

Lipoprotein-Associated Phospholipase A₂ Activity Is a Marker of Risk But Not a Useful Target for Treatment in Patients With Stable Coronary Heart Disease

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Background—We evaluated lipoprotein-associated phospholipase A₂ (Lp-PLA₂) activity in patients with stable coronary heart disease before and during treatment with darapladib, a selective Lp-PLA₂ inhibitor, in relation to outcomes and the effects of darapladib in the STABILITY trial.

Methods and Results—Plasma Lp-PLA₂ activity was determined at baseline (n=14 500); at 1 month (n=13 709); serially (n=100) at 3, 6, and 18 months; and at the end of treatment. Adjusted Cox regression models evaluated associations between Lp-PLA₂ activity levels and outcomes. At baseline, the median Lp-PLA₂ level was 172.4 μmol/min per liter (interquartile range 143.1–204.2 μmol/min per liter). Comparing the highest and lowest Lp-PLA₂ quartile groups, the hazard ratios were 1.50 (95% CI 1.23–1.82) for the primary composite end point (cardiovascular death, myocardial infarction, or stroke), 1.95 (95% CI 1.29–2.93) for hospitalization for heart failure, 1.42 (1.07–1.89) for cardiovascular death, and 1.37 (1.03–1.81) for myocardial infarction after adjustment for baseline characteristics, standard laboratory variables, and other prognostic biomarkers. Treatment with darapladib led to a ≈65% persistent reduction in median Lp-PLA₂ activity. There were no associations between on-treatment Lp-PLA₂ activity or changes of Lp-PLA₂ activity and outcomes, and there were no significant interactions between baseline and on-treatment Lp-PLA₂ activity or changes in Lp-PLA₂ activity levels and the effects of darapladib on outcomes.

Conclusions—Although high Lp-PLA₂ activity was associated with increased risk of cardiovascular events, pharmacological lowering of Lp-PLA₂ activity by ≈65% did not significantly reduce cardiovascular events in patients with stable coronary heart disease, regardless of the baseline level or the magnitude of change of Lp-PLA₂ activity.

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Inflammatory activity is an integral component of the development of atherosclerosis and its complications.¹ Biomarkers indicating myocardial dysfunction (eg, troponin, natriuretic peptides), renal dysfunction (eg, cystatin C), and inflammatory activity (eg, C-reactive protein) are risk

indicators of future cardiovascular events in healthy persons and in patients with established coronary heart disease (CHD).^{2–5} Higher levels of lipoprotein-associated phospholipase A₂ (Lp-PLA₂) activity have also been associated with an increased risk of coronary events in healthy elderly persons

Table 1. Demographics, Baseline Characteristics, and Biomarker Levels in Relation to Quartile Groups of Baseline Lp-PLA₂ Activity

Variables	Baseline Lp-PLA ₂ Activity				P Value	P Value for Trend
	Q1 (<143.1) n=3617	Q2 (143.1–172.4) n=3631	Q3 (172.4–204.2) n=3615	Q4 (≥204.2) n=3637		
Lp-PLA ₂ , μmol/min/L	125 (108–135)	158 (151–166)	187 (180–195)	230 (216–253)	<0.0001	<0.0001
Assigned treatment						
Placebo	1817 (50.2)	1766 (48.6)	1779 (49.2)	1859 (51.1)	0.1544	0.4342
Darapladib	1800 (49.8)	1865 (51.4)	1836 (50.8)	1778 (48.9)		
Age, y	65 (60–71)*	65 (60–71)	65 (59–71)	64 (57–71)	<0.0001	<0.0001
Sex, male	2490 (68.8)	2916 (80.3)	3127 (86.5)	3291 (90.5)	<0.0001	<0.0001
Geographic region					<0.0001	<0.0001
Asia-Pacific	821 (22.7)	602 (16.6)	534 (14.8)	609 (16.7)		
Eastern Europe	712 (19.7)	826 (22.7)	922 (25.5)	958 (26.3)		
North America	873 (24.1)	910 (25.1)	961 (26.6)	1061 (29.2)		
South America	207 (5.7)	204 (5.6)	231 (6.4)	226 (6.2)		
Western Europe	1004 (27.8)	1089 (30.0)	967 (26.7)	783 (21.5)		
Weight, kg	79 (68–91)	83 (73–94)	84 (74–95)	85 (74–96)	<0.0001	<0.0001
Current smoker	466 (12.9)	555 (15.3)	677 (18.7)	926 (25.5)	<0.0001	<0.0001
Hypertension	2627 (72.6)	2608 (71.8)	2569 (71.1)	2560 (70.4)	0.1275	0.0311
Diabetes mellitus	1678 (46.4)	1459 (40.2)	1272 (35.2)	1185 (32.6)	<0.0001	<0.0001
Statin at randomization	3575 (98.8)	3577 (98.5)	3508 (97.0)	3439 (94.6)	<0.0001	<0.0001
High-intensity statin	268 (7.4)	270 (7.4)	242 (6.7)	227 (6.2)	0.1271	0.0201
Prior myocardial infarction	2079 (57.5)	2146 (59.1)	2167 (59.9)	2197 (60.4)	0.0583	0.0018
Prior PCI or CABG	2731 (75.5)	2735 (75.3)	2677 (74.1)	2661 (73.2)	0.0715	0.0035
Polyvascular disease	491 (13.6)	535 (14.7)	529 (14.6)	649 (17.8)	<0.0001	<0.0001
LDL cholesterol, mmol/L	1.7 (1.3–2.1)	1.9 (1.6–2.3)	2.2 (1.8–2.7)	2.6 (2.1–3.3)	<0.0001	<0.0001
HDL cholesterol, mmol/L	1.3 (1.1–1.5)	1.2 (1.0–1.4)	1.2 (1.0–1.4)	1.1 (0.9–1.3)	<0.0001	<0.0001
Triglycerides, mmol/L	1.3 (1.0–1.8)	1.5 (1.1–2.0)	1.6 (1.1–2.2)	1.7 (1.3–2.4)	<0.0001	<0.0001
Hemoglobin, g/L	138 (129–146)	142 (133–151)	145 (137–153)	148 (139–156)	<0.0001	<0.0001
eGFR, mL/min [†]	75 (62–87)	74 (61–86)	74 (62–86)	74 (61–87)	0.5690	0.9025
C-reactive protein, mg/L	2.8 (1.3–3.1)	2.8 (1.4–3.1)	2.8 (1.5–3.2)	3.0 (1.5–3.4)	<0.0001	<0.0001
Interleukin 6, ng/L	1.2 (0.6–2.9)	1.3 (0.6–2.9)	1.3 (0.7–3.1)	1.6 (0.8–3.6)	<0.0001	<0.0001
High-sensitivity troponin T, ng/L	9.0 (5.9–13.6)	9.4 (6.4–14.1)	9.4 (6.3–14.2)	9.4 (6.2–15.0)	<0.0001	<0.0001
NT-proBNP, ng/L	174 (85–372)	181 (87–386)	186 (81–361)	174 (78–402)	0.0762	0.5699
Cystatin C, mg/L	1.0 (0.9–1.2)	1.0 (0.9–1.2)	1.0 (0.9–1.2)	1.0 (0.9–1.2)	<0.0001	<0.0001
GDF-15, ng/L	1280 (923–1824)	1250 (902–1798)	1216 (905–1746)	1266 (927–1821)	0.0003	0.0913

Categorical variables are shown as number (percentage); continuous variables are presented as median (interquartile range). CABG indicates coronary artery bypass grafting; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor 15; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Lp-PLA₂, lipoprotein-associated phospholipase A₂; NT-proBNP, N-terminal proB-type natriuretic peptide; PCI, percutaneous coronary intervention; Q, quartile.

[†]eGFR was estimated using the Chronic Kidney Disease Epidemiology Collaboration formula.

Table 2. Multivariable Analysis of Demographics, Baseline Characteristics and Biomarkers Significantly Associated With Baseline Lp-PLA₂ Activity

Background Characteristic	Adjusted Difference in Mean*	95% CI*	P Value
Western Europe vs North America	-19.81	(-21.58; -18.03)	<0.0001
Eastern Europe vs North America	-17.09	(-18.97; -15.21)	<0.0001
Asia-Pacific vs North America	-16.42	(-18.48; -14.36)	<0.0001
South America vs North America	-14.91	(-17.81; -12.02)	<0.0001
Female vs male	-15.69	(-17.60; -13.77)	<0.0001
Diabetes mellitus	-6.640	(-8.092; -5.188)	<0.0001
HDL cholesterol, 0.1 mmol/L increase	-4.333	(-4.554; -4.113)	<0.0001
Diagnosis of hypertension	-2.066	(-3.503; -0.6295)	0.0048
Body mass index, 1 kg/m ² increase	-0.218	(-0.361; -0.074)	0.0030
Triglycerides, 0.1 mmol/L increase	-0.071	(-0.139; -0.003)	0.0389
GDF-15, 10% increase	0.401	(0.252; 0.551)	<0.0001
Hemoglobin, 10 g/L increase	0.589	(0.536; 0.641)	<0.0001
Age, 10-year increase	0.908	(0.005; 1.812)	0.0489
Cystatin C, 10% increase	1.159	(0.771; 1.547)	<0.0001
Polyvascular disease	1.919	(0.169; 3.671)	0.0317
Current smoker vs never smoked	2.094	(0.049; 4.139)	0.0447
LDL cholesterol, 0.1 mmol/L increase	3.118	(3.040; 3.196)	<0.0001

GDF-15 indicates growth differentiation factor 15; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Lp-PLA₂, lipoprotein-associated phospholipase A₂.

*Mean and 95% CI for adjusted difference in mean Lp-PLA₂ activity level estimated in relation to the respective change in each background characteristic.

and in patients with stable CHD.⁶⁻⁹ There is some evidence that Lp-PLA₂ might be part of the atherosclerotic process and may contribute to plaque destabilization through inflammatory activity in the atherosclerotic lesions.¹⁰⁻¹² Consequently, it is important to further evaluate the independent prognostic value of Lp-PLA₂ activity as a risk marker for individual nonfatal and fatal cardiovascular events in large long-term

prospective studies of patients with CHD in addition to considering information provided by other prognostic biomarkers.

Darapladib is a selective Lp-PLA₂ inhibitor¹³ that reduces Lp-PLA₂ activity in plasma¹⁴ and in the atherosclerotic plaque.^{15,16} Experiments in hypercholesterolemic diabetic swine¹⁷ and in an angiographic and intravascular ultrasound study in patients¹⁸ showed that darapladib prevented progression of the necrotic core, assumed to be the vulnerable component of atherosclerotic lesions. In the present STabilization of Atherosclerotic plaque By Initiation of darapLadib TherapY (STABILITY) trial (ClinicalTrials.gov identifier NCT00799903), darapladib 160 mg daily did not significantly reduce the primary composite end point of cardiovascular death, myocardial infarction (MI), or stroke in patients with stable CHD (hazard ratio [HR] 0.94, 95% CI 0.85-1.03, *P*=0.20) but nominally reduced the rate of the secondary end point, major coronary events (coronary death, MI, or urgent coronary revascularization; HR 0.90, 95% CI 0.82-1.00, *P*=0.045).¹⁹ The present predefined ancillary study evaluated the independent prognostic value of baseline Lp-PLA₂ activity concerning cardiovascular events, the reduction of Lp-PLA₂ activity by darapladib, and the associations between baseline and changes in Lp-PLA₂ activity and clinical outcomes.

Methods

Trial Design, Patients, Treatments, and Follow-up

The STABILITY trial was a prospective double-blind randomized trial evaluating the efficacy and safety of darapladib 160 mg, a specific inhibitor of Lp-PLA₂, compared with placebo concerning cardiovascular outcomes in patients with stable CHD.¹⁹ In brief, the study randomized 15 828 patients from 39 countries with stable CHD, defined as prior MI, prior coronary revascularization, or multivessel CHD confirmed by coronary angiography. Patients also had to meet at least 1 of the following cardiovascular risk criteria: age ≥60 years; diabetes mellitus requiring pharmacotherapy; high-density lipoprotein cholesterol <1.03 mmol/L; current or previous smoker, defined as ≥5 cigarettes per day on average; significant renal dysfunction (estimated glomerular filtration rate ≥30 and <60 mL/min per 1.73 m² or urine albumin:creatinine ratio of ≥30 mg albumin per gram of creatinine); or polyvascular disease (CHD and cerebrovascular disease or CHD and peripheral arterial disease). The patients were followed with regular outpatient visits for a median of 3.7 years. The primary outcome was the composite of cardiovascular death, nonfatal MI, or nonfatal stroke. Secondary outcomes were the composites of major coronary events (ie, fatal and nonfatal MI, death from CHD, or urgent coronary revascularization for myocardial ischemia) and the

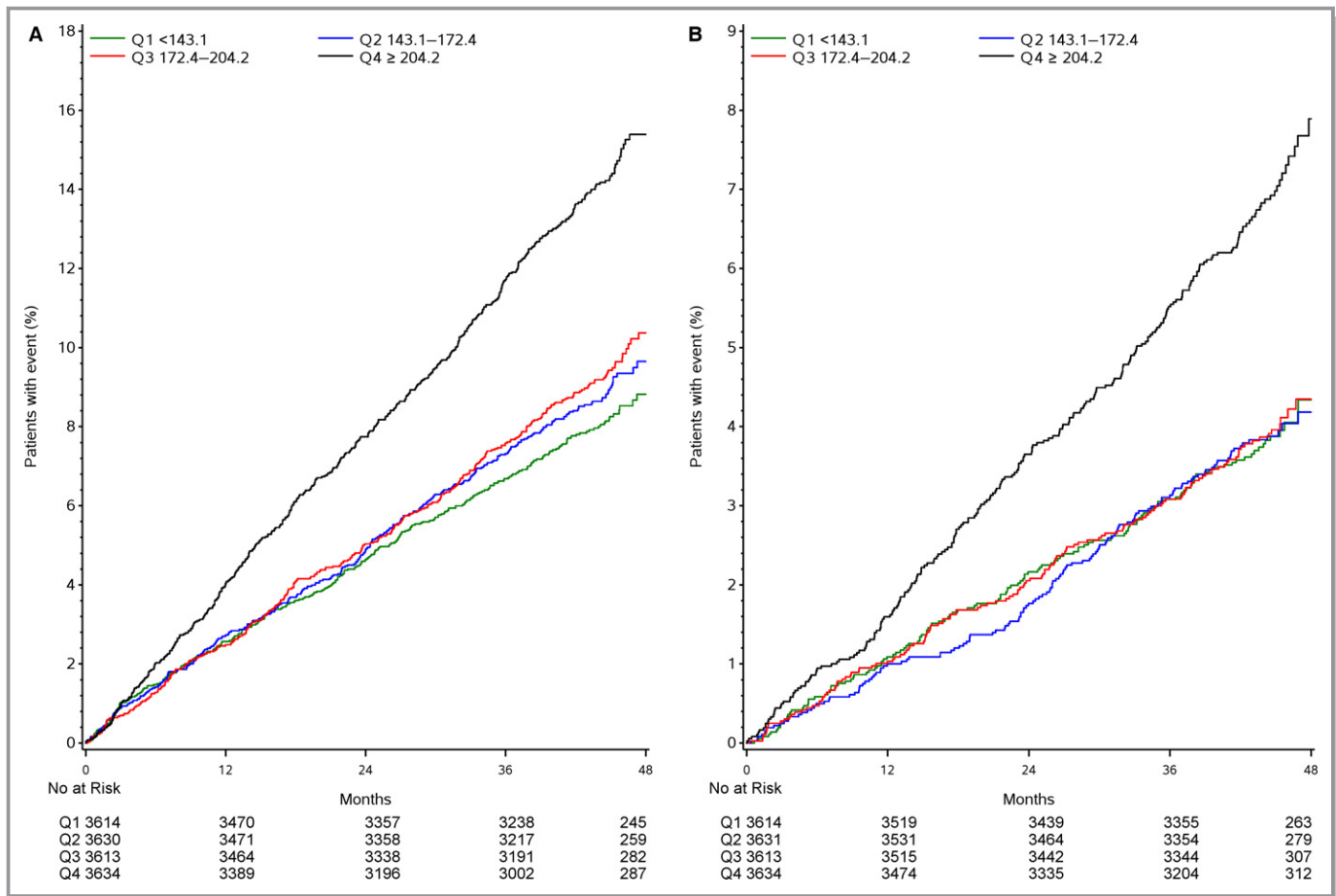


Figure 1. Quartile groups of baseline Lp-PLA₂ activity (in $\mu\text{mol}/\text{min}$ per liter) in relation to outcomes by Kaplan–Meier analysis. A, Major adverse cardiac events (cardiovascular death, myocardial infarction, or stroke). B, Cardiovascular death. Q indicates quartile; Lp-PLA₂; lipoprotein-associated phospholipase A₂.

composite of total coronary events, which also included all coronary revascularizations. Additional secondary end points were the individual components of the primary composite end point, hospitalization for heart failure, and all-cause mortality. Components of cardiovascular death included death from unknown cause, fatal MI, fatal stroke, complications of a cardiac procedure, arrhythmia, congestive heart failure or shock, other vascular cause of death, and sudden death. All suspected end points were documented and reported by STABILITY study investigators and were adjudicated by an independent clinical events committee. All participants provided informed consent, and the study was approved by the relevant institutional review committee in each participating country.

Samples and Biochemical Methods

Blood samples were obtained from the majority of patients at baseline and 24 ± 2 hours after the last intake of study drug at months 1, 3, 6, and 18, and at the end of treatment. Plasma

aliquots were stored in central repositories at -70°C until biochemical analysis was performed. Measurements of Lp-PLA₂ activity and other biomarkers were performed in 14 500 patients at baseline for whom samples were available and suitable for assay. For 13 709 of these patients, Lp-PLA₂ activity was also measured after 1 month. In addition, the change in Lp-PLA₂ activity over a longer time period was evaluated in a random sample of 100 patients with plasma samples available from all follow-up visits. The sample size of 100 patients was based on the assumption of a mean difference in percentage change from baseline between treatment groups in Lp-PLA₂ activity of between 25% and 65%, with a common standard deviation of 25%, a 2-sided type I error rate (α level) of 5% and power of 90%. In total, 100 participants were selected with the goal of having ≈ 50 participants from each treatment group to describe the long-term inhibition of Lp-PLA₂ activity by the randomized treatment.

The Lp-PLA₂ activity was measured in an automated enzyme assay system (PLAC Test for Lp-PLA₂ Activity;

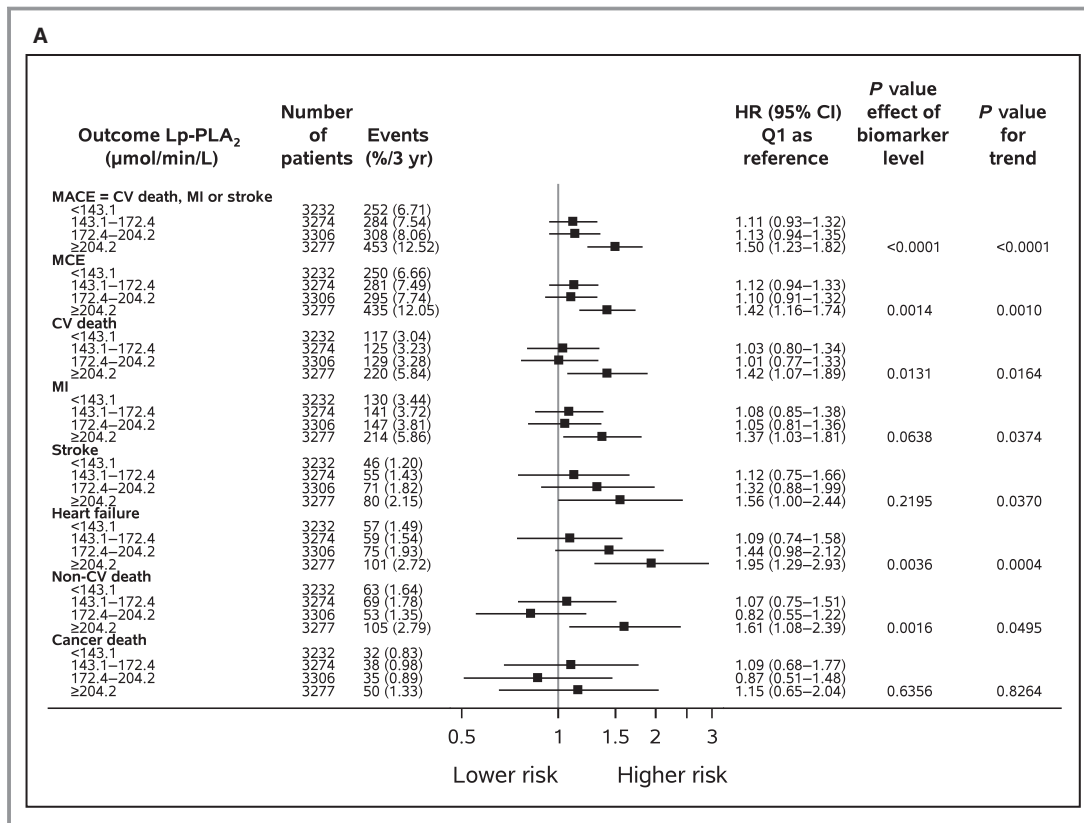


Figure 2. Baseline Lp-PLA₂ activity (μmol/min per liter) quartile groups in relation to outcomes in (A) all patients, (B) in women, and (C) in men, adjusted for demographics, baseline characteristics, routine biochemical variables, and prognostic biomarkers. The x-axis presents a logarithmic scale. MACE indicates CV death, MI, or stroke; MCE indicates coronary heart disease death, MI, or urgent coronary revascularization. Event rates are Kaplan–Meier rates. Adjustment variables: randomized treatment, geographic region, age, sex, body mass index, current smoking, hypertension, diabetes mellitus, prior MI, prior coronary revascularization, multivessel coronary heart disease, polyvascular disease, significant renal dysfunction, routine biochemical variables (hemoglobin, white blood cell count, estimated glomerular filtration rate [Chronic Kidney Disease Epidemiology Collaboration], low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides), and prognostic biomarkers (N-terminal proB-type natriuretic peptide, high-sensitivity cardiac troponin T, cystatin C, high-sensitivity C-reactive protein, and interleukin 6). CV indicates cardiovascular; HR, hazard ratio; Lp-PLA₂, lipoprotein-associated phospholipase A₂; MACE, major adverse cardiac events; MCE, major coronary events; MI, myocardial infarction; Q, quartile.

Diadexus) with a colorimetric substrate that is converted on hydrolysis by the phospholipase enzyme. The analytical sensitivity (limit of quantitation) of the assay is 10 μmol/min per liter. Within-run and within-laboratory variability were determined by testing 4 human plasma samples and 2 controls with Lp-PLA₂ activities ranging from 113 to 315 μmol/min per liter. Samples were assayed in duplicate twice a day over 20 days and with 3 kit lots using 1 instrument. Total precision coefficients of variation for each reagent lot and sample were <3%. Several dilution series were prepared from plasma samples with known high and low Lp-PLA₂ activity levels and were tested with 3 kit lots. Linearity with a deviation of ≤10% was demonstrated from 10 to 382 μmol/min per liter, which is considered the measuring range of the assay. The Lp-PLA₂ activity measurements were

performed by the manufacturer (Diadexus) on samples blinded for study treatment. The levels of high-sensitivity cardiac troponin T, N-terminal proB-type natriuretic peptide, growth differentiation factor 15 (precommercial assay),²⁰ and cystatin C were determined by electrochemiluminescence immunoassays using a Cobas Analytics e601 system (Roche Diagnostics), performed at the Uppsala Clinical Research Center Laboratory at Uppsala University in Sweden. High-sensitivity C-reactive protein was analyzed using the CardioPhase high-sensitivity C-reactive protein (Dade Behring) 2-site particle-enhanced immunonephelometry sandwich assay. The routine biochemical analyses and high-sensitivity C-reactive protein assay were performed at a central laboratory with standardized methods (Quest Diagnostics Clinical Laboratories, Inc).

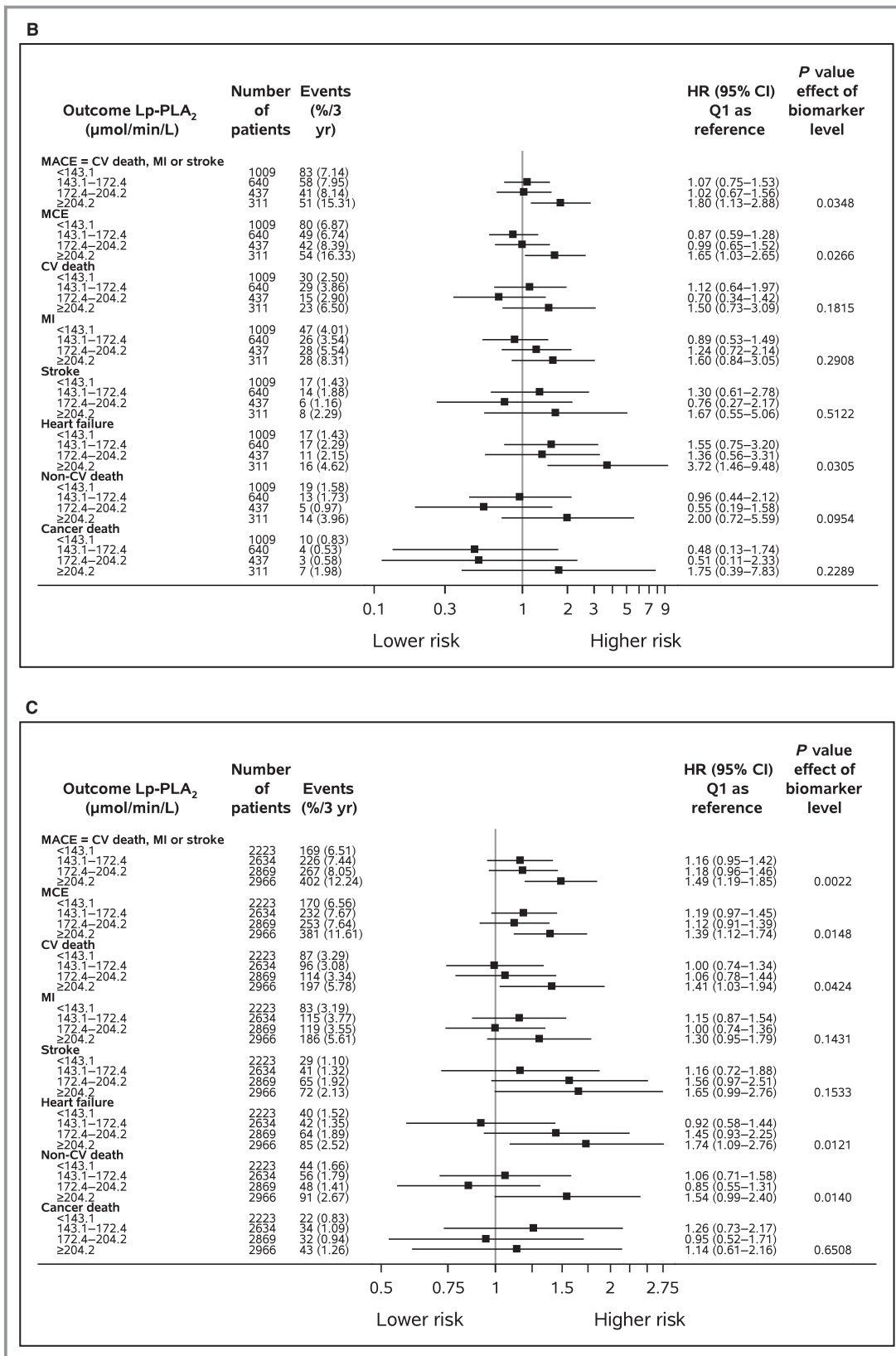


Figure 2. Continued

Table 3. C-Indices and Reclassification Statistics When Adding Information on the Lp-PLA₂ Activity Level to the Full Model of Baseline Characteristics and Other Biomarkers

Outcome	C-Index (95% CI) –Lp-PLA ₂	C-Index (95% CI) +Lp-PLA ₂	Event NRI	Nonevent NRI	NRI
MACE	0.675 (0.659–0.691)	0.678 (0.662–0.694)	–0.074	0.248	0.175
MCE	0.658 (0.641–0.674)	0.659 (0.643–0.676)	–0.074	0.238	0.164
Cardiovascular death	0.756 (0.733–0.780)	0.757 (0.734–0.781)	–0.046	0.237	0.191
MI	0.665 (0.642–0.687)	0.667 (0.645–0.689)	–0.099	0.239	0.140
Stroke	0.680 (0.646–0.714)	0.686 (0.652–0.720)	0.084	0.050	0.134
Heart failure	0.832 (0.804–0.860)	0.833 (0.805–0.861)	0.167	0.081	0.249
Non-cardiovascular death	0.740 (0.708–0.772)	0.743 (0.711–0.775)	0.051	0.125	0.176
Cancer death	0.757 (0.716–0.798)	0.758 (0.717–0.799)	0.194	–0.029	0.165
Total death	0.732 (0.714–0.751)	0.735 (0.716–0.753)	–0.005	0.207	0.202

The full model contained the following data: randomized treatment, geographic region, age, sex, body mass index, current smoking, hypertension, diabetes mellitus, prior myocardial infarction, prior coronary revascularization, multivessel coronary heart disease, polyvascular disease, significant renal dysfunction, routine biochemical variables (hemoglobin, white blood cell count, estimated glomerular filtration rate [Chronic Kidney Disease Epidemiology Collaboration], low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides), and prognostic biomarkers (N-terminal proB-type natriuretic peptide, high-sensitivity cardiac troponin T, cystatin C, high-sensitivity C-reactive protein, and interleukin 6). Lp-PLA₂ indicates lipoprotein-associated phospholipase A₂; MACE, major adverse cardiac events; MCE, major coronary events; MI, myocardial infarction; NRI, net reclassification index.

Statistics

Lp-PLA₂ activity at baseline

Demographics and other baseline characteristics were summarized by quartile group of the baseline Lp-PLA₂ activity level. The univariable relationships between Lp-PLA₂ activity and background variables were evaluated using the chi-square and Kruskal–Wallis tests for categorical and continuous variables, respectively. Multivariable analyses were used to evaluate the independent associations between baseline Lp-PLA₂ activity and other variables, using linear regression models in which continuous variables were included as linear or log-transformed variables, as appropriate. Baseline Lp-PLA₂ activity as a predictor of outcomes was evaluated using Kaplan–Meier estimates of the cumulative risk to the first occurrence of an event for the Lp-PLA₂ quartile groups. The independent associations between baseline Lp-PLA₂ activity levels and outcomes were evaluated using adjusted Cox regression models in which the HR and 95% CI were calculated using the lowest quartile group of Lp-PLA₂ activity (quartile 1) as the reference and performing a trend analysis across quartile groups. The predefined adjustment variables for the statistical analyses were randomized treatment, geographic region, age, sex, body mass index, current smoking, hypertension, diabetes mellitus, prior MI, prior coronary revascularization, multivessel CHD, polyvascular disease, significant renal dysfunction, levels of standard biomarkers (hemoglobin, white blood cell count, estimated glomerular filtration rate [Chronic Kidney Disease Epidemiology Collaboration], low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides),

and levels of prognostic biomarkers (high-sensitivity cardiac troponin T, N-terminal proB-type natriuretic peptide, growth differentiation factor 15, high-sensitivity C-reactive protein, interleukin 6), which were entered as quartiles after log transformation. A Cox proportional hazards model with treatment group (darapladib or placebo), baseline Lp-PLA₂ activity categorized by quartile group, and treatment group Lp-PLA₂ activity interaction as independent variables was used to test whether the treatment effect differed in relation to Lp-PLA₂ activity level. The relevance of the Lp-PLA₂ activity interacting with the effect of treatment was evaluated based on the significance of interaction statistics. Data were handled by complete-case analyses with no imputation for missing values. No correction for multiplicity was performed. The analyses of associations between the Lp-PLA₂ activity and baseline characteristics, other biomarkers and outcomes, and changes of Lp-PLA₂ activity over time were prespecified in the statistical analysis plans.

Lp-PLA₂ activity over time

The HRs and *P* values for the association of changes in Lp-PLA₂ activity after 1 month and clinical outcomes were estimated using a Cox proportional hazards regression model with baseline Lp-PLA₂ activity and change from baseline to month 1 in Lp-PLA₂ activity as the covariate, stratified by treatment. In the 100-participant cohort, Lp-PLA₂ activity over time was characterized using the generalized estimating equations model with participants fitted as a repeated effect, and it included the following terms: treatment, visit, baseline Lp-PLA₂ activity, treatment by visit, and baseline by visit

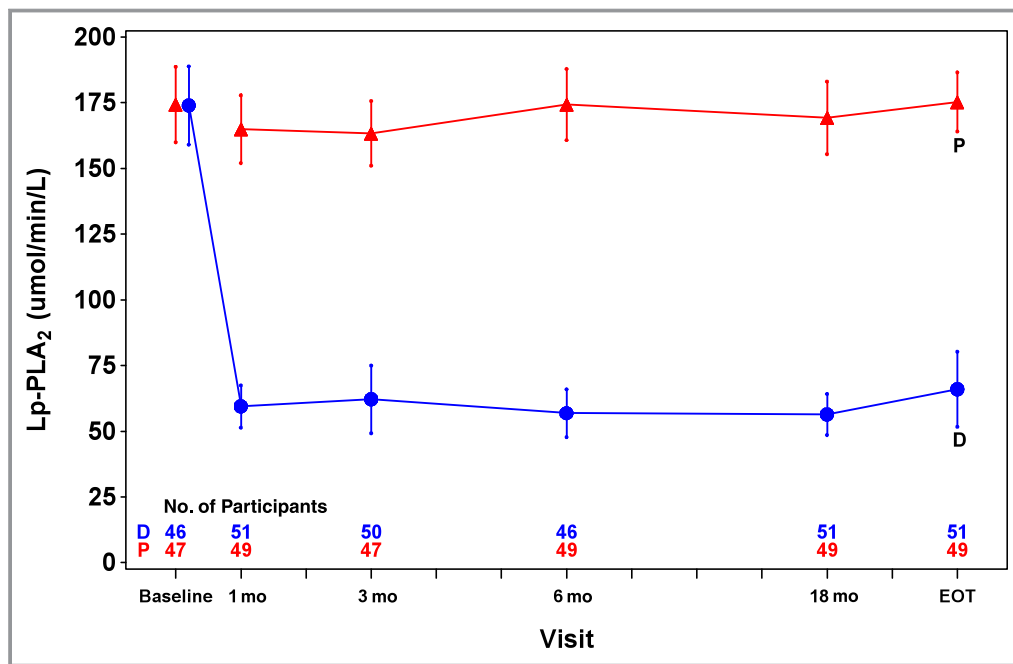


Figure 3. Lp-PLA₂ activity (µmol/min per liter) over time during treatment with darapladib and placebo in a subset of 100 patients with available plasma samples for Lp-PLA₂ activity measurements until end of follow-up. Symbols illustrate means and CIs for the respective darapladib (blue) and placebo (red) groups at baseline; at follow-up visits at 1, 3, 6, and 18 months; and at the EOT visit. D, darapladib; EOT, end of treatment; Lp-PLA₂, lipoprotein-associated phospholipase A₂; P, placebo.

interactions. Point estimates, 95% CIs, and *P* values for the mean differences between patients treated with darapladib and placebo at months 1, 3, 6, and 18, and at the end of treatment were calculated using this generalized estimating equations model.

Results

For the 14 500 patients with Lp-PLA₂ activity measurements, baseline characteristics were representative of the overall STABILITY population and balanced between the randomized treatment groups (Table 1). There were no differences in rates of outcome events between patients with and without Lp-PLA₂ activity measurements. The distribution of the Lp-PLA₂ activity levels did not deviate from normality (median 172 µmol/min per liter [interquartile range 143–204 µmol/min per liter]). There were statistically significant differences for the majority of the baseline characteristics and levels of other biomarkers when stratifying patients by baseline Lp-PLA₂ quartile groups (Table 1). The following factors showed independent and relevant associations with higher Lp-PLA₂ activity in multivariable analyses: male sex, North American origin, current smoking, higher low-density lipoprotein cholesterol level, and lower high-density lipoprotein cholesterol level. Diabetes mellitus was associated with lower Lp-PLA₂ activity (Table 2).

In the present cohort, 661 (4.6%) cardiovascular deaths, 695 (4.8%) MIs, and 280 (1.9%) strokes occurred. Of the 661 cardiovascular deaths, 142 deaths of unknown cause were not verified as noncardiovascular and thus were included among the cardiovascular deaths. In total, there were 1444 (10.0%) first primary outcome events and 1404 (9.7%) major coronary events. The outcome events were accrued at a stable rate during follow-up (Figure 1). There was a significant difference in event rates between the highest quartile group of baseline Lp-PLA₂ activity in comparison to the lower 3 quartile groups, among which no significant differences in event rates were observed (Figures 1 and 2). The higher event rates in the highest Lp-PLA₂ quartile group were observed for all types of events, namely, the primary composite of cardiovascular death, MI, or stroke; the secondary composite of major coronary events; and the individual events cardiovascular death, MI, stroke, hospitalization for heart failure, and cardiovascular and total mortality. The elevated event rates of all of these events in the highest Lp-PLA₂ quartile group were maintained after adjusting for baseline characteristics and conventional laboratory tests. In addition, after adjustment for the prognostic biomarkers N-terminal proB-type natriuretic peptide, high-sensitivity cardiac troponin T, growth differentiation factor 15, cystatin C, high-sensitivity C-reactive protein, and interleukin 6, the increased risks of the composite events, hospitalization for heart failure, cardiovascular death, and total death remained,

Table 4. Change in Lipoprotein-Associated Phospholipase A₂ Activity After 1 Month by Subgroup in Patients Assigned to Darapladib

Subgroup	Darapladib		
	n	Mean Change	Mean Percentage Inhibition
Prior MI			
Yes	4063	-111.8	63.0
No	2773	-112.9	64.3
Prior coronary revascularization			
Yes	5306	-112.7	64.0
No	1815	-111.1	62.0
Multivessel CHD			
Yes	974	-116.7	65.0
No	5862	-111.5	63.3
Time from CHD event to randomization			
Recent	1628	-110.0	63.1
Remote	5229	-113.0	63.7
Age ≥60 yr			
Yes	5055	-112.5	64.5
No	1781	-111.7	60.7
Diabetes mellitus requiring pharmaceutical management			
Yes	2289	-110.4	65.5
No	4547	-113.2	62.5
HDL-C <40 mg/dL			
Yes	2285	-118.9	63.1
No	4549	-109.0	63.7
Current/previous smoker			
Yes	1344	-113.2	60.1
No	5465	-112.0	64.4
Significant renal dysfunction			
Yes	2068	-117.5	66.3
No	4768	-110.0	62.3
Polyvascular disease			
Yes	1045	-115.3	63.9
No	5791	-111.7	63.4
No. of additional predictors of cardiovascular risk			
1	2376	-108.4	62.0
2	2371	-112.2	63.3
≥3	2066	-117.0	65.5
Race group collapsed			
White	5533	-112.2	62.6
Nonwhite	1303	-112.5	67.2
Region			
North America	1756	-115.6	64.0
Eastern Europe	1632	-109.6	60.4
Western Europe	1833	-110.0	63.7

Continued

Table 4. Continued

Subgroup	Darapladib		
	n	Mean Change	Mean Percentage Inhibition
South America	403	-114.0	62.2
Asia-Pacific	1212	-114.0	67.2
Group in United States			
US	1331	-116.0	63.8
Non-US	5505	-111.4	63.4
Sex			
Male	5623	-114.8	63.1
Female	1213	-100.8	65.4
Blood pressure			
High	3409	-113.8	64.3
Target	3425	-110.8	62.8
eGFR, mL/min/1.73 m²			
<60	937	-121.0	67.3
≥60	5888	-111.0	62.9
LDL-C, mmol/L			
<1.80	2401	-97.1	63.3
≥1.80 to <2.50	2723	-111.8	63.6
≥2.58	1805	-132.5	63.6
Baseline Lp-PLA₂ activity			
Tertile 1	2273	-78.3	61.4
Tertile 2	2342	-110.4	64.0
Tertile 3	2221	-149.0	65.2
High-sensitivity C-reactive protein, mg/L			
<1.0	2516	-108.9	63.9
1.0 to 3.0	2359	-114.4	63.9
>3.0	1667	-115.5	62.9
Family history of CHD			
Yes	1750	-113.4	63.0
No	5069	-111.8	63.7
Body mass index, kg/m²			
<25	1380	-113.5	66.3
25 to <30	2910	-113.6	63.6
≥30	2536	-110.1	61.9
Waist/hip ratio			
Level 1	851	-110.8	65.3
Level 2	1681	-113.9	64.9
Level 3	4234	-112.1	62.6
Aspirin use			
Yes	6293	-111.7	63.4
No	568	-118.9	64.5
Statin use			
Yes	6657	-111.7	63.5
No	179	-133.9	64.4

Continued

Table 4. Continued

Subgroup	Darapladib		
	n	Mean Change	Mean Percentage Inhibition
Beta blocker use			
Yes	5393	−111.6	63.2
No	1443	−114.7	64.8
P2Y12 use			
Yes	2267	−110.4	63.9
No	4569	−113.2	63.3
ACEI/ARB use			
Yes	5291	−112.7	63.9
No	1545	−110.9	62.3

ACEI indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CHD, coronary heart disease; HDL-C, high-density lipoprotein cholesterol; MI, myocardial infarction.

whereas associations with the individual ischemic events MI or stroke were somewhat attenuated. After complete adjustment, the HRs were 1.50 (95% CI 1.23–1.82) for the primary composite end point, 1.42 (95% CI 1.16–1.74) for major coronary events, 1.95 (95% CI 1.29–2.93) for hospitalization for heart failure, 1.42 (95% CI 1.07–1.89) for cardiovascular death, 1.47 (95% CI 1.17–1.84) for total death, 1.37 (95% CI 1.03–1.81) for MI, and 1.56 (95% CI 1.00–2.44) for stroke when comparing the highest and lowest Lp-PLA₂ quartile groups (Figure 2A). These findings were similar in men and women, without any interaction with sex (Figure 2B and 2C). The addition of Lp-PLA₂ activity levels to the model including all clinical characteristics, risk factors, and other biomarkers provided no significant change of the c-index and only a modest improvement of the net reclassification index for most events based on better discrimination of patients without increased risk (Table 3).

At 1 month, the Lp-PLA₂ activity was reduced from a median of 172 to 57 μmol/min per liter (interquartile range 42–75 μmol/min per liter, mean reduction 112 μmol/min per liter, mean percentage reduction 64%) in the darapladib group compared with a median decrease from 173 to 164 μmol/min per liter (interquartile range 136–196 μmol/min per liter, mean reduction 9 μmol/min per liter, mean percentage reduction 4%) in the placebo group. Darapladib treatment produced a persistent ≈65% relative reduction in Lp-PLA₂ activity from the first on-treatment measurement at 1 month until study termination, whereas the placebo group had no significant change in Lp-PLA₂ activity (Figure 3). The changes in Lp-PLA₂ activity were consistent in all subgroups based on clinical characteristics or biomarkers (Table 4).

In the highest Lp-PLA₂ quartile group, there was no significant reduction in the primary composite end point of

cardiovascular death, MI, or stroke (HR 0.86, 95% CI 0.72–1.03), whereas the secondary composite end point of major coronary events showed a statistically significant reduction (HR 0.82, 95% CI 0.68–0.98); however, there were no significant interactions between quartile groups of baseline Lp-PLA₂ activity and the effects of darapladib (Figures 4 and 5). Finally, there were no significant associations between either the level of Lp-PLA₂ activity at 1 month or the reduction in Lp-PLA₂ activity and any of the outcome events in the trial (Tables 5 through 8).

Discussion

This prespecified analysis of the STABILITY trial in patients with stable CHD followed for 3.7 years on a background of optimal medical therapy verified that Lp-PLA₂ activity is a marker of overall cardiovascular risk and total mortality in patients with stable CHD, in accordance with previous studies.^{6–9} Dyslipidemia, male sex, current smoking, and living in the North American region were associated with higher Lp-PLA₂ activity, in agreement with other observations.²¹ After adjusting for both baseline characteristics and other prognostic biomarkers, Lp-PLA₂ activity remained a significant marker of composite cardiovascular events, hospitalization for heart failure, and cardiovascular and total mortality, whereas the associations with new ischemic events such as MI and stroke were attenuated. The finding of an independent association between Lp-PLA₂ activity and composite cardiovascular events is in accordance with most^{6–9,22–27} although not all²⁸ previous studies of stable patients at high risk for or with established CHD. The rather weak independent association with a raised risk of cardiovascular events when adjusting for other biomarkers might be a corollary to the lack of association between Lp-PLA₂ activity and outcomes in patients with acute coronary syndromes having pronounced elevations of other prognostic biomarkers.²⁹

Darapladib treatment persistently reduced Lp-PLA₂ activity by ≈65% in all patient groups, consistent with other findings.^{16,18,29} Reduction in Lp-PLA₂ activity was not associated with any significant effect on the primary composite outcome of cardiovascular death, MI, or stroke in the STABILITY trial overall or with any subgroup based on the Lp-PLA₂ activity level at baseline. This lack of effect in the overall trial and in all Lp-PLA₂ activity subgroups is in agreement with the findings from the SOLID trial of patients with recent acute coronary syndromes.²⁹ In addition, in the present study, there were no significant associations between any outcome and the magnitude of reduction in Lp-PLA₂ activity level by darapladib treatment. These accumulated experiences show that although high Lp-PLA₂ activity was associated with an increased risk of cardiovascular events, pharmacological lowering of Lp-PLA₂ activity was not a useful

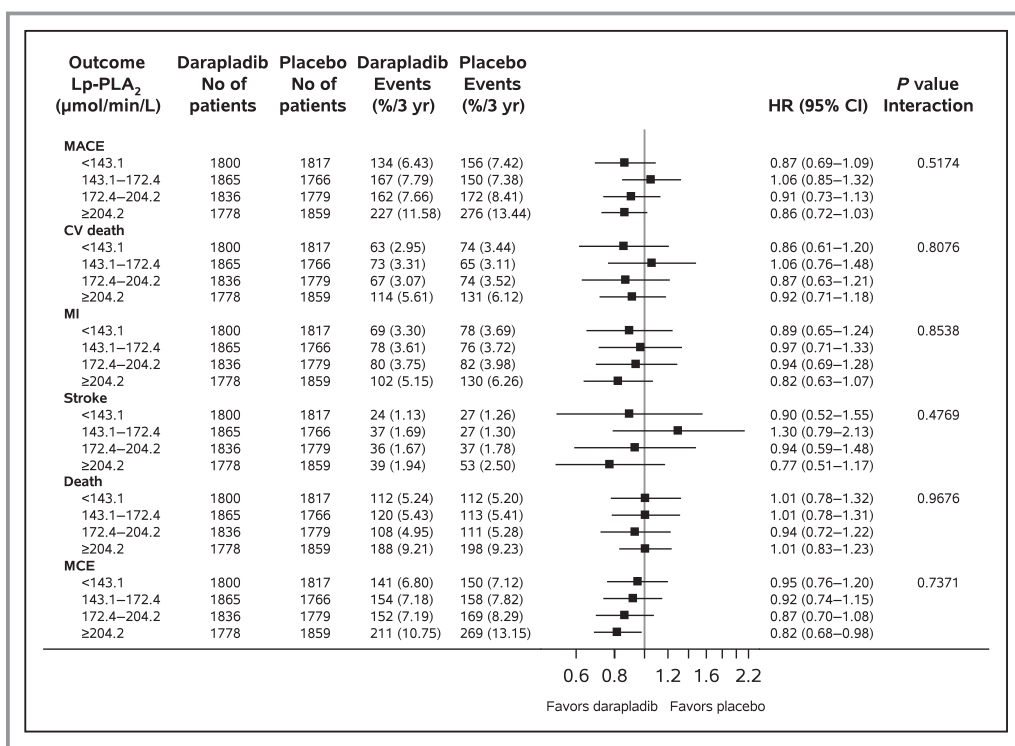


Figure 4. Effect of darapladib compared with placebo in relation to baseline Lp-PLA₂ activity (µmol/min per liter) quartile groups concerning all outcomes. MACE specifies CV death, MI, or stroke; MCE specifies coronary heart disease death, MI, or urgent coronary revascularization. Event rates are Kaplan–Meier rates. CV indicates cardiovascular; HR, hazard ratio; Lp-PLA₂, lipoprotein-associated phospholipase A₂; MACE, major adverse cardiac events; MCE, major coronary events; MI, myocardial infarction.

treatment target for prevention of cardiovascular events in patients with stable CHD.

Prior to the current study, several lines of evidence supported Lp-PLA₂ activity as a risk indicator of adverse outcomes both in patients with stable CHD and in the general population in addition to being potentially involved in the development of atherosclerosis and vulnerable plaque at the tissue level.^{6–9,22–27} Furthermore, in 2 case–control studies, natural deficiency of Lp-PLA₂ activity due to carriage of the V279F-null allele in the Lp-PLA₂ gene was associated with a lower risk of developing CHD.³⁰ In a recent study in European CHD patients, a single-nucleotide polymorphism of the Lp-PLA₂ gene was found to be associated with both increased Lp-PLA₂ activity in plasma and a raised risk of MI.³¹ Lp-PLA₂ has also been shown to be highly concentrated in unstable and ruptured atherosclerotic plaques and to be strongly expressed in macrophages in lesions prone to rupture.¹⁶ Direct inhibition of Lp-PLA₂ activity prevented progression to advanced coronary atherosclerotic lesions in diabetic and hypercholesterolemic swine.¹⁷ Finally, in a phase II trial, darapladib appeared to stabilize the necrotic core size and plaque burden in coronary atherosclerotic plaques compared with placebo¹⁸; therefore, it was a logical hypothesis that inhibition of Lp-PLA₂

activity with darapladib might lead to clinical benefit, especially in patients with high Lp-PLA₂ activity at baseline and/or a pronounced reduction by the treatment.²⁷ Nevertheless, this current study, based on 3 to 5 years darapladib treatment and Lp-PLA₂ activity levels that were available for ≈14 500 patients before and during treatment and with a consistent ≈65% reduction of the Lp-PLA₂ activity in all patient groups, showed no associated effects of darapladib on clinical outcomes. This finding clearly refutes any clinically important effects of darapladib for prevention of cardiovascular events in patients with CHD.

The current findings corroborate other studies showing that patients with stable CHD and high Lp-PLA₂ activity have a raised risk of cardiovascular events and total mortality. The increase in risk with Lp-PLA₂ activity was not linear but was most pronounced in the ≈25% of patients with the highest levels. The increased risk associated with high Lp-PLA₂ activity was independent of demographics, clinical characteristics, and conventional biochemical risk indicators such as dyslipidemia, dysglycemia, anemia, and renal dysfunction. When adjusting for cardiac, inflammatory, and renal function biomarkers, the independent associations between Lp-PLA₂ activity and cardiovascular and total mortality and

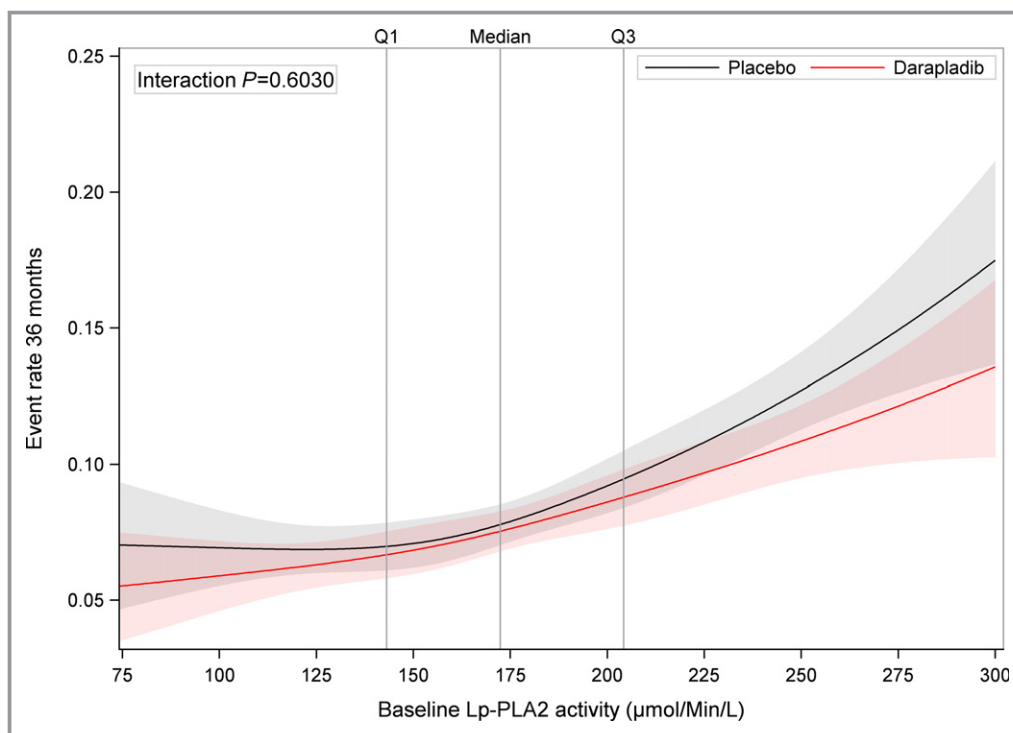


Figure 5. Continuous Lp-PLA₂ activity ($\mu\text{mol}/\text{min}$ per liter) at baseline in relation to major adverse cardiac events (cardiovascular death, myocardial infarction, or stroke) during treatment with darapladib (red line) and placebo (black line) using restricted cubic splines using a Cox proportional hazards model including treatment group, LpPLA₂, and treatment by LpPLA₂ interaction as covariates. Lp-PLA₂, lipoprotein-associated phospholipase A₂; Q, quartile.

hospitalization for heart failure remained, whereas the associations with ischemic events were attenuated. Similarly, there was no association between Lp-PLA₂ activity and cardiovascular outcomes in patients with acute coronary syndromes, in which the majority have elevated cardiac and inflammatory markers.²⁹ Without an associated indication for a specific treatment, it is unlikely that Lp-PLA₂ activity will be used for identification of patients at high risk, considering the competition with other already generally available and more specific prognostic biomarkers. In the present study, as in a previous study,³² there was an inverse relationship between Lp-PLA₂ activity and diabetes mellitus. In that study, diabetes mellitus was associated with a redistribution of Lp-PLA₂ activity from low- to high-density lipoprotein particles.³² These complex interactions among Lp-PLA₂ activity, glucose, and lipoprotein metabolism might need to be considered in future studies of the importance of Lp-PLA₂ activity for cardiovascular events in different patient populations.

Conclusions

Based on the experiences from 3 to 5 years of darapladib treatment and available Lp-PLA₂ activity levels before and

during treatment in 14 500 patients with stable CHD, high pretreatment Lp-PLA₂ activity was associated with an increased risk of cardiovascular events; however, pharmacological lowering of Lp-PLA₂ activity did not significantly reduce cardiovascular events, regardless of the baseline level or degree of reduction of Lp-PLA₂ activity.

Appendix

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Table 5. Changes in Lp-PLA₂ Activity at 1 Month* Compared With Baseline in Relation to Outcomes in the Respective Placebo and Darapladib Groups

Outcome Events		Placebo Change			Darapladib Change			Change From Baseline	
		n	Mean	%	n	Mean	%	HR for 10-U Increase (95% CI)	P Value
MACE [†]	Yes	706	−10.9	5.1	646	−121.7	65.5	0.99 (0.97–1.01)	0.258
	No	6167	−8.5	4.1	6190	−111.3	63.3		
MCE [‡]	Yes	699	−9.7	4.4	614	−120.4	65.1	1.00 (0.98–1.02)	0.919
	No	6174	−8.7	4.1	6222	−111.5	63.3		
Total coronary events	Yes	1106	−9.1	4.1	976	−117.2	64.3	1.01 (0.99–1.02)	0.451
	No	5767	−8.7	4.2	5860	−111.5	63.4		
Cardiovascular death	Yes	323	−12.0	5.5	291	−122.4	65.4	0.99 (0.96–1.01)	0.335
	No	6550	−8.6	4.1	6545	−111.8	63.4		
Myocardial infarction	Yes	341	−9.1	4.3	311	−121.6	66.0	0.99 (0.97–1.02)	0.571
	No	6532	−8.8	4.2	6525	−111.8	63.4		
Stroke	Yes	136	−13.2	6.3	129	−117.7	64.6	0.99 (0.96–1.03)	0.724
	No	6737	−8.7	4.1	6707	−112.2	63.5		
Hospitalization for heart failure	Yes	160	−10.4	4.5	136	−127.5	66.8	0.97 (0.94–1.01)	0.122
	No	6713	−8.7	4.2	6700	−112.0	63.4		
All-cause mortality	Yes	498	−11.3	5.0	478	−120.9	65.4	0.99 (0.97–1.01)	0.223
	No	6375	−8.6	4.1	6358	−111.6	63.4		

Event rates are Kaplan–Meier rates. Lp-PLA₂ indicates lipoprotein-associated phospholipase A₂; MACE, major adverse cardiac events; MCE, major coronary events.

*The analyses are adjusted for baseline Lp-PLA₂ and stratified by randomized treatment. The changes from baseline results are identical to the Month 1 results as both are adjusted for the baseline value.

[†]MACE: cardiovascular death, myocardial infarction, or stroke.

[‡]MCE: coronary heart disease death, myocardial infarction, or urgent coronary revascularization.

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Table 6. Range of Change of Lp-PLA₂ Activity Level From Baseline to 1 Month in All Patients With Lp-PLA₂ Measurements at 1 Month (Including Both Darapladib and Placebo Groups) Per Decile Group

Decile	Change From Baseline Lp-PLA ₂ (μmol/min/L)
1	≤−146.1
2	>−146.1 to ≤−121.4
3	>−121.4 to ≤−101.9
4	>−101.9 to ≤−80.9
5	>−80.9 to ≤−46.1
6	>−46.1 to ≤−21.8
7	>−21.8 to ≤−10.9
8	>−10.9 to ≤−2.1
9	>−2.1 to ≤8.6
10	>8.6

Lp-PLA₂ indicates lipoprotein-associated phospholipase A₂.

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Table 7. Range of Percentage Change of Lp-PLA₂ Activity Level at 1 Month Compared With Baseline in All Patients With Lp-PLA₂ Measurements at 1 Month (Including Both Darapladib and Placebo Groups) Per Decile Group

Decile	Percentage Inhibition of Lp-PLA ₂ (μmol/min/L)
1	≤−5.3
2	>−5.3 to ≤1.3
3	>1.3 to ≤6.5
4	>6.5 to ≤12.3
5	>12.3 to ≤25.7
6	>25.7 to ≤55.3
7	>55.3 to ≤63.4
8	>63.4 to ≤69.5
9	>69.5 to ≤76.2
10	>76.2

Lp-PLA₂ indicates lipoprotein-associated phospholipase A₂.

Table 8. Range of Lp-PLA₂ Activity Level at 1 Month in All Patients With Lp-PLA₂ Measurements at 1 Month (Including Both Darapladib and Placebo Groups) Per Decile Group

Decile	Levels at 1 Month Lp-PLA ₂ (μmol/min/L)
1	≤37.9
2	>37.9 to ≤50.9
3	>50.9 to ≤63.3
4	>63.3 to ≤78.5
5	>78.5 to ≤106.9
6	>106.9 to ≤134.7
7	>134.7 to ≤156.4
8	>156.4 to ≤178.1
9	>178.1 to ≤205.6
10	>205.6

Lp-PLA₂ indicates lipoprotein-associated phospholipase A₂.

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