Effect of Darapladib on Plasma Lipoprotein-Associated Phospholipase A₂ Activity in Japanese Dyslipidemic Patients, With Exploratory Analysis of a PLA₂G7 Gene Polymorphism of Val279Phe

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Background: Lipoprotein-associated phospholipase A₂ (Lp-PLA₂) is being evaluated as a therapeutic target for treatment of atherosclerosis. This is the first study to examine the effects of darapladib, a novel selective Lp-PLA₂ inhibitor, on Lp-PLA₂ activity in Japanese dyslipidemic patients with/without the Val279Phe (V279F) single-nucleotide polymorphism (SNP) of the PLA₂G7 gene. Exploratory analysis to examine the effects of V279F on Lp-PLA₂ inhibition of darapladib was also performed.

Methods and Results: This was a 4-week, multicenter, randomized, double-blind, placebo-controlled, parallel-group, dose-ranging trial of darapladib in 107 Japanese patients with dyslipidemia receiving statins. Patients were randomized to placebo (n=25), darapladib 40 mg (n=28), 80 mg (n=28), or 160 mg (n=26). All darapladib doses produced sustained dose-dependent inhibition of Lp-PLA₂ activity of approximately 49%, 58%, and 67%, respectively (P<0.001 for all comparisons). The inhibitory effect achieved a plateau by 1 week. Patients with the V279F homogenous mutation who have no circulating levels of Lp-PLA₂ were excluded from the study. The Lp-PLA₂ activity was inhibited in both homozygous wild-type and heterozygote genotypes of the V279F polymorphism subjects to a similar extent, although the heterogeneous mutation has almost half the level of Lp-PLA₂ activity compared with that of wild-type in Japanese people. The most common adverse events were odor related. No major safety concerns were noted.

Conclusions: Darapladib produced sustained inhibition of Lp-PLA₂ activity in Japanese dyslipidemic patients with/without the V279F SNP of Lp-PLA₂. (Circ J 2013; 77: 1518–1525)

Key Words: Atherosclerosis; Darapladib; Dyslipidemia; Lipoprotein-associated phospholipase A₂; V279F

Atherosclerosis, the most common cause of myocardial infarction, stroke, and cardiovascular death, is an inflammatory disease. Elevated circulating low-density lipoprotein (LDL) is well known to be a precursor of atherosclerosis and a risk factor for acute coronary syndrome.¹⁻⁴ When LDL is trapped in the subintimal space, apolipoprotein B (apoB) facilitates several steps in the oxidation of cholesterol, which signals the upregulation of adhesion molecules and cell surface chemoattractants that recruit monocytes and macrophages into the nascent atheroma. Macrophages play a key role in the ingestion of cholesterol, resulting in the release of free fatty acids and lysophospholipids, which provide metabolites for various inflammatory pathways. Phospholipase-driven inflammation is associated with endothelial dysfunction, plaque formation, and coronary artery disease (CAD), especially with acute coronary syndrome.⁵⁻⁷ LDL oxidation results in a biochemical modification affecting phospholipid and apoB components. Lipoprotein-associated phospholipase A₂ (Lp-PLA₂), also known as platelet-activating factor acetylhydrolase (PAF-AH), is an enzyme that has pro-inflammatory properties thought to be involved during LDL oxidation in the development and progression of atherosclerosis,⁸⁻¹⁰ and is currently being evaluated as a new therapeutic target.⁸

Lp-PLA₂ hydrolyzes oxidized phospholipids generated during the oxidation of LDL, and leads to formation of pro-inflammatory products,⁶ such as lysophosphatidylcholine and oxidized non-esterified fatty acid. In contrast to other PLA₂ enzymes, Lp-PLA₂ acts preferentially on water-soluble polar...
phospholipids with oxidatively truncated sn-2 chains, lacking enzymatic activity on naturally occurring long-chain fatty acids in phospholipids found in cellular membranes. The prevalence of 279FF was reported to be 0.4%, 1.2%, and 3% in the Chinese, Korean, and Japanese populations, respectively, but this variant is rare in non-Asian populations.

Darapladib is a novel, selective, reversible, orally active inhibitor of Lp-PLA2 activity, discovered by GlaxoSmithKline (GSK). Darapladib does not inhibit the other secretory PLA2 including sPLA2-IIA. It is in development by GSK for prevention of major adverse cardiovascular events in 2 large outcomes studies. In 1 non-clinical study, using a preclinical model of atherosclerosis in pigs with diabetes mellitus and hypercholesterolemia, despite the development of sustained severe hypercholesterolemia, selective inhibition of Lp-PLA2 by darapladib resulted in a significant decrease in atherosclerotic coronary lesion development in comparison to controls. The primary route of elimination is via the feces and the compound is eliminated both as intact darapladib as well as oxidative metabolites, which are modified primarily with CYP3A4.

Two international Phase II studies have been completed. One study examined the effects of darapladib on biomarkers of cardiovascular risk in 959 CAD and CAD-risk equivalent patients who were previously randomized to atorvastatin 20 mg or 80 mg and then randomized to oral darapladib 40, 80, 160 mg, or placebo for 12 weeks. Overall dose-dependent inhibition of Lp-PLA2 activity was sustained over the study period and was present in both atorvastatin dose groups, at different baseline LDL cholesterol (LDL-C; < or ≥70 mg/dl), and high-density lipoprotein cholesterol (HDL-C) < or ≥40 mg/dl. The other study (IBIS-2 study) compared the effects of 12-month treatment with darapladib 160 mg daily or placebo on coronary atheroma deformability and hs-CRP in 330 patients with angiographically documented coronary disease. The results showed that darapladib inhibits plasma Lp-PLA2 activity in a dose-dependent manner; no major safety concern was noted. In the IBIS-2 study, inhibition of Lp-PLA2 with darapladib also prevented necrotic core expansion of coronary plaque as measured on intravascular ultrasound.

The present study is the first to examine the effects of darapladib on plasma Lp-PLA2 activity and to assess the safety and tolerability of darapladib in Japanese dyslipidemic patients. In addition, the effect of the V279F heterozygote on the inhibition activity of darapladib is investigated.

**Methods**

**Patients**

The subjects included dyslipidemic patients aged 20–80 years on statin therapy with no change in lipid-lowering medication or dose during the 4 weeks before randomization. Exclusion criteria included recent (<6 months prior to screening) cardiovascular event and/or vascular procedure; Lp-PLA2 activity <10 nmol·min⁻¹·ml⁻¹; poorly controlled dyslipidemia (LDL-C ≥160 mg/dl); poorly controlled hypertension (blood pressure ≥160 mmHg systolic and/or ≥100 mmHg diastolic); severe renal dysfunction (estimated glomerular filtration rate [eGFR] <30 ml·min⁻¹·1.73 m⁻²; eGFR [ml·min⁻¹·1.73 m⁻²]=0.741×175×Age⁻0.203×Cr¹.154; [x0.742 if patient is female]); chronic heart failure; or previous exposure to darapladib.

The study was approved by Institutional Review Boards of clinical trial sites. The study was conducted following the latest version of Declaration of Helsinki. All participants provided written informed consent.

**Study Design**

This was a 4-week, multicenter, randomized, double-blind, placebo-controlled, parallel-group, dose-ranging trial of darapladib in patients with dyslipidemia receiving statin therapy. The stratified randomization with plasma Lp-PLA2: activity was conducted in screened patients to receive placebo or enteric-coated darapladib 40 mg, 80 mg, or 160 mg tablets once a day in a ratio of 1:1:1:1.

The study consisted of visit 1 (screening visit; weeks –6 to –2), visit 2 (baseline visit at which patients were randomized to treatment with darapladib or placebo; week 0), visits 3, 4, 5 (treatment visit; weeks 1, 2, 4), and visit 6 (follow-up visit; week 7). Patients were instructed to take the study medication once daily with food, generally after breakfast, to avoid risk of taking the study medication under a condition of low gastric pH. Patients were asked to swallow the tablets without chewing, because the tablets were enteric-coated.

**Endpoints**

The primary efficacy endpoint was change from baseline to week 4 in plasma Lp-PLA2: activity (log-transformed). The secondary endpoints were percent inhibition of Lp-PLA2: activity in plasma at week 4, and each-visit changes from baseline of Lp-PLA2: activity and percent inhibition of Lp-PLA2: activity.

To examine the effects of darapladib on plasma Lp-PLA2: activity, blood samples were taken, apart from pharmacogenetics samples, at each visit between screening and follow-up. Plasma Lp-PLA2: activity was measured using colorimetric assay.

For exploratory endpoints, whole blood was taken from patients to detect a gene polymorphism of V279F for examination of the effect of darapladib on the V279F heterozygous (279FFV) population. The effect of darapladib on biomarkers such as plasminogen activating inhibitor type 1 (PAI-1), hs-CRP, interleukin 6 (IL-6), P-selectin and urinary 11-dehydrothromboxane B2 was also evaluated.

Safety endpoints included the incidence and severity of adverse events (AEs); change from baseline in clinical laboratory data and urinalysis at week 2, week 4, and follow-up visit; change from baseline in 12-lead electrocardiogram (ECG) at week 4; and change from baseline in vital signs (blood pressure, heart rate) at each visit.

**Statistical Analysis**

The primary efficacy analysis group was the full analysis set (FAS): randomized patients except those who received no dose of study drug and those for whom Lp-PLA2: activity was not measured or evaluable. The secondary efficacy analysis
For each biomarker, the ANCOVA-adjusted baseline value was used for log-transformed change from baseline to week 4.

The number and percentage of serious AEs (SAEs), AEs leading to discontinuation of investigational product, and other safety endpoints were summarized by treatment group.

**Results**

**Subject Baseline Characteristics**

A total of 107 patients were randomized to placebo (n=25), darapladib 40 mg (n=28), 80 mg (n=28), or 160 mg (n=26). All 107 patients were included in the FAS and safety group. They received standard care for dyslipidemia and other diseases including diabetes mellitus and hypertension. The patients’ lifestyle did not change during the 4-week treatment period. Patient demographic characteristics are listed in Table 1. The V279F polymorphism genotype was generally similar across the treatment groups. There were 82/107 patients (77%) with the homozygous wild-type (279VV) and 25/107 (23%) with the heterozygous (279VF) genotype. There was no homozygous mutant (FF) subject because 3 patients who had Lp-PLA2 activity ≤10 nmol·min⁻¹·ml⁻¹ were excluded at group, the per-protocol set (PPS), excluded patients with major protocol violations. The safety group included patients receiving at least 1 dose of study drug.

The primary efficacy analysis compared each dose of darapladib vs. placebo for the change from baseline in log-transformed Lp-PLA2 activity to week 4. A parametric analysis of covariance (ANCOVA) model included baseline Lp-PLA2 activity as a covariate. Each of the 3 comparisons between active groups and placebo was made at the overall 2-sided significance level of 5% with adjustment for multiplicity using Dunnett correction. Missing values of Lp-PLA2 activity after randomization were imputed using last observation carried forward. The sensitivity analysis to assess the robustness of the primary analysis was conducted using the PPS and observed case analysis, which is the non-imputation method for missing values.

For secondary efficacy analysis, to examine the dose response of darapladib on inhibition of plasma Lp-PLA2 activity, change from baseline in log-transformed plasma Lp-PLA2 activity to week 4 was analyzed using ANCOVA with contrast methods at the 1-sided 2.5% significance level. Subgroup analyses of V279F polymorphism were conducted by ANCOVA.

**Table 1. Demographic and Baseline Subject Characteristics**

| Characteristics          | Placebo (n=25) | Darapladib 40 mg (n=28) | Darapladib 80 mg (n=28) | Darapladib 160 mg (n=26) | Total (n=107) |
|--------------------------|----------------|-------------------------|-------------------------|--------------------------|---------------|
| Sex                      |                |                         |                         |                          |               |
| Female                   | 17 (68)        | 17 (61)                 | 18 (64)                 | 13 (50)                  | 65 (61)       |
| Male                     | 8 (32)         | 11 (39)                 | 10 (36)                 | 13 (50)                  | 42 (39)       |
| Age category (years)     |                |                         |                         |                          |               |
| <65                      | 14 (56)        | 15 (54)                 | 21 (75)                 | 18 (69)                  | 68 (64)       |
| ≥65                      | 11 (44)        | 13 (46)                 | 7 (25)                  | 8 (31)                   | 39 (36)       |
| <75                      | 25 (100)       | 27 (96)                 | 26 (93)                 | 25 (96)                  | 103 (96)      |
| ≥75                      | 0              | 1 (4)                   | 2 (7)                   | 1 (4)                    | 4 (4)         |
| Age (years)              | 59.5±11.71     | 59.8±10.17               | 58.3±9.54               | 58.3±10.48               | 59.0±10.34    |
| Body weight (kg)         | 60.8±9.65      | 63.5±13.327              | 60.8±10.77              | 63.4±13.988              | 62.1±11.995   |
| BMI (kg/m²)              | 24.3±2.868     | 24.6±3.203               | 23.4±2.96               | 24.2±3.471               | 24.1±3.122    |
| Height (cm)              | 158.0±8.09     | 159.8±9.58               | 160.5±9.90              | 161.0±9.52               | 159.9±9.26    |
| TC (mmol/L)              | 5.2±0.70       | 5.2±0.64                 | 5.3±0.71                | 4.8±0.57                 |               |
| LDL-C (mmol/L)           | 3.1±0.59       | 2.9±0.61                 | 3.2±0.71                | 2.8±0.46                 |               |

Data given as mean±SD or n (%). BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol.

**Table 2. Plasma Lp-PLA2 Activity Change From Baseline to Week 4**

| Treatment          | Visit          | Geometric mean | Adj. ratio† | vs. placebo (95% CI)‡ | P-value |
|--------------------|----------------|----------------|--------------|------------------------|---------|
| Placebo (n=25)     | Baseline       | 127.56         | 0.961        |                        |         |
|                    | Week 4/withdrawal | 122.56        |              |                        |         |
| Darapladib 40 mg   | Baseline       | 125.62         | 0.494        | 0.514 (0.449–0.590)    | <0.001  |
| (n=28)             | Week 4/withdrawal | 62.12         |              |                        |         |
| Darapladib 80 mg   | Baseline       | 136.06         | 0.404        | 0.421 (0.367–0.483)    | <0.001  |
| (n=28)             | Week 4/withdrawal | 54.97         |              |                        |         |
| Darapladib 160 mg  | Baseline       | 124.84         | 0.313        | 0.326 (0.284–0.375)    | <0.001  |
| (n=26)             | Week 4/withdrawal | 39.12         |              |                        |         |

ANCOVA was performed on the log-transformed data and back-transformed to provide statistics in original scale. Dunnett correction was used to adjust multiplicity. †Adjusted geometric mean ratio from baseline to week 4 of each of the darapladib groups. ‡Adjusted geometric mean ratio of each of the darapladib groups compared with the placebo. CI, confidence interval; Lp-PLA2, lipoprotein-associated phospholipase A2.
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All darapladib doses (40 mg, 80 mg, and 160 mg) produced sustained inhibition of Lp-PLA2 activity in a dose-dependent fashion (approximately 49%, 58%, and 67% inhibition, respectively; P<0.001 for all comparisons) from baseline to week 4 (Table 2). On the follow-up visit, Lp-PLA2 activity level returned to the baseline level (Figure). Sensitivity analysis using the observed case dataset for the FAS and PPS came to the same conclusions and showed robustness of primary analysis.

Secondary Analysis All darapladib doses showed sustained inhibition in plasma Lp-PLA2 activity over time. The dose

**Table 3. Plasma Lp-PLA2 Activity Change vs. V279F Subgroup**

| Category/Treatment          | Visit          | Geometric mean | Adj. ratio† | vs. placebo (95% CI)‡ | P-value   |
|----------------------------|----------------|----------------|-------------|-----------------------|-----------|
| Homozygous wild-type (VV)  |                |                |             |                       |           |
| Placebo (n=18)             | Baseline       | 146.88         | 0.957       |                       |           |
|                           | Week 4/withdrawal | 140.30        |             |                       |           |
| Darapladib 40 mg (n=22)    | Baseline       | 145.62         | 0.488       | 0.510 (0.434–0.599)   | <0.001    |
|                           | Week 4/withdrawal | 70.86         |             |                       |           |
| Darapladib 80 mg (n=23)    | Baseline       | 152.98         | 0.396       | 0.414 (0.353–0.485)   | <0.001    |
|                           | Week 4/withdrawal | 60.80         |             |                       |           |
| Darapladib 160 mg (n=19)   | Baseline       | 148.82         | 0.316       | 0.330 (0.280–0.390)   | <0.001    |
|                           | Week 4/withdrawal | 47.04         |             |                       |           |
| Heterozygote (VF)          |                |                |             |                       |           |
| Placebo (n=7)              | Baseline       | 88.74          | 1.010       |                       |           |
|                           | Week 4/withdrawal | 86.59         |             |                       |           |
| Darapladib 40 mg (n=6)     | Baseline       | 73.09          | 0.510       | 0.505 (0.378–0.676)   | <0.001    |
|                           | Week 4/withdrawal | 38.32         |             |                       |           |
| Darapladib 80 mg (n=5)     | Baseline       | 79.36          | 0.435       | 0.431 (0.321–0.578)   | <0.001    |
|                           | Week 4/withdrawal | 34.56         |             |                       |           |
| Darapladib 160 mg (n=7)    | Baseline       | 77.49          | 0.303       | 0.301 (0.229–0.394)   | <0.001    |
|                           | Week 4/withdrawal | 23.73         |             |                       |           |

ANCOVA was performed on the log-transformed data and back-transformed to provide statistics in original scale. Dunnett correction was used to adjust multiplicity. †Adjusted geometric mean ratio from baseline to week 4 of each of the darapladib groups. ‡Adjusted geometric mean ratio of each of the darapladib groups compared with the placebo. CI, confidence interval; Lp-PLA2, lipoprotein-associated phospholipase A2.
deemed exploratory.

Data for biomarkers are given in Table 4. PAI-1 in the darapladib 160-mg group had a significant reduction (P<0.001) compared with placebo. For the inflammatory biomarker hs-CRP, there was a reduction only in the darapladib 80-mg group (P=0.012) compared with placebo. Also, IL-6 showed a decreasing trend in the darapladib 80-mg and 160-mg groups compared with placebo. The platelet activation biomarker response of darapladib on inhibition of plasma Lp-PLA2 was analyzed using ANCOVA with contrast method at the 1-sided 2.5% significance level. Linear trend was the best fit of contrast for the dose-response analysis.

Exploratory Analysis There was no difference in proportional change of plasma Lp-PLA2 activity between the 279VV and 279VF subjects (Table 3), in both genotypes showing a significant effect vs. placebo. Analyses of the genotypes were deemed exploratory.

| Table 4. Biomarker Change From Baseline to Week 4 |
|-----------------------------------------------|
|                                  | n  | Adj. ratio† | vs. placebo (95% CI)‡ | P-value |
| PAI-1 (ng/ml)                      |    |             |                          |         |
| Placebo                           | 25 | 1.247       |                          |         |
| Darapladib 40 mg                  | 27 | 1.032       | 0.828 (0.673–1.019)     | 0.074   |
| Darapladib 80 mg                  | 28 | 1.047       | 0.840 (0.685–1.029)     | 0.091   |
| Darapladib 160 mg                 | 25 | 0.837       | 0.671 (0.542–0.832)     | <0.001  |
| hs-CRP (mg/L)                     |    |             |                          |         |
| Placebo                           | 23 | 1.396       |                          |         |
| Darapladib 40 mg                  | 26 | 1.203       | 0.862 (0.560–1.327)     | 0.496   |
| Darapladib 80 mg                  | 25 | 0.797       | 0.571 (0.371–0.879)     | 0.012   |
| Darapladib 160 mg                 | 22 | 1.335       | 0.956 (0.613–1.492)     | 0.842   |
| IL-6 (ng/L)                       |    |             |                          |         |
| Placebo                           | 16 | 1.141       |                          |         |
| Darapladib 40 mg                  | 19 | 0.866       | 0.760 (0.508–1.135)     | 0.176   |
| Darapladib 80 mg                  | 17 | 0.782       | 0.686 (0.456–1.032)     | 0.070   |
| Darapladib 160 mg                 | 15 | 0.770       | 0.675 (0.439–1.036)     | 0.072   |
| P-selectin (μg/L)                 |    |             |                          |         |
| Placebo                           | 24 | 1.041       |                          |         |
| Darapladib 40 mg                  | 24 | 1.014       | 0.974 (0.855–1.110)     | 0.694   |
| Darapladib 80 mg                  | 27 | 0.981       | 0.942 (0.830–1.070)     | 0.354   |
| Darapladib 160 mg                 | 22 | 1.004       | 0.965 (0.844–1.103)     | 0.597   |
| U-TxB2 (pg/ml)                    |    |             |                          |         |
| Placebo                           | 25 | 1.185       |                          |         |
| Darapladib 40 mg                  | 27 | 1.193       | 1.007 (0.697–1.454)     | 0.971   |
| Darapladib 80 mg                  | 28 | 1.178       | 0.994 (0.689–1.434)     | 0.974   |
| Darapladib 160 mg                 | 26 | 1.426       | 1.203 (0.828–1.748)     | 0.329   |

ANOVA was performed on the log-transformed data and back-transformed to provide statistics in original scale. Dunnett correction was used to adjust multiplicity. †Adjusted geometric mean ratio from baseline to week 4 of each of the darapladib groups. ‡Adjusted geometric mean ratio of each of the darapladib groups compared with the placebo. CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; PAI-1, plasminogen activating inhibitor type 1; U-TxB2, urinary 11-dehydrothromboxane B2.

| Table 5. Most Frequent Treatment-Emergency AEs† |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Treatment-emergency AE | Placebo (n=25) | Darapladib 40 mg (n=28) | Darapladib 80 mg (n=28) | Darapladib 160 mg (n=26) |
| Any event        | 10 (40)         | 18 (64)         | 15 (54)         | 16 (62)         |
| Abnormal feces   | 0 (0)           | 5 (18)          | 6 (21)          | 5 (19)          |
| Nasopharyngitis  | 4 (16)          | 3 (11)          | 4 (14)          | 5 (19)          |
| Urine odor abnormal | 0 (0)          | 2 (7)           | 5 (18)          | 4 (15)          |
| Diarrhea         | 1 (4)           | 1 (4)           | 2 (7)           | 3 (12)          |
| Eczema           | 2 (8)           | 1 (4)           | 1 (4)           | 2 (8)           |
| Headache         | 1 (4)           | 2 (7)           | 2 (7)           | 0 (0)           |
| Skin odor abnormal | 0 (0)          | 2 (7)           | 0 (0)           | 1 (4)           |
| Abdominal pain upper | 0 (0)         | 0 (0)           | 0 (0)           | 2 (8)           |
| Constipation     | 0 (0)           | 2 (7)           | 0 (0)           | 0 (0)           |
| Muscle spasms    | 0 (0)           | 0 (0)           | 2 (7)           | 0 (0)           |

†Any AE that occurred in more than 1 subject in any group. AE, adverse event.
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(P-selectin and U-Tx-B2) showed no significant change in all the darapladib treatment groups compared with placebo (Table 4).

Safety
Darapladib was generally well-tolerated. All AEs were reported as being mild or moderate in intensity. Table 5 lists the most frequently reported AEs (incidence ≥2%) in any treatment group in the safety group. The most frequently reported AEs in the darapladib groups were odor-related disorders, primarily abnormal feces.

A total of 1/25 patients (4%) in the placebo group, 5/28 (18%) in the darapladib 40-mg group, 6/28 (21%) in the darapladib 80-mg group, and 7/26 (27%) in the darapladib 160-mg group experienced AEs that were considered related to study drug by the investigator.

There were no deaths reported during the study and follow-up. One subject in the darapladib 40-mg treatment group experienced a non-fatal SAE (pulmonary embolism) and was withdrawn from the study. The SAE was not considered to be related to darapladib by the investigator.

There was no evidence of a trend for dose-related effects in any laboratory parameter. In all treatment groups, the mean changes from baseline were small and were not of clinical importance.

No clinically meaningful change in vital signs over the period of study were reported, except for blood pressure increase in the placebo group, which was reported as an AE. No pathognomonic findings in ECG results were seen during the study.

Discussion
Several large prospective epidemiological studies have suggested a positive association between plasma Lp-PLA2: level and CVD risk. Lp-PLA2 plays a role during LDL oxidation and has been recognized as a predictor of CVD events, providing risk information beyond that provided by conventional CVD risk factors. Furthermore, its potential as a therapeutic target for CVD risk reduction has been proposed. The present study is the first to evaluate the efficacy and safety of darapladib, a novel inhibitor of Lp-PLA2 activity, in Japanese dyslipidemic patients receiving statin therapy, and to evaluate the influence of the V279F variant of the PLA2G7 gene on the effect of darapladib.

All darapladib doses (40 mg, 80 mg, and 160 mg) produced sustained inhibition of Lp-PLA2 activity at approximately 49%, 58%, and 67%, respectively (P<0.001 for all comparisons). A linear dose response of darapladib on inhibition of plasma Lp-PLA2 activity was observed. The inhibitory effect of plasma Lp-PLA2 activity achieved a plateau by 1 week. The results are similar to those reported by Mohler et al, whose study recruited patients in 15 countries. In that 12-week study, inhibition of Lp-PLA2 activity was sustained at approximately 43%, 55%, and 66% for darapladib 40, 80, and 160 mg, respectively (P<0.001 for all comparisons) vs. placebo.

Lp-PLA2 binds to the carboxyl terminus of human apolipoprotein B, and approximately 80% of circulating Lp-PLA2 is associated with apolipoprotein B-containing lipoproteins. The remaining Lp-PLA2 is less firmly associated with phospholipid moiety of HDL-C and does not bind to apolipoprotein A-I. Higher Lp-PLA2 mass or activity is found in proatherogenic small dense LDL-C and electonegative LDL-C particles. Previous studies have also shown that various hypolipidemic drugs (eg, statins, fenofibrate, ezetimibe) decrease plasma Lp-PLA2 mass or activity due to LDL-C lowering, without a direct effect on macrophage expression of the enzyme. In general, statin treatment alone (eg, pravastatin, fluvastatin, simvastatin, atorvastatin) has been associated with approximately 20–30% reduction in the measurement of Lp-PLA2 (mass or activity) in stable CVD patients. Similarly, other lipid-modifying drugs, such as ezetimibe and fenofibrate, modestly lower Lp-PLA2 (mass or activity).

Mohler et al reported that darapladib produced substantial additional reductions in Lp-PLA2 activity when added to intensive atorvastatin therapy (up to 66%). This effect was largely independent of atorvastatin dose and preserved in clinically relevant strata of LDL-C and HDL-C values. The present study also showed that darapladib gave additional reductions in Lp-PLA2 activity of up to 67% compared to the placebo group when added to statin therapy (atorvastatin, 43%; rosuvastatin, 27%; pravastatin, 26%; simvastatin, 4%; pitavastatin, 1%).

In the present study, patients whose Lp-PLA2 activity was ≤10 nmol · min⁻¹ · ml⁻¹ at screening were regarded as lacking Lp-PLA2 activity, hence indicative of 279FF, and were excluded. Consequently, there were 82 patients (77%) with 279VV and 25 (23%) with 279VF. Of the several polymorphisms of the PLA2G7 gene, the V279F homogenous mutation is known to lack Lp-PLA2 activity. Jang et al found that the V279F variant in the PLA2G7 gene led to significant loss of enzyme activity in heterozygous subjects and no appreciable enzyme activity in homozygous subjects. The mechanism of the deficiencies of plasma Lp-PLA2 activity were suggested to be due to a loss-of-function mutation (V279F, exon 9, position 994; G3T; G>T) in the Lp-PLA2 gene, because Val-279 conserved in plasma Lp-PLA2 lies between the active site Ser-273 and Asp-296 residues in a region that is critical for proper folding of the enzyme.

It has been reported that the mutant allele was present in 27% of the Japanese population as heterozygotes and in 4% as homozygotes. In contrast, no heterozygous or homozygous deficient subjects were identified in a random North American population and were rare in the Caucasian hapmap samples and absent in the African samples (www.hapmap.org). In addition, some reports showed that Lp-PLA2 activity in the 279VF population was approximately half of that in the 279VV population, and that 279FF completely lacked Lp-PLA2 activity.

It should be noted that, in the present study, darapladib significantly reduced plasma Lp-PLA2 activity in comparison with placebo in both the 279VV and 279VF genotype subgroups. Moreover, there was no significant difference in change of plasma Lp-PLA2 activity in both the 279VF and 279VV subgroups after 4 weeks of treatment. This suggests that darapladib may have the potential to reduce risk of atherosclerosis and subsequent cardiovascular events even in individuals with the 279VF genotype that have almost half the level of Lp-PLA2 activity compared to that of the wild type. A further phase III studies, to evaluate for the incidence of major adverse cardiovascular events in chronic CAD and acute coronary syndrome patients, are now going on. We await the results on cardiovascular outcome.

Several cardiovascular biomarkers were evaluated within a limited number of patients and a short period in this study, so the possible effect on cardiovascular biomarkers provided only exploratory data and may not be robust. The on-going phase III studies may identify the effect on CV biomarkers correctly.
No major safety concerns were noted, and darapladib appeared to be generally well-tolerated in patients. The majority of AEs were not related to the study medication. The most common AEs with a probable relationship to study drug were odor related. Odor-related events were not unexpected, in line with previous studies in which odor-related AEs have been associated with repeated doses of darapladib. None of the odor-related events resulted in withdrawal of patients from the study.

**Study Limitations**
This was a phase II study designed to examine the efficacy and safety of darapladib in a small group of patients. The effects of darapladib on clinically important outcomes are currently unknown; whether the inhibitory effect of darapladib shown in the present study leads to clinical efficacy in the prevention of major adverse cardiovascular events in patients with CAD or acute coronary syndrome is being examined in 2 ongoing international phase III outcomes studies. If darapladib is proven to be effective in preventing major cardiovascular events and the progression of atherosclerotic disease, then it would be also applicable to the heterozygous (VF) genotype, which has almost half the level of Lp-PLA2 activity compared with that of the wild type.

**Conclusion**
Darapladib produced sustained inhibition of Lp-PLA2 activity with no major safety problems in Japanese dyslipidemic patients with or without a V279F variant who were receiving GlaxoSmithKline K.K. Japan.

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