plaque burden as a measure of their potential benefits. This approach is supported by observations that measurements of coronary plaque burden using IVUS are associated with cardiovascular outcomes [23]. With evidence of enhanced systemic cholesterol efflux capacity in preliminary studies [10], we anticipated that RVX-208 might promote incremental regression of atherosclerotic plaque on serial IVUS evaluation compared with background therapy. However, RVX-208 did not demonstrate an incremental benefit on plaque burden and was accompanied by liver enzyme elevations compared with placebo.

The lack of additional efficacy of RVX-208 is disappointing and surprising, given promising earlier findings. In fact, the lipid changes observed with RVX-208 were

Table 2 Median (interquartile range) percentage change in lipid and inflammatory laboratory values in placebo- and RVX-208-treated patients

Parameter	Placebo ($n = 80$)		RVX-208 ($n = 243$)		†P value between groups
	Percent change	†P value vs. BL	Percent change	†P value vs. BL	
Total cholesterol (mmol/L)	−11.1 (−22.4 to 8.6)	0.001	−5.9 (−19.5 to 9.1)	<0.001	0.30
LDL cholesterol (mmol/L)	−17.9 (−31.6 to 0)	<0.001	−15.8 (−31.0 to 4.3)	<0.001	0.55
HDL cholesterol (mmol/L)	9.1 (−8.3 to 22.2)	<0.001	11.1 (0 to 25.0)	<0.001	0.24
Triglycerides (mmol/L)	−2.9 (−22.4 to 43.6)	0.19	4.0 (−24.2 to 33.0)	0.04	0.77
Non-HDL cholesterol (mmol/L)	−14.3 (−32.2 to 7.9)	<0.001	−12.0 (−28.2 to 6.7)	<0.001	0.50
Apolipoprotein B (g/L)	−11.5 (−26.9 to 4.7)	<0.001	−6.1 (−21.8 to 6.8)	<0.001	0.23
Apolipoprotein A-I (g/L)	10.6 (1.3 to 18.4)	<0.001	12.8 (1.1 to 22.5)	<0.001	0.18
Total HDL particles (μmol/L)[a]	6.3 (−1.1 to 20.1)	<0.001	10.0 (−0.5 to 23.2)	<0.001	0.13
Small HDL particles (μmol/L)[a]	6.0 (−7.5 to 22.7)	0.003	5.5 (−8.9 to 22.5)	<0.001	0.98
Large HDL particles (μmol/L)[a]	38.0 (−6.3 to 75.5)	<0.001	38.1 (0 to 95.8)	<0.001	0.69
hsCRP (mg/L)	−33.8 (−59.8 to 23.7)	0.08	−32.7 (−67.1 to 16.9)	<0.001	0.65

BL baseline, *HDL* high-density lipoprotein, *hsCRP* high-sensitivity C-reactive protein, *LDL* low-density lipoprotein

[a] Nuclear magnetic resonance spectroscopy-derived HDL particle measures

† P value based on analysis of covariance on rank-transformed data with treatment group as a factor and baseline value as a covariate

Table 3 Measures of atheroma burden at baseline and nominal change in placebo- and RVX-208-treated patients

Parameter	Placebo ($n = 73$)	RVX-208 ($n = 208$)	P value[a]
Baseline atheroma burden			
Percent atheroma volume (%)	36.2 (30.2 to 44.8)	38.1 (33.4 to 44.3)	0.11
Total atheroma volume (mm^3)	154.8 (118.1 to 209.7)	199.9 (154.4 to 258.3)	<0.001
Atheroma volume at most diseased 10-mm sub-segment (mm^3)	50.7 (37.1 to 70.4)	61.6 (41.9 to 82.9)	0.05
Nominal change in atheroma burden			
Percent atheroma volume (%)	−0.3 (–1.7 to 0.9); $P = 0.23$[b]	−0.4 (−1.8 to 1.3); $P = 0.08$[b]	0.81
Total atheroma volume (mm^3)	−3.8 ± 12.6; $P = 0.01$[b]	−4.2 ± 17.2; $P < 0.001$[b]	0.86
Atheroma volume at most diseased 10-mm sub-segment (mm^3)	−1.3 (–5.4 to 1.9); $P = 0.01$[b]	−2.2 (−6.1 to 1.7); $P < 0.001$[b]	0.79
Percentage of patients demonstrating any regression			
Percent atheroma volume	41 (56.2)	117 (56.3)	0.99
Total atheroma volume	40 (54.8)	115 (55.3)	0.94

Data are presented as median (interquartile range), n (%), or as least-squared mean ± standard error

[a] P value for comparison between groups. For nominal change in atheroma burden, the P value was generated from an analysis of covariance on rank-transformed data with treatment group as a factor and baseline value as a covariate

[b] P value for comparison from baseline

similar to those reported in prior studies, yet they paralleled changes in the placebo group, resulting in no incremental benefit on plaque burden. Despite prior evidence of enhanced cholesterol efflux capacity [10] and accumulation of larger, cholesterol-rich HDL particles [9], we observed no incremental effect on HDL-associated measures compared with placebo. This is particularly striking since patients had low HDL cholesterol levels at baseline. The lack of a robust incremental impact on systemic measures of HDL presaged the failure of RVX-208 to promote greater plaque regression in well-treated patients. This finding may possibly reflect either a lack of efficacy of RVX-208 or the inability to improve on benefits produced by statins and other background therapies. It should be particularly noted that the lipid changes in the placebo group were unexpected and may have contributed to the lack of benefit on plaque burden with RVX-208 in this study.

Enhanced endogenous expression of apoA-I is an attractive approach to the therapeutic modification of HDL

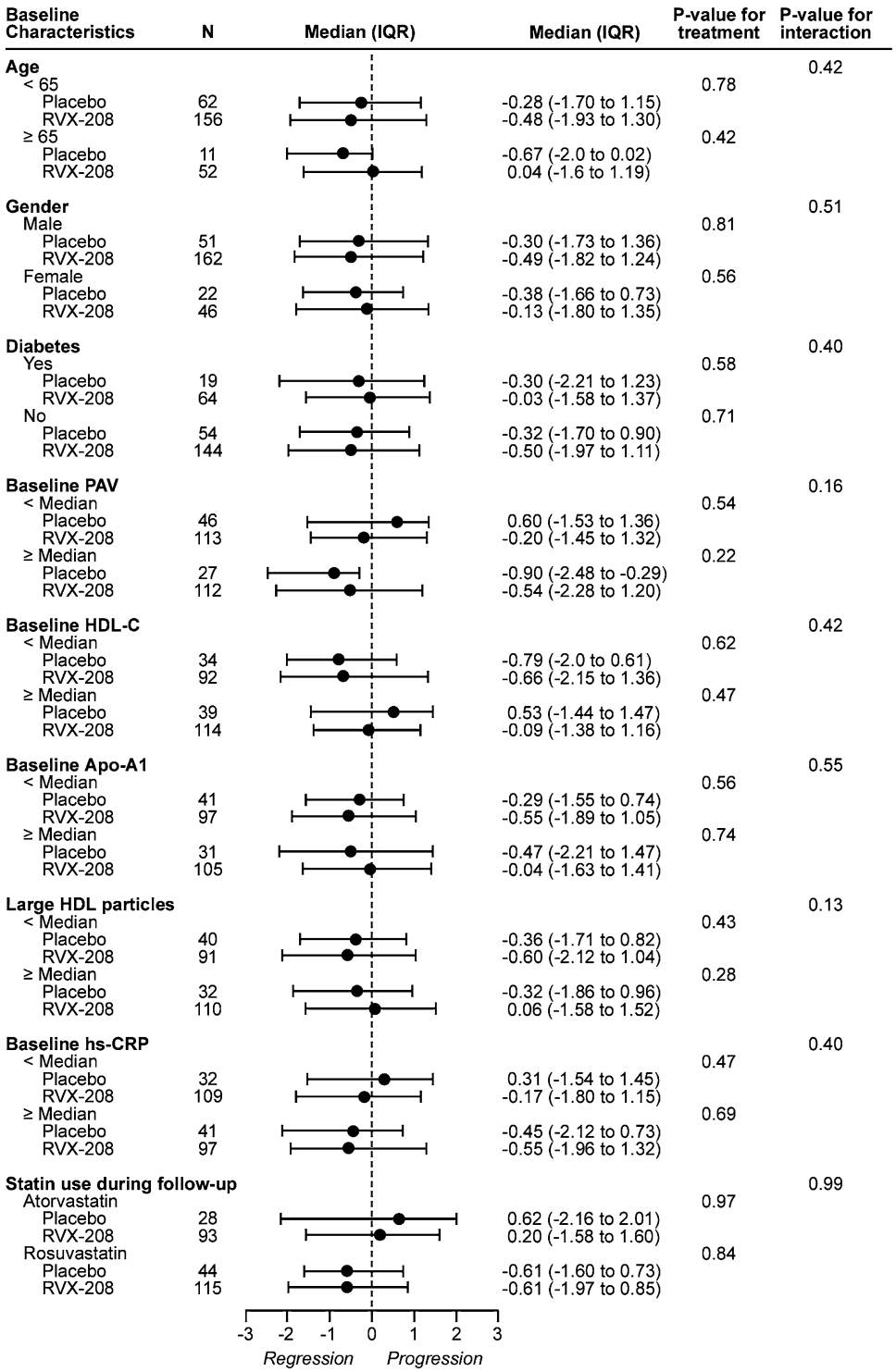

Fig. 2 Prespecified subgroup analysis of the primary endpoint, change in percent atheroma volume from baseline in the RVX-208 treatment group to week 26. *Apo* apolipoprotein, *HDL* high-density lipoprotein, *HDL-C* HDL-cholesterol, *hs-CRP* high-sensitivity C-reactive protein, *IQR* interquartile range, *PAV* percent atheroma volume

function. Finding an agent that selectively upregulates hepatic apoA-I expression, without effects on other proteins, has proven daunting. The lipid changes in previous studies of RVX-208 were consistent with enhanced lipid mobilization. However, given the lack of any discernible incremental effect on HDL with RVX-208 in ASSURE I, the absence of a favorable impact on atherosclerotic plaque should not be interpreted as a failure of the HDL hypothesis. Ongoing clinical trials will evaluate the potential cardiovascular efficacy of other mechanisms that target HDL. While it is possible that HDL may be present in a dysfunctional form in patients with coronary disease and a high prevalence of concomitant risk factors [24], whether this may have contributed to any lack of incremental

Table 4 Reasons for discontinuation from the study, laboratory abnormalities, and cardiovascular events in placebo- and RVX-208-treated patients

Parameter	Placebo ($n = 80$)	RVX-208 ($n = 243$)	P value
Discontinuation from study			
Discontinuation	4 (5.0)	19 (7.8)	0.40
Reason for discontinuation			
Adverse event	1 (1.3)	1 (0.4)	
Lost to follow-up	0	2 (0.8)	
Withdrawal of consent	2 (2.5)	14 (5.8)	
Death	1 (1.3)	0	
Other	0	2 (0.8)	
Laboratory abnormalities			
ALT/AST >3 × ULN	0	17 (7.1)	0.009
Bilirubin >2 × ULN	0	0	1.00
CK >3 × ULN	0	3 (1.3)	0.58
Creatinine >1.5 × ULN	0	2 (0.9)	1.00
Cardiovascular events			
Major adverse cardiovascular events	11 (13.8)	18 (7.4)	0.09
Death	1 (1.3)	0	0.25
Myocardial infarction	1 (1.3)	4 (1.6)	1.00
Coronary revascularization	7 (8.8)	11 (4.5)	0.17
Hospitalization for unstable angina or heart failure	3 (3.8)	5 (2.1)	0.41

Data are presented as n (%) unless otherwise indicated

Four patients did not have follow-up ALT/AST data and 11 patients did not have follow-up CK or creatinine data

ALT alanine transaminase, *AST* aspartate transaminase, *CK* creatine kinase, *ULN* upper limit of normal

benefit on plaque burden is uncertain. The prior observations that RVX-208 increases large HDL particles would suggest that HDL is intact, although this requires ongoing validation.

Molecular investigations revealed that RVX-208 increased hepatic apoA-I expression via bromodomain and extra-terminal protein inhibition. Resulting derepression of the genetic sequence coding for apoA-1 led to an increase in protein synthesis [25]. As a result, this compound represents the first epigenetic foray into the metabolic treatment of cardiovascular disease. It will be of interest to see whether other epigenetic therapeutic approaches to the modification of cardiometabolic risk proceed into clinical development.

A number of limitations of the current study should be noted. All patients presented for a clinically indicated coronary angiogram. Whether similar findings would have been observed in asymptomatic individuals is unknown. The study was shorter in duration than previous IVUS evaluations of oral therapies. Whether a favorable impact of RVX-208 might be observed during a longer period of follow-up is beyond the scope of this study. The current analysis evaluated the impact of RVX-208 on plaque volume and not plaque morphology. Patients requiring treatment with the highest doses of atorvastatin and rosuvastatin were excluded, thus the impact of RVX-208 in combination with potent statin therapy, which promotes marked disease regression [26], remains unknown. While no significant differences in patient characteristics were observed between the groups at baseline, the potential effect of residual confounding cannot be excluded. We did observe that several baseline measures of plaque burden were greater in the RVX-208-treated group. Previous studies have demonstrated greater regression in patients with higher baseline plaque volume [27]. Accordingly, a greater degree of regression with RVX-208 might have been anticipated in this setting, but was not observed. The ASSURE study was not powered to definitively evaluate the impact of RVX-208 on cardiovascular events, which would require evaluation via a large clinical outcomes trial.

In the quarter century following the introduction of statins to clinical practice, the search to identify new strategies to achieve greater cardiovascular risk reductions has been intensive yet unsuccessful. Ongoing residual risk [28] with current approaches and intolerance in some patients [29] emphasizes the need to develop new agents to reduce cardiovascular risk. In the current study, we did not observe an incremental effect on protective lipid parameters and atherosclerotic plaque burden with RVX-208. The

search to identify a strategy that promotes HDL functionality and improve cardiovascular outcomes continues.

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Investigators: Argentina: Sanatorio Allende Hipolito, Córdoba (E. Moreyra, MD), Hospital Privado, Córdoba (M. Ballarino, MD), Instituto de Cardiología de Corrientes, Corrientes Capital (J. Baccaro, MD), Sanatorio Parque S. A. Privado,Córdoba (H. Luquez, MD), Instituto de diagnóstico y tratamiento de afecciones cardiovasculares, La Plata (D. Grinfeld, MD), ICBA Instituto Cardiovascular de Buenos Aires, Ciudad de Buenos Aires (L. Padilla, MD), Instituto DAMIC SRL, Córdoba (A. Lorenzatti, MD), Sanatorio San Lucas, San Isidro (G. Marchetti, MD), Clinica Chutro SRL, Córdoba (H. Jure, MD), Sanatorio Güemes,Ciudad de Buenos Aires (M. Bettinotti, MD); Belgium: Universitair Ziekenhuis Antwerpen, Edegem (C. Vrints, MD), Ziekenhuis Oost Limburg, Genk (M. Vrolix, MD), Universite Catholique de Louvain, Brussels (J. Renkin, MD), Centre Hospitalier Universitaire de Charleroi, Charleroi (J. Lalmand, MD); Brazil: Instituto do Coração do Triângulo Mineiro, Uberlândia (R. Vieira Botelho, MD), Hospital Cardiológico Costantini, Curitiba (Costantino Roberto Costantini Frack, MD), Instituto do Coração, São Paulo (P. Lemos, MD), Santa Casa de Misericórdia de Porto Alegre, Porto Alegre (V. Correia deLima, MD), Hemodinâmica Meridional, Cariacica (B. Moulin Machado, MD), Centro de Pesquisa em Cardiologia da Via Médica, Goiânia (W. Kunz Sebba Barroso de Souza, MD), Hospital do Coração do Brasil, Brasília (A. Gomes Taques Fonseca, MD), Instituto Dante Pazzanese de Cardiologia, São Paulo (J. de Ribamar Costa Junior, MD); Hungary: Szent-Györgyi Albert Klinikai Központ, Szeged (I. Ungi, MD), Semmelweis Egyetem Kardiológiai Közpon, Budapest (B. Merkely, MD), Pécsi Tudományegyetem, Pécs (I. Horvath, MD), Fővárosi Önkormányzat Bajcsy-Zsilinszky Kórház, Budapest (B. Nagybaczoni, MD), Honvédkórház - Állami Egészségügyi Központ, Budapest (R. Kiss, MD), Budai Irgalmasrendi Kórhá, Budapest (A. Zsoldos, MD); Netherlands: Onze Lieve Vrouwe Gasthuis, Amsterdam, (J. P. Herrman, MD), Isala Klinieken, Zwolle (M. Gosselink, MD), St. Antonius Ziekenhuis, Niewegein (B. Rensing, MD), Catharina Ziekenhuis, Eindhoven (C. Joost Botman, MD), Maasstad Ziekenhuis, Rotterdam (P. Smits, MD), Maastricht Universitair Medisch Centrum, Maastricht (A. Moens, MD), Canisius Wilhelmina Ziekenhuis, Nijmegen (A. J. M. Oude Ophuis, MD), Medisch Spectrum Twente, Enschede (C. Von Birgelen, MD), Medisch Centrum Alkmaar, Alkmaar (A. A. C. M. Heestermans, MD); Poland: Samodzielna Pracownia Hemodynamiki, Krakow (D. Dudek, MD), Klinika Kardiologii Inwazyjnej, Warszawa (R. Gil, MD), Zakład Kardiologii Inwazyjnej, Katowice (A. Ochala, MD), Oddział Strukturalnych Chorób Serca Samodzielny Publiczny Szpital Kliniczny, Katowice (G. Smolka, MD), Instytut Kardiologii, Warszawa (A. Witkowski, MD); Russia: Acad. V. I. Shumakov, Moscow (V. Chestukhin, MD), St. Petersburg State Healthcare Institution, Saint Petersburg (L. Shcheglova, MD),

Russian Cardiology Research-and-Production Complex, Moscow (V. Kukharchuk MD, M. Ruda, MD, and F.Ageev, MD), 3 Central Military Clinical Hospital, Moscow (V. Ivanov, MD), Tyumen Department of South-Ural Scientific Center of RAMS, Tyumen (S. Shalaev, MD), A. N. Bakulev of RAMS Cardiosurgery Institution, Moscow (S. Matskeplishvili, MD), Scientific Research Cardiology Institute of Sibirian Branch of RAMS,Tomsk (O. Koshelskaya, MD), FSI Federal Center of Heart, Blood and Endocrinology, St. Petersburg (M. Karpenko, MD), FSI State Scientific Research Center of Preventive Medicine of Rosmedtechnologies, Moscow (V. Mazaev, MD), Orenburg State Medical Academy of Roszdrav, Orenburg (R. Sayfutdinov, MD); Spain: Hospital de Meixoeiro, Vigo (A. Iniguez, MD), Hospital Clinico Universitario Virgen de la Victoria, Málaga (J. Maria Hernandez, MD), Hospital de Cabueñes, Gijón (J. Rondan, MD), Hospital Universitari Germans Trias i Pujol, Barcelona (J. Mauri, MD), Hospital Universitario La Paz, Madrid (R. Moreno, MD), Hospital de Galdakao-Usansolo, Galdakao (J. Ramon Rumoroso, MD), Hospital General Universitario Santa Lucia, Cartagena (F. Pico, MD), Hospital Universitari de Bellvitge, Barcelona (A. Cequier, MD), Hospital Clinico Universitario San Carlos, Madrid (F. Alfonso, MD), Hospital Clínico Universitario de Santiago, Santiago de Compostela (R. Trillo, MD), Hospital Vall d'Hebrón, Barcelona (B. Blanco, MD), Hospital Universitario Marqués de Valdecilla, Santander (J. Zueco, MD).

Author contributions Drs. Nicholls and Nissen and the Cleveland Clinic Coordinating Center for Clinical Research had full and independent access to all of the data in the study. Dr. Nicholls takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs. Nicholls and Nissen and Ms. Wolski and Borgman were responsible for study concept and design. Drs. Nicholls, Puri, Uno, Herrman, Merkely, and Kataoka and Ms. Borgman take responsibility for data acquisition. Drs. Nicholls, Nissen, Ballantyne, Barter, Brewer, Kastelein, Puri, Uno, Kataoka, Hu, Herrman, and Merkely and Ms. Wolski and Ms. Borgman analysed and interpreted the data. Drs. Nicholls and Nissen and Ms. Wolski drafted the manuscript. Drs. Nicholls, Nissen, Ballantyne, Barter, Brewer, Kastelein, Puri, Uno, Kataoka, Hu, Herrman, and Merkely and Ms. Wolski and Ms. Borgman provided critical revision of the manuscript for important intellectual content. For the purpose of the academic interpretation of the study, Ms. Wolski and Dr. Hu performed all primary statistical analyses of the study that were used for the manuscript. Ms. Wolski is an employee of the Cleveland Clinic Coordinating Center for Clinical Research. Dr. Hu is a faculty member within the Department of Quantitative Health Sciences at the Cleveland Clinic Lerner College of Medicine of Case Western Reserve University. Drs. Nicholls and Nissen obtained funding for the study. Ms. Borgman provided administrative support.

Compliance with Ethical Standards

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Role of the Sponsor The sponsor, Resverlogix Corporation, participated actively in designing the study, developing the protocol, and providing logistical support during the trial. Monitoring of the study was performed by a contract research organization, inVentiv Health Inc., under contract with the sponsor. The sponsor maintained the trial database. Statistical analysis was performed by statisticians employed by inVentiv, although the analysis employed by the manuscript represent those performed by the academic statisticians (Ms. Wolski, Dr. Hu). After completion of the trial, as specified in the study contract, a complete copy of the database was transferred to the Cleveland Clinic Coordinating Center for Clinical Research, where analyses were verified by independent statisticians, Kathy Wolski, MPH and Bo Hu, PhD. The manuscript was prepared by the corresponding author and modified after consultation with co-authors. The sponsor was permitted to review the manuscript and suggest changes, but the final decision on content was exclusively retained by the academic authors.

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