Effects of the Direct Lipoprotein-Associated Phospholipase A_2 Inhibitor Darapladib on Human Coronary Atherosclerotic Plaque

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Background—Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is expressed abundantly in the necrotic core of coronary lesions, and products of its enzymatic activity may contribute to inflammation and cell death, rendering plaque vulnerable to rupture.

Methods and Results—This study compared the effects of 12 months of treatment with darapladib (an oral Lp-PLA_2 inhibitor, 160 mg daily) or placebo on coronary atheroma deformability (intravascular ultrasound palpography) and plasma high-sensitivity C-reactive protein in 330 patients with angiographically documented coronary disease. Secondary end points included changes in necrotic core size (intravascular ultrasound radiofrequency), atheroma size (intravascular ultrasound gray scale), and blood biomarkers. Background therapy was comparable between groups, with no difference in low-density lipoprotein cholesterol at 12 months (placebo, 88±34 mg/dL; darapladib, 84±31 mg/dL; $P=0.37$). In contrast, Lp-PLA_2 activity was inhibited by 59% with darapladib ($P<0.001$ versus placebo). After 12 months, there were no significant differences between groups in plaque deformability ($P=0.22$) or plasma high-sensitivity C-reactive protein ($P=0.35$). In the placebo-treated group, however, necrotic core volume increased significantly (4.5±17.9 mm^3; $P=0.009$), whereas darapladib halted this increase (−0.5±13.9 mm^3; $P=0.71$), resulting in a significant treatment difference of −5.2 mm^3 ($P=0.012$). These intraplaque compositional changes occurred without a significant treatment difference in total atheroma volume ($P=0.95$).

Conclusions—Despite adherence to a high level of standard-of-care treatment, the necrotic core continued to expand among patients receiving placebo. In contrast, Lp-PLA_2 inhibition with darapladib prevented necrotic core expansion, a key determinant of plaque vulnerability. These findings suggest that Lp-PLA_2 inhibition may represent a novel therapeutic approach. **(*Circulation*. 2008;118:1172-1182.)**

Key Words: atherosclerosis ■ drugs ■ imaging ■ lipoprotein-associated phospholipase A_2

Despite intensive management of conventional risk factors, many patients continue to experience recurrent coronary events.[1] Most acute coronary events arise from initially non–flow-limiting stenoses that often are underestimated by angiography.[2,3] The salient features of culprit lesions resulting in fatal myocardial infarction include the

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disruption of a thin fibrous cap, the presence of a large lipid-rich necrotic core, and an extensive inflammatory cell infiltrate.[4] Recent intravascular ultrasound (IVUS)–based regression trials could not address these important lesion characteristics because of inherent limitations in IVUS technology, although they have demonstrated a relationship between changes in the overall plaque volume and plasma low-density lipoprotein cholesterol (LDL-C) levels.[5,6] Because the current standard of care for high-risk cardiovascular patients already mandates intensive LDL-C lowering, intravascular imaging that could assess changes in plaque biomechanical or compositional characteristics may be particularly helpful in evaluating the mechanisms of future events or the efficacy of new pharmacological agents that act via novel mechanisms.[7]

Editorial p 1120
Clinical Perspective p 1182

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is a circulating enzyme produced and secreted by inflammatory cells centrally involved in atherosclerosis,[8–11] bound predominantly to apolipoprotein B–containing lipoproteins, and highly expressed in the necrotic core of atherosclerotic lesions.[12,13] Although this enzyme was first described as the platelet-activating factor acetylhydrolase, it has much broader substrate specificity.[14] Lp-PLA_2 rapidly degrades oxidatively modified phospholipids in LDL-C, leading to formation of proinflammatory and cytotoxic products.[8,15,16] Because enhanced cell death and impaired clearance of apoptotic bodies are thought to be key mechanisms for necrotic core expansion,[17] Lp-PLA_2 inhibition may favorably affect rupture-prone lesions.

The objective of this study was to explore the effects of treatment with a direct Lp-PLA_2 inhibitor (darapladib) on coronary plaque deformability, composition, and size. In addition, the effects of Lp-PLA_2 inhibition on several biomarkers and clinical safety parameters were assessed.

Methods

Study Design

The Integrated Biomarkers and Imaging Study-2 trial was an international, multicenter, randomized, double-blind, placebo-controlled study of patients with angiographically confirmed coronary heart disease. The trial was designed by the Steering Committee (Appendix) in collaboration with the sponsor. Institutional review boards at each center approved the protocol, and patients provided written informed consent.

Patient Population

Patients ≥18 years of age undergoing cardiac catheterization for acute coronary syndrome (ACS; ST-segment elevation myocardial infarction or non–ST-segment elevation myocardial infarction) or non-ACS (eg, chronic stable angina or troponin-negative resting chest pain) were eligible. The protocol specified 50% of randomized patients to have troponin-positive ACS. Key exclusion criteria were planned surgical revascularization, stroke in the past 6 months, chronic hepatic disorder or abnormal alanine aminotransferase, bilirubin (alanine aminotransferase >2.5 or bilirubin >1.5 times the upper limit of normal), serum creatinine >2.0 mg/dL, blood pressure >160/100 mm Hg, poorly controlled diabetes mellitus (HbA_{1c} >10%), severe heart failure or left ventricular ejection fraction <30%, and current life-threatening condition. Patients were ineligible if angiography demonstrated left main coronary stenosis >50% or their coronary anatomy was inappropriate for IVUS. Within 10 days of qualifying cardiac catheterization, eligible patients were randomized to oral doses of darapladib 160 mg (GlaxoSmithKline, King of Prussia, Pa) or placebo once daily (1:1 ratio) for the 12-month treatment. The randomization was stratified according to ACS status and center.

IVUS Imaging

After intracoronary nitroglycerin administration, the vessel was imaged with a 20-MHz IVUS catheter (Eagle-Eye, Volcano Therapeutics, Rancho Cordova, Calif) during a 0.5-mm/s continuous motorized pullback (R-100 pullback device). The nonintervened studied segment had to be at least 40 mm long and exhibit <50% stenosis by angiography. All imaging data were stored digitally in a dedicated console (In-Vision Gold, Volcano Therapeutics, Rancho Cordova, Calif). Patients who received at least 1 dose of the investigational agent were scheduled to undergo IVUS of the same study vessel at 12 months. Patients who required urgent cardiac catheterization <12 months but >6 months after randomization had their imaging data included in the analysis. Details regarding imaging methodologies are provided in the online Data Supplement and are illustrated schematically in Figure 1.

IVUS-Based End-Point Definitions

IVUS-Based Palpography

The density of high strain per 10 mm (the coprimary end point) was defined as the number of cross sections with strain values ≥0.9% divided by the number of all analyzable cross sections in the region of interest and normalized for 10 mm.[7]

IVUS-Based Radiofrequency Analysis

The necrotic core volume was calculated by multiplying the mean necrotic core area of the region of interest by its length. Necrotic core values were expressed in cubic millimeters or as the percent of total radiofrequency (RF)-derived plaque volume. Other components of the plaque (dense calcium, fibrofatty tissue, and fibrous tissue) were calculated in the same manner.

IVUS Gray Scale

Total atheroma volume and percent atheroma volume were calculated. The latter was derived by dividing the total atheroma volume by the total vessel volume and multiplying by 100. Of note, total atheroma volume derived from IVUS gray scale contains both atherosclerotic plaque and media.

Biomarkers

Blood samples were collected at baseline and after randomization. Plasma high-sensitivity C-reactive protein (hsCRP; coprimary end point) was measured as described previously.[18] Plasma Lp-PLA_2 activity was measured by a colorimetric method with an intra-assay precision of 1.7% and interassay precision of 4.8%.[11,18] Details regarding other biomarkers are provided in the online Data Supplement.

Clinical Safety

Patient safety was assessed through adverse event reporting, physical examinations, ECG monitoring, and clinical laboratory tests. After randomization, patients visited the study centers at 1 and 3 months and every 3 months thereafter.

To detect any potential signal of harm, the Independent Data Monitoring Committee met every 3 months, by teleconference or in person, to review the unblinded safety data. The Independent Data Monitoring Committee deliberations took place entirely separately and independently of the Steering Committee or the sponsor (Appendix).

Statistical Analysis

Continuous variables for imaging end points were reported as mean±SD. Within-treatment-group changes from baseline were

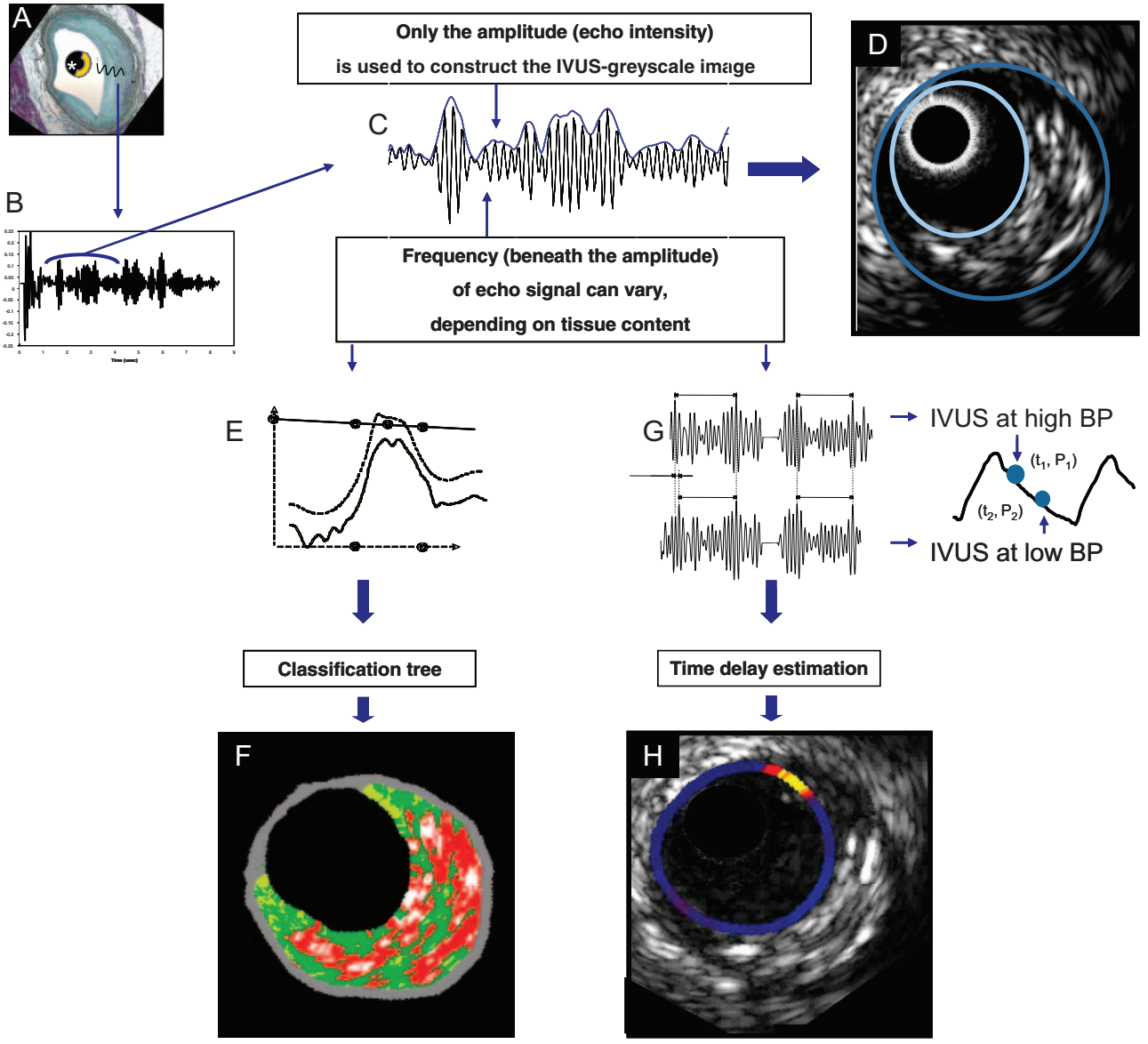

Figure 1. A, The ultrasound signal is generated in a piezoelectric crystal (*) that transmits and receives sound waves. B, Ultrasound reflected by the tissue deforms crystal, generating RF signal. C, Gray-scale IVUS is derived from the amplitude of RF signal, discarding information beneath the peaks of the signal. D, Changes in the electric field of the piezoelectric crystal caused by ultrasound reflection are used to generate a gray-tone image. E, IVUS RF analysis uses several additional spectral parameters to identify 4 plaque components. F, Plaque components that are identified are dense calcium (white), fibrous (green), fibrofatty (greenish-yellow), and necrotic core (red). G, IVUS palpography takes advantage of RF signals generated by the artery being deformed by blood pressure (BP). Using analysis of RF signals at "low" and "high" BP, the strain (deformation) in the inner layer of atheroma is determined. H, This strain is quantified and superimposed on the IVUS image at the lumen/vessel wall boundary. Note that high strain (yellow) is found at the shoulders of the eccentric plaque.

evaluated by paired *t* tests, and treatment comparisons of changes from baseline in IVUS were analyzed with ANCOVA adjusted for ACS status, pooled country, baseline value, and segment length. Treatment differences were expressed as the adjusted mean, 95% CI, and probability value. Biomarkers were log transformed and analyzed with last-observation-carried-forward ANCOVA and repeated-measures mixed modeling adjusted for ACS status, pooled country, visit, and interaction between visit and treatment. Treatment differences were expressed as adjusted percentage change, 95% CI, and probability value. No multiplicity adjustments were applied; all probability values are presented 2 sided.

The sample size was determined with an estimation approach because the effects of an Lp-PLA$_2$ inhibitor on novel imaging parameters were unknown. The SDs of changes for high-strain density (IVUS palpography) and hsCRP in a similar patient population were used, with additional consideration given to the expected number of nonevaluable IVUS imagings.[7] With this approach, by recruiting ≈300 patients, the study could estimate the treatment effects on the reduction of plaque deformability or hsCRP with good precision; reductions of ≈20% were anticipated to reach statistical significance.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Between November 16, 2005, and August 16, 2006, we randomized 330 patients. We assigned 155 patients to pla-

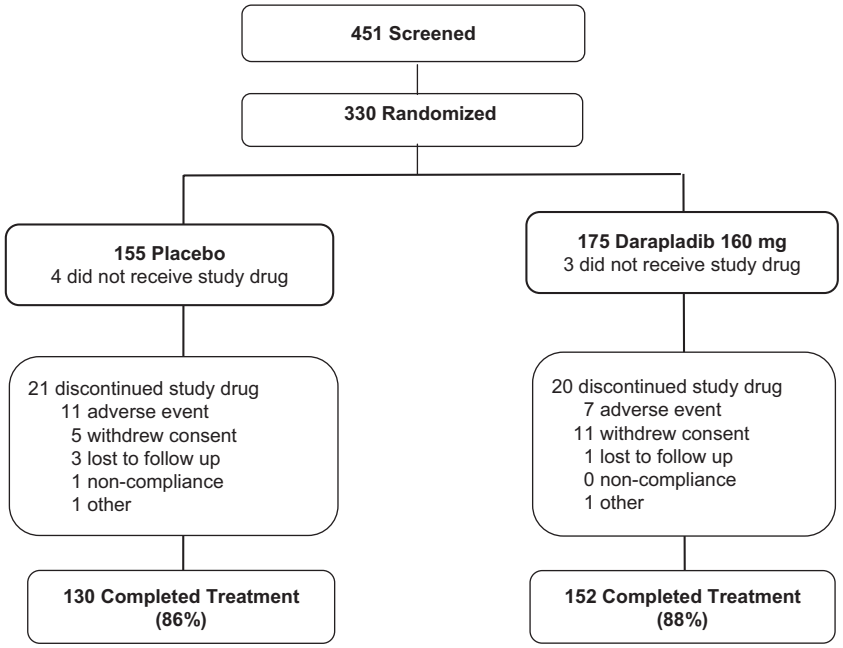

Figure 2. Patient disposition flow chart. Completed treatment refers to patients who received ≥11 months of treatment.

cebo and 175 patients to darapladib (Figure 2). As shown in Table 1, both groups were well matched except for a higher prevalence of hypertension among those receiving darapladib. At randomization, there was a high level of adherence to guideline-mandated treatments, including statin use in ≈90% of patients.

Imaging Efficacy Results

Of randomized patients who received at least 1 dose of placebo or darapladib, those with analyzable baseline and follow-up IVUS after a minimum of 6 months of treatment constituted the imaging population. The median time to follow-up IVUS was 364 days in the placebo and 364 days in the darapladib group, with repeat IVUS at ≥11 months after randomization in 97% and 96% patients, respectively.

IVUS Palpography

At baseline, the density of high strain per 10 mm was comparable between placebo-treated (n=115) and darapladib-treated (n=131) patients (0.44±0.64 versus 0.43±0.63, respectively). At follow-up, the between-group comparison of the change from baseline in density of high strain (coprimary end point) was not statistically significant (−0.08; 95% CI, −0.21 to 0.05; P=0.22). Details regarding prespecified sensitivity analysis, including only vessels with high strain at baseline, are provided in the online Data Supplement.

In the 10-mm subsegments with the highest baseline density of high strain per 10 mm (placebo, 1.22±1.56; darapladib, 1.21±1.62), both groups showed significant reductions after 12 months (placebo, 35%, P=0.001; darapladib, 33%, P=0.002), but the difference between groups was not significant (P=0.87).

IVUS RF Analysis

At baseline, necrotic core volume measurements were comparable between treatment groups in the entire region of interest and in the worst 10-mm subsegment (Table 2).

In the entire region of interest, necrotic core volume increased significantly during the study period among those receiving placebo (4.5±17.9 mm^3; P=0.009) but remained unchanged in the darapladib group (−0.5±13.9 mm^3; P=0.71 for within-group comparison). When the change from baseline was compared between groups, there was a significant treatment effect in favor of darapladib (−5.2 mm^3; 95% CI, −9.2 to −1.1; P=0.012). The nominal changes in necrotic core expressed as percent of RF-derived atheroma volume showed consistent treatment effects (Table 2).

In the 10-mm subsegment containing the largest necrotic core volume, there also was a significant treatment effect in favor of darapladib (−2.2 mm^3; 95% CI, −3.6 to −0.8; P=0.003; Table 2).

Figure 3 illustrates the high degree of consistency in treatment effect of darapladib compared with placebo in several important clinical subgroups.

When the individual plaque components were analyzed as a percent of IVUS RF–derived atheroma, an increase in necrotic core was paralleled by a decrease in fibrous tissue. In a comparison of the 2 groups, the differences in necrotic core (P=0.047) were accompanied by the reciprocal changes in fibrous tissue (P=0.021) (Figure 4). These findings suggest that in the placebo group a larger amount of fibrous tissue was converted into necrotic core, whereas in the darapladib group this process was significantly attenuated. The Table in the online Data Supplement provides data for all plaque components.

Gray-Scale IVUS

At baseline, total atheroma volume was comparable between the placebo-treated (n=118) and darapladib-treated (n=143) groups (placebo, 313±149 mm^3; darapladib, 327±189 mm^3; P=0.51). At 12 months, atheroma volume decreased by −4.9±32.7 mm^3 in the placebo group (P=0.10) and −5.0±28.0 mm^3 in the darapladib group (P=0.033).

Table 1. Baseline Characteristics

	Placebo (n=151)	Darapladib (n=172)
Clinical characteristics		
Age, y	57.3±10.9	59.4±9.8
Men, n (%)	126 (83)	140 (81)
Body mass index, kg/m^2	27.8±3.8	27.5±4.0
Diabetes mellitus, n (%)	22 (15)	22 (13)
Hypertension, n (%)	89 (59)	115 (67)
Low HDL cholesterol (<40 mg/dL), n (%)	40 (26)	45 (26)
Hypercholesterolemia, n (%)	95 (63)	108 (63)
Current smoker, n (%)	57 (38)	64 (37)
Prior medical history, n (%)		
Prior myocardial infarction	49 (32)	51 (29)
Prior coronary revascularization	47 (31)	50 (29)
Peripheral artery disease	7 (5)	17 (10)
Prior stroke	3 (2)	4 (2)
Index hospitalization, n (%)		
ACS	74 (49)	87 (51)
STEMI	35 (23)	40 (23)
Non-STEMI	39 (26)	47 (27)
PCI during index hospitalization	122 (81)	130 (76)
Cardiovascular medications at randomization, n (%)		
Aspirin	138 (91)	149 (87)
Clopidogrel or ticlopidine	122 (81)	136 (79)
Any antiplatelet medication	150 (>99)	170 (99)
ACE inhibitors or ARBs	88 (58)	101 (59)
β-Blockers	119 (79)	138 (80)
Statins	134 (89)	157 (91)
Laboratory values		
Cholesterol, mg/dL		
Total	187.3±47.6	182.3±43.2
LDL	108.2±41.4	103.6±37.4
HDL	46.8±11.2	48.0±12.4
Triglycerides, mg/dL		
Median	141	136
IQR	97–202	96–193
hsCRP, mg/L		
Geometric mean	2.4	2.4
95% CI	1.9, 3.1	1.9, 3.0
Lp-PLA$_2$ activity, μmol · min^{-1} · L^{-1}		
Geometric mean	159	160
95% CI	152, 167	153, 167
Blood pressure, mm Hg		
Systolic	125.7±16.9	128.0±16.1
Diastolic	75.2±10.1	75.6±9.9
Study vessel*		
LAD, n (%)	44 (36)	56 (39)
LCx, n (%)	32 (26)	37 (26)
RCA, n (%)	45 (37)	51 (35)
Diameter stenosis, %†	28.0 (10.5)	26.6 (10.3)
Mean lumen diameter, mm†	2.9±0.5	3.0±0.6

HDL indicates high-density lipoprotein; STEMI, ST-segment–elevation myocardial infarction; PCI, percutaneous coronary intervention; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; IQR, interquartile range; LAD, left anterior descending artery; LCx, left circumflex artery; and RCA, right coronary artery. Values are presented as mean±SD unless otherwise specified. To convert to mmol/L, multiply values of cholesterol by 0.02586 and triglycerides by 0.0113.

*Imaging evaluable population: placebo, 121 patients; darapladib, 146 patients.

†Quantitative coronary angiography: placebo, 121 patients; darapladib, 144 patients.

Table 2. Necrotic Core Measurements Based on IVUS RF Analysis

	Placebo (n=110)	Darapladib (n=129)	P
Region of interest, mm	48±16	49±16	
Necrotic core volume, mm^3*			
Baseline			
Mean±SD	21.5±21.9	22.8±24.5	
Median	14.8	13.6	
Interquartile range	6.1 to 28.2	7.3 to 32.4	
Follow-up			
Mean±SD	26.0±25.3	22.4±25.8	
Median	15.7	16.3	
Interquartile range	8.1 to 36.4	5.6 to 27.1	
Change from baseline			
Mean±SD	4.5±17.9	−0.5±13.9	
P†	0.009	0.71	
Least-squares mean (95% CI)‡	−5.2 (−9.2 to −1.1)		0.012
Necrotic core of total plaque, %*			
Baseline			
Mean±SD	13.1±7.6	13.4±6.5	
Median	12.6	12.5	
Interquartile range	6.8 to 16.8	9.1 to 17.5	
Follow-up			
Mean±SD	15.7±8.8	13.9±7.7	
Median	14.7	12.6	
Interquartile range	8.5 to 22.1	7.8 to 18.7	
Change from baseline			
Mean±SD	2.5±9.6	0.5±7.6	
P†	0.006	0.453	
Least-squares mean (95% CI) ‡	−2.0 (−3.9 to −0.0)		0.047
Necrotic core in the worst 10 mm, mm^3			
Baseline			
Mean±SD	9.2±8.1	9.1±7.6	
Median	7.2	6.8	
Interquartile range	3.5 to 11.8	3.2 to 12.7	
Follow-up			
Mean±SD	10.1±9.2	7.9±6.7	
Median	7.3	5.7	
Interquartile range	3.3 to 16.0	2.7 to 11.2	
Change from baseline			
Mean±SD	0.9±6.6	−1.2±4.9	
P†	0.162	0.008	
Least-squares mean (95% CI)‡	−2.2 (−3.6 to −0.8)		0.003

*Values denote measurements in the entire region of interest.

†Paired *t* test (within-group comparison).

‡ANCOVA adjusted for ACS, pooled country, baseline value, and matched segment length (between-group comparison).

The between-group comparison of change from baseline was not significant ($P=0.95$). The percent atheroma volume also was similar between groups (placebo, 42.2±10.0%; darapladib, 40.7±10.1%; $P=0.24$) at baseline. The within-group changes and between-group differences were not significant ($P=0.90$). Likewise, atheroma volume showed no treatment effect in the worst 10-mm subsegment (data not shown).

Biomarkers and Other Laboratory Assessments

At 12 months, hsCRP was 1.0 mg/L (95% CI, 0.8 to 1.2) among those receiving placebo (n=140) and 0.9 mg/L (95% CI, 0.8 to 1.1) in the darapladib group (n=162). Nonsignificant reductions in hsCRP were observed with repeated-measures analysis (−15%; 95% CI, −36 to 11; $P=0.22$). These results were consistent with the last-observation-carried-forward analysis and observed data at 12 months (hsCRP was lower in the darapladib group by −12% [95% CI, −32 to 15; $P=0.35$] and −22% [95% CI, −41 to 3; $P=0.08$], respectively). A significantly higher percentage of patients, however, achieved very low levels of hsCRP (<1 mg/L) on darapladib (62%) than did those on placebo (45%) (odds ratio, 1.99; 95% CI, 1.22 to 3.24; $P<0.008$).

Plasma Lp-PLA_2 activity was significantly reduced by darapladib at all time points. At 12 months, Lp-PLA_2 activity was 153 $\mu mol \cdot min^{-1} \cdot L^{-1}$ (95% CI, 147 to 159) in the placebo and 62 $\mu mol \cdot min^{-1} \cdot L^{-1}$ (95% CI, 58 to 65) in the darapladib group. This corresponds to −59% (95% CI, −62 to −56; $P<0.001$) inhibition of Lp-PLA_2 activity compared with placebo (repeated-measures analysis and last-observation-carried-forward analysis). The concentrations of other biomarkers (interleukin-6, myeloperoxidase, intracellular adhesion molecule-1, oxidized phospholipid/apolipoprotein B, and matrix metalloproteinase-9 activity) did not change significantly.

Approximately 94% patients continued statins at the end of the treatment period. The 12-month LDL-C was 88±34 mg/dL in the placebo group and 84±31 mg/dL in the darapladib group ($P=0.37$). Plasma high-density lipoprotein cholesterol also was unaffected by treatment (48±11 mg/dL in the placebo; 50±13 mg/dL in the darapladib group; $P=0.18$).

Adverse Events, Clinical Safety, and Outcomes

Table 3 summarizes adverse events in both treatment groups. A higher incidence of malodor (mainly feces or urine) was reported with darapladib (16%) compared with placebo (3%) but was an uncommon cause of withdrawal from the study (darapladib, 2%).

Vital signs were similar except for the mean on-treatment systolic blood pressure, which was higher in the darapladib group by 3.0 mm Hg (95% CI, 0.3 to 5.7; $P=0.031$) when analyzed with the repeated-measures analysis adjusted for baseline values. After 12 months, systolic blood pressure was 129.6±17.2 mm Hg in the placebo and 133.3±18.5 mm Hg in the darapladib group. A posthoc analysis of the intraaortic blood pressure at the time of follow-up cardiac catheterization showed no differences between groups (placebo,

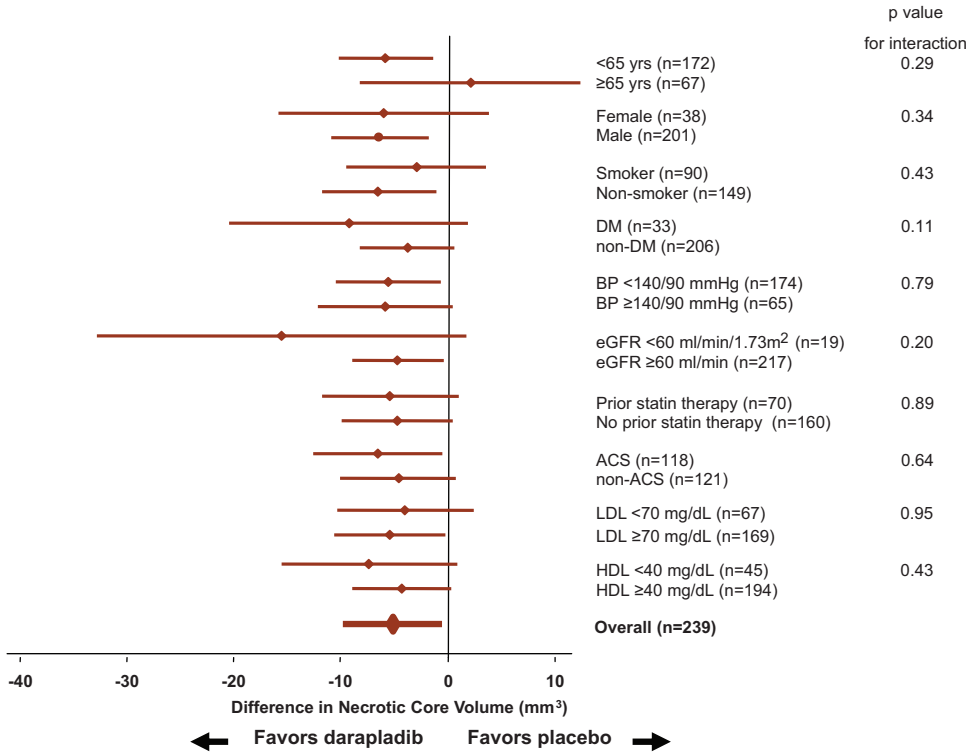

Figure 3. Treatment differences in necrotic core volume across clinical subgroups. The results are displayed as adjusted mean treatment difference and 95% CI. LDL-C and high-density lipoprotein (HDL) cholesterol values refer to on-treatment levels. DM indicates diabetes mellitus; eGFR, estimated glomerular filtration rate.

124.1±23.8 mm Hg; darapladib, 121.8±23.0 mm Hg; $P=0.19$).

There was no evidence of significant differences in platelet biomarkers (P-selectin, sCD40L, and urinary 11-dehydro-thromboxane B_2) measured at multiple time points (3, 6, and 12 months) except for higher levels of CD40L at 12 months (placebo, 181 pg/mL [95% CI, 150 to 217]; darapladib, 258 pg/mL [95% CI, 208 to 320]; $P=0.024$). No imbalance in clinical events related to increased platelet activity was observed (Table 3).

Discussion

The main finding of this study was that darapladib, a direct Lp-PLA_2 inhibitor, prevented necrotic core expansion, whereas patients receiving placebo showed significant progression despite standard-of-care treatment, with average LDL-C levels <90 mg/dL. The results suggest that human coronary atherosclerosis is a dynamic process with potential for replacement of fibrous tissue by necrotic core. Prolonged Lp-PLA_2 inhibition halted this process, indicating a direct effect on human atheroma that is distinct from current pharmacological therapies.

Lp-PLA_2 and Necrotic Core Formation

The mere presence of Lp-PLA_2 in high-risk plaques does not necessarily establish its causal role in lesion propensity for rupture. However, Lp-PLA_2 inhibition, with the subsequent reduction of its product, lysophosphatidylcholine, has reduced inflammation and cell death in studies in vitro.[12,15,16] Furthermore, darapladib decreased intraplaque lysophosphatidylcholine content, attenuated the expression of inflammatory genes, and reduced necrotic core in a porcine model of coronary atherosclerosis.[19] In humans, treatment with darapladib not only inhibited intraplaque Lp-PLA_2 activity but also reduced activity of the intracellular proteases (caspase-3 and caspase-8) that are responsible for apoptotic cell death.[20] Therefore, the finding from the present study, that darapladib interferes with necrotic core expansion, is consistent with the overall biological hypothesis and supports a proatherogenic role for Lp-PLA_2.

Intravascular Imaging of Coronary Atherosclerosis

Until recently, no intravascular imaging modality has been capable of assessing the composition of coronary atheroma in a precise manner. IVUS RF analysis has been validated in postmortem pressure-perfused human coronary arteries and in the specimens retrieved by coronary atherectomy and carotid endarterectomy.[21–24] The present study provides the first longitudinal observation of the atherosclerotic process among patients receiving standard-of-care treatment. We demonstrate continued necrotic core expansion in patients receiving placebo, whereas darapladib halted this process, with a consistent effect across several subgroups. Because atherosclerosis is a highly heterogeneous process, we also confirmed statistically significant regression of necrotic core volume in the worst 10-mm subsegments (Table 2).

The IVUS palpography failed to detect a significant effect on biomechanical properties of coronary plaques during darapladib treatment. An unexpectedly high percentage of patients without high strain (37%) at baseline may have

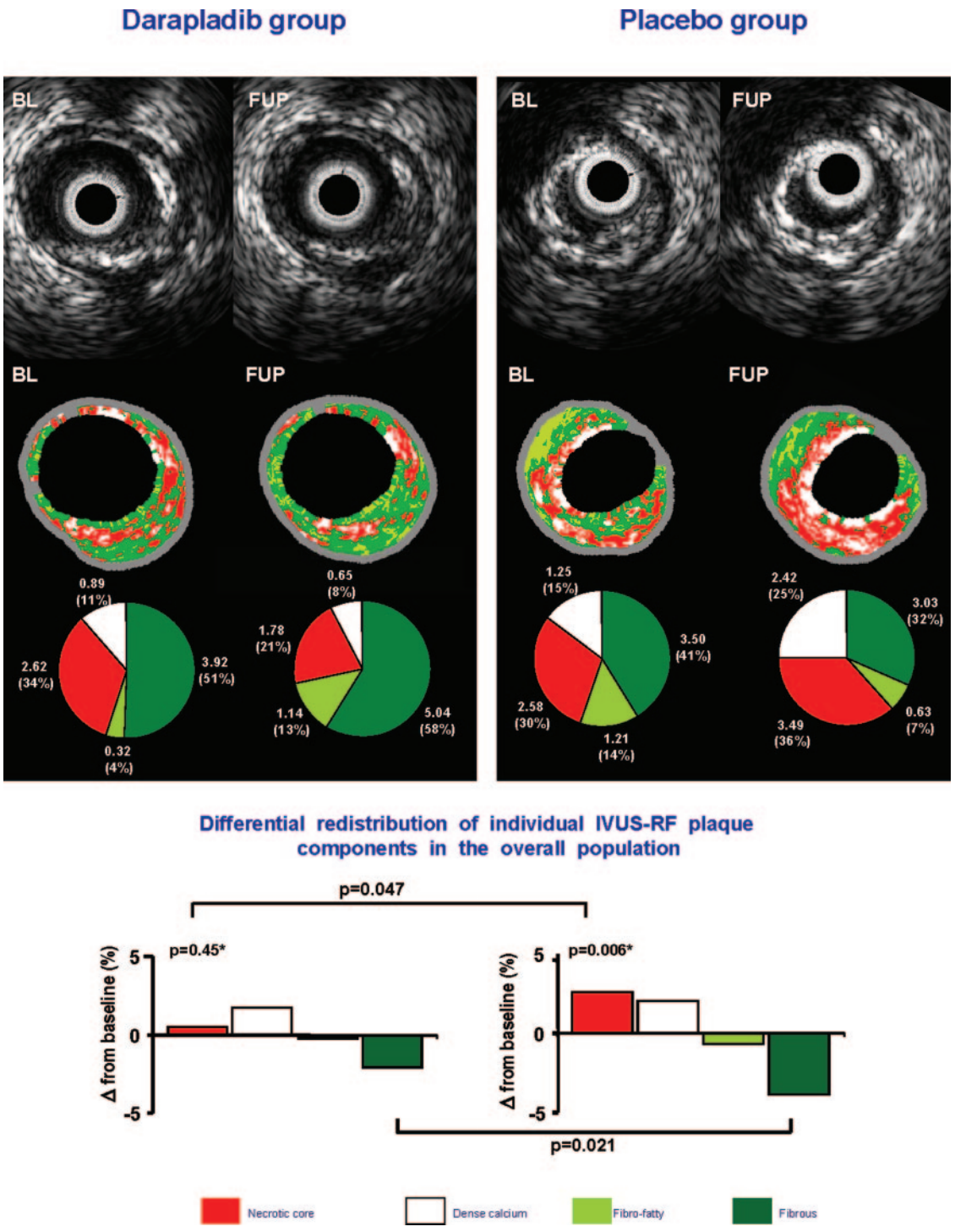

Figure 4. Top, Representative examples of plaque composition in the darapladib- and placebo-treated groups. The gray-scale frames and corresponding IVUS RF frames are shown. The pie charts provide quantitative display of individual plaque components in the cross sections. Values are in millimeters squared (and percent of RF-derived atheroma area). In the darapladib-treated patient, a decrease in necrotic core is seen. In the placebo-treated patient, an increase in necrotic core is demonstrated. Bottom, Differential changes in plaque components from IVUS RF analysis in the overall placebo and darapladib groups. Results are expressed as mean change from baseline (BL), with individual components expressed as percent of RF-derived atheroma volume. FUP indicates follow-up. *Within-group change from baseline.

reduced the statistical power needed to demonstrate significant differences between treatment groups.[7] Supporting this hypothesis is the prespecified sensitivity analysis demonstrating a significant reduction in high strain in the darapladib group ($P=0.009$) when only patients with highly deformable plaque at baseline were analyzed.

Despite the relatively short treatment duration, the changes in volumetric measurements using IVUS gray scale were

Table 3. Adverse Events, Clinical Outcomes, and Laboratory Abnormalities

Variable	Placebo (n=151)	Darapladib (n=172)
Adverse events,* n (%)		
Serious adverse event	46 (30)	49 (28)
Any adverse event	109 (72)	121 (70)
Any adverse event leading to withdrawal	11 (7)	7 (4)
MACE event, n (%)		
Composite of first MACE (CV death, MI, stroke, coronary revascularization)	29 (19)	29 (17)
All components of MACE,† n (%)		
Death	0	0
Myocardial infarction	7 (5)	4 (2)
Stroke	1 (<1)	1 (<1)
Coronary revascularization	29 (19)	28 (16)
Other CV events,† n (%)		
Coronary restenosis	20 (13)	18 (10)
In-stent thrombosis	2 (1)	1 (<1)
Hospitalization for myocardial ischemia	13 (9)	11 (6)
Blood pressure–related events, n (%)		
Investigator-reported hypertensive adverse events	4 (3)	3 (2)
Blood pressure ≥140/90 mm Hg	95 (63)	117 (68)
Systolic blood pressure ≥140 mm Hg	89 (59)	112 (65)
Laboratory abnormalities,‡ n (%)		
Alanine aminotransferase ≥3 times ULN	1 (<1)	2 (1)
Aspartate aminotransferase ≥3 times ULN	0	1 (<1)
Total bilirubin ≥1.5 times ULN	5 (3)	1 (<1)
Alkaline phosphatase ≥2 times ULN	0	0

MACE indicates major adverse cardiac events; CV, cardiovascular; MI, myocardial infarction; and ULN, upper limit of normal. Adverse events were collected during treatment phase and up to 28 days after discontinuation of the study medication.

*The safety population consisted of patients who received at least 1 dose of placebo or darapladib.

†Patients with >1 event are counted more than once.

‡All counts refer to number of patients.

comparable to those observed in other regression trials with similar on-treatment LDL-C values.[25] IVUS gray scale has been recognized as an established method to assess regression/progression of atherosclerosis, but it relies exclusively on plaque volume measurements.[5,6,25] The present study demonstrates that IVUS RF analysis detects changes in atheroma composition in the absence of detectable overall plaque volume changes. We cannot exclude the possibility that a longer treatment duration (eg, >12 months) may have allowed an even more pronounced effect on necrotic core and ultimately atheroma volume.

Darapladib and Biomarker Response

hsCRP, a broad marker of systemic inflammatory burden, showed only trends toward lower values in the darapladib group, similar to a recent report.[18] The noxious effects of Lp-PLA_2 depend on intraplaque concentrations of its substrate (ie, oxidized phospholipid in modified LDL-C) and in situ Lp-PLA_2 concentration, which may explain the predominantly local effects of darapladib without a more profound systemic impact.[26] A high adherence to statin therapy also contributed to lowering hsCRP (and other inflammatory biomarkers), thus minimizing the opportunity for further reductions with darapladib.

Clinical Safety

Although the study was underpowered to address the effects of darapladib on cardiovascular outcomes, there was no imbalance in reported clinical outcomes. The higher sCD40L level in the darapladib group at 12 months is probably not of clinical relevance because only much higher values predict ischemic events.[27] Of note, other platelet biomarkers (P-selectin and 11-dehydro-thromboxane B_2) did not differ between treatment groups. Importantly, there was no excess of clinical events associated with increased platelet reactivity.

Higher systolic blood pressure in the darapladib group was not consistent with the results of a prior clinical study and requires careful attention in future studies.[18] Additional comparison of the intraarterial blood pressure also revealed no differences between groups.

Study Limitations

This was an exploratory study that used novel imaging IVUS modalities to assess plaque deformability, composition, and size. The clinical relevance of plaque compositional changes observed with sustained, long-term Lp-PLA_2 inhibition requires confirmation in event-driven outcomes trials.

Our observations focus on a small segment of the coronary arterial tree and might not fully represent disease progression elsewhere. Finally, the approach undertaken in this and other IVUS trials has not assessed changes in the precise plaque phenotype that may portend clinical risk (eg, thin-cap fibroatheroma).

Conclusions

This study demonstrated that necrotic core expansion occurs despite contemporary cardiovascular therapies, even in the absence of overall plaque size increase. This unabated necrotic core expansion could be responsible, in part, for the recurrent cardiovascular events in high-risk patients. Lp-PLA_2 inhibition with darapladib halted this process and therefore may represent a new approach for the treatment of atherosclerosis if the benefit of this intervention is confirmed by the results of future event-driven outcomes trials.

Appendix

Integrated Biomarker and Imaging Study-2 Steering Committee: Patrick W. Serruys (chairman; Rotterdam, the Netherlands), Gerrit-Anne van Es (Rotterdam, the Netherlands), Christian Hamm (Bad Nauheim, Germany), William Wijns (Aalst, Belgium), Andrew Zalewski (nonvoting member; King of Prussia, Pa), and Andrew Zambanini (nonvoting member; Middlesex, UK).

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Participating centers (number of patients enrolled): Austria: Hanusch Krankenhaus, Georg Gaul (6). Belgium: Centre Hospitalier Universitaire Sart-Tilman, Victor Legrand (10); ZNA Campus Middelheim, Stefan Verheye (25); Cardiovascular Center, Aalst, William Wijns (14). Czech Republic: Všeobecná Fakultní Nemocnice, Michael Aschermann (23). Denmark: Skejby University Hospital, Hans Erik Bøtker (18). Germany: West German Heart Center, Raimund Erbel (7); Kerckhoff Klinik, Christian Hamm (7); Universitätsklinikum Heidelberg, Stefan Hardt, Helmut Kücherer (1); Universitätsklinikum München, Volker Klauss (14), Universitätsklinikum Ulm, Wolfgang Koenig (9); Segeberger Kliniken, Gert Richardt (3). The Netherlands: Medisch Spectrum Twente, Clemens von Birgelen (14); Medisch Centrum Leeuwarden, Adrianus Johannes van Boven (12); Catharina Hospital and Catherine R&D, Herman Rolf Michels (14), Erasmus Medical Center, Patrick Serruys (20); Medisch Centrum Rijnmond Zuid, Pieter Smits (11). Norway: Haukeland Sykehus, Oyvind Bleie (20). Poland: Upper Silesian Heart Center, Pawel Buszman (40); Szpital Uniwersytecki, Dariusz Dudek (19). Spain: Hospital Marques de Valdecilla, Thierry Colman (9); Hospital Clinico San Carlos, Carlos Macaya (9). Switzerland: Kantonsspital Luzern, Paul Erne (25).

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Disclosures

D. D'Amico, T. Hutchinson, Dr Zambanini, and Dr Zalewski are GlaxoSmithKline employees. Dr Vince is employed by and owns equity in Volcano Corp. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

Despite intensive management of conventional risk factors, coronary events continue to recur at an unacceptably high rate. Thus, novel therapeutic options for treating coronary heart disease are needed. Inhibition of the enzyme lipoprotein-associated phospholipase A_2 (Lp-PLA_2) with darapladib has emerged as a potential approach to addressing residual risk of cardiovascular events by directly targeting high-risk coronary atheroma. This multicenter study used intravascular ultrasound–derived techniques to characterize coronary atheroma in patients receiving a standard-of-care treatment. The results demonstrate that the necrotic core, a key determinant of plaque vulnerability, continued to increase among patients receiving placebo despite a high level of adherence to recommended therapies. This result is consistent with a proatherogenic role for Lp-PLA_2 that is postulated to contribute to plaque vulnerability. In contrast, treatment with the direct Lp-PLA_2 inhibitor darapladib added to standard of care prevented necrotic core expansion. Pharmacological intervention with darapladib exerted favorable effects on coronary atheroma consistent with plaque stabilization. These findings suggest that direct Lp-PLA_2 inhibition may represent a novel therapeutic approach to reducing residual risk in patients with coronary heart disease.