Randomized trial of an inhibitor of secretory phospholipase A₂ on atherogenic lipoprotein subclasses in statin-treated patients with coronary heart disease

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Aims To investigate the effects of secretory phospholipase A₂ (sPLA₂) inhibition on plasma lipoproteins. Secretory phospholipase A₂ isoenzymes promote atherosclerosis by mechanisms that include lipoprotein modification, retention, and oxidation.

Methods and results Phospholipase Levels And Serological Markers of Atherosclerosis II (PLASMA II) is a Phase II, randomized, double-blind, placebo-controlled parallel-arm study of two once-daily doses of the novel sPLA₂ inhibitor, 1-H-indole-3-glyoxamide or varespladib methyl (Anthera Pharmaceuticals, Hayward, CA, USA). One hundred and thirty-five stable coronary heart disease patients were treated with either varespladib methyl 250 mg once daily, varespladib methyl 500 mg once daily, or placebo for 8 weeks. Varespladib methyl treatment resulted in statistically significant dose-dependent reductions that were different from placebo in sPLA₂ concentration, low-density lipoprotein (LDL) cholesterol, and non-high-density lipoprotein (HDL) cholesterol. When compared with placebo, varespladib methyl 500 mg once daily reduced LDL cholesterol by 15% (\( P < 0.001 \)), non-HDL cholesterol by 15% (\( P = 0.001 \)), total very LDL (VLDL) particle concentration by 14% (\( P = 0.022 \)), and small VLDL particle concentration by 24% (\( P = 0.030 \)). Relative to baseline, varespladib methyl 500 mg once daily reduced total LDL particle concentration (7% \( P = 0.002 \)) and small LDL particle concentration (11% \( P = 0.014 \)).

Conclusion Reductions in atherogenic lipoproteins suggest that varespladib methyl 500 mg once daily may be an effective anti-atherosclerotic agent.

Trial registered at ClinicalTrials.gov, identifier: NCT00525954.

Keywords Secretory phospholipase A₂, CHD risk, Low-density lipoprotein cholesterol, Non-high-density lipoprotein cholesterol, Lipoprotein subclasses, Inflammation

Introduction High risk of recurrent cardiovascular events in patients receiving evidence-based therapies have prompted investigations into therapies that reduce vascular inflammation and improve stability of vulnerable plaques in an effort to further reduce the risk of cardiovascular events. Secretory phospholipase A₂ (sPLA₂) represents a family of enzymes that have been identified as a potential target of therapy. Secretory phospholipase A₂ isoenzymes Groups IIA (sPLA₂-IIA), V (sPLA₂-V), and X (sPLA₂-X) promote atherosclerosis by multiple mechanisms that include lipoprotein modification, retention, and oxidation. Experimental animal models confirm the importance of sPLA₂ inhibition as these studies demonstrate reduced atheroma volume and improved stability of atherosclerotic plaques. Varespladib methyl is an sPLA₂ inhibitor in human subjects with specificity towards sPLA₂-IIA (IC₅₀: 9–14 nM), sPLA₂-V (IC₅₀: 77 nM), and sPLA₂-X (IC₅₀: 15 nM). The effect of

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varespladib methyl treatment (50–500 mg twice daily for 8 weeks) on sPLA₂ concentration, lipid, and lipoprotein in patients with stable coronary heart disease (CHD) had been reported previously. Briefly, varespladib methyl treatment reduced low-density lipoprotein (LDL) cholesterol primarily through lowering of small LDL particles, and the magnitude of these changes was larger in statin-treated patients vs. patients not taking a statin. The current study was designed to examine the effects of once-daily dosing of varespladib methyl (A-002, Anthera Pharmaceuticals, Hayward, CA, USA), on sPLA₂ concentration, plasma lipids, and lipoproteins, and inflammatory biomarkers in stable CHD patients in whom the use of statins was more consistent with standards of care than reported in PLASMA I. The rationale for using once-daily dosing was primarily based on patient convenience since varespladib methyl is envisioned for use in conjunction with a statin that is also given once daily. Thus, PLASMA II was designed to determine the dosage of varespladib methyl for use in a larger safety and efficacy Phase III clinical trial.

Methods
Phospholipase Levels And Serological Markers of Atherosclerosis II (PLASMA II) is a Phase II, randomized, double-blind, placebo-controlled parallel-arm study of two different once-daily doses of the novel specific sPLA₂ inhibitor, 1-H-indole-3-glyoxamide or varespladib methyl (NCT00525954). A total of 135 patients were recruited with stable CHD defined as previous myocardial infarction (>12 weeks earlier), unstable angina (>6 weeks earlier), evidence of coronary artery disease by arteriography, or a revascularization procedure. Patients were randomly allocated in equal numbers to one of the three arms of the study: varespladib methyl 250 mg, varespladib methyl 500 mg, or placebo. A total of 138 subjects were recruited, 135 subjects received at least one dose of the study medication, and 124 subjects completed the 8-week study. Three subjects withdrew consent before they were given any study medication. Eleven subjects discontinued due to adverse reactions (Figure 1).

All patients provided written informed consent and the study protocol was approved by local and national Ethics Committees in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines.

Biochemical analysis
Group IIA sPLA₂ concentration was analysed by quantitative two-site enzyme immunoassay (CLASS Laboratories, University of Michigan, Ann Arbor, MI, USA). The intra- and inter-assay coefficients of variation (CVs) at low, intermediate, and high sPLA₂-IIA concentrations ranged from 8.3 to 25.7% and 6.7 to 9.2% respectively, and the lower limit of detection for the assay was 0.1 pmol/L. Plasma lipids, chemistry panels, and high-sensitivity C-reactive protein levels were measured by standard procedures (Quest Diagnostics, Van Nuys, CA, USA). Low-density lipoprotein cholesterol concentrations were calculated. The intra- and inter-assay CVs for plasma lipid concentrations were: total cholesterol 0.6 and 1.5%, respectively; high-density lipoprotein (HDL) cholesterol 1.3 and 1.7%, respectively; and triglycerides 1.9 and 1.4%, respectively. The intra- and inter-assay CVs for high-sensitivity C-reactive protein were 7.0 and 4.3%, respectively, and the lower limit of detection was 0.2 mg/L. Lipoprotein subclass profiles were measured with an automated nuclear magnetic resonance spectrosocpic assay (LipoScience, Inc., Raleigh, NC, USA). The respective intra- and inter-assay CVs for the three very LDL (VLDL) subclasses were 1.4–12.8 and 3.1–11.7%; for the large and small LDL subclasses, 4.3–11.9 and 5.3–13.2%; and for the three HDL subclasses, 2.7–5.6 and 3.0–5.9%, respectively. The nuclear magnetic resonance (NMR) analysis involves deconvolution of a composite plasma NMR signal to give the amplitudes of the signals from the various lipoprotein subclasses which contribute to it (i.e. the whole is assumed to be the sum of its parts). If one (or more) subclasses are deduced computationally to make no contribution to the plasma signal, its concentration is reported as zero. A meaningful detection limit cannot be determined because the detectability of a particular subclass signal depends on the variable concentrations of many other subclasses whose signals overlap with the signal of interest. Oxidized LDL (ox-LDL) was measured by two-site enzyme-linked immunosorbent assay (Mercodia, Figure 1 CONSORT diagram. *Data from last observation carried forward.
Uppsala, Sweden) using the specific antibody 4E6 and a second mono-
clonal generated against a distinct epitope. The intra- and inter-assay
CVs for ox-LDL were 4.4 and 6.9%, respectively, and the lower limit
of detection for the assay was <1 mIU/L.

**Statistical analysis**

Primary efficacy analysis was the comparison of changes from baseline
to Week 8 on sPLA2-IIA concentration between the pooled varespla-
dib methyl group and placebo. On the basis of data from the previous
study PLASMA,13 20 subjects per group were required to detect a
reduction in sPLA2-IIA concentration of >80% between varespladib
and placebo treatment groups with 90% power and α = 0.05. We
included additional subjects in order to obtain more safety data on
the once-daily dosing regimen of varespladib methyl. For testing
within treatment groups, the paired t-test was used to compare each
value at Week 8 to the value at baseline. If normality assumptions
failed, the Wilcoxon signed-rank test was used. Per cent changes
were first calculated as individual per cent change for each subject
and then summarized using descriptive statistics. Treatment differences
are expressed as mean differences with standard errors. SAS software
version 9.1.3 (SAS Institute, Inc., Cary, NC, USA) was used to perform
data analysis.

Secondary efficacy analyses included pair-wise comparisons of each
dose of varespladib methyl and comparison of changes from baseline
to Week 8 between each varespladib methyl group and the placebo
group. Summary descriptive statistics were derived for individual
time points and for change and per cent change from baseline.

Adverse events were coded using the MedDra dictionary version
10.0 and categorized by body system and preferred term, by intensity,
and by causal relationship to the study agent. All adverse events
were classified by body system and preferred term. All adverse events
were summarized using descriptive statistics. Treatment differences
were primarily due to reductions in small HDL particles.

When compared with placebo, varespladib methyl reduced
tutable (n = 89) Placebo (n = 46)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Varespladib methyl 250 mg (n = 45)</th>
<th>Varespladib methyl 500 mg (n = 44)</th>
<th>Overall varespladib methyl</th>
<th>Placebo (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67 ± 9</td>
<td>67 ± 11</td>
<td>67 ± 10</td>
<td>64 ± 12</td>
</tr>
<tr>
<td>Sex (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31 (69)</td>
<td>32 (73)</td>
<td>63 (71)</td>
<td>35 (76)</td>
</tr>
<tr>
<td>Female</td>
<td>14 (31)</td>
<td>12 (27)</td>
<td>26 (29)</td>
<td>11 (24)</td>
</tr>
<tr>
<td>Caucasian (%)</td>
<td>43 (96)</td>
<td>41 (93)</td>
<td>84 (94)</td>
<td>42 (91)</td>
</tr>
<tr>
<td>BMIb (kg/m²)</td>
<td>31 ± 5</td>
<td>31 ± 5</td>
<td>31 ± 5</td>
<td>30 ± 6</td>
</tr>
<tr>
<td>ASA (%)</td>
<td>37 (83)</td>
<td>40 (91)</td>
<td>77 (87)</td>
<td>34 (74)</td>
</tr>
<tr>
<td>β-Blockers (%)</td>
<td>26 (58)</td>
<td>26 (59)</td>
<td>52 (88)</td>
<td>27 (59)</td>
</tr>
<tr>
<td>ACE-inhibitors (%)</td>
<td>16 (36)</td>
<td>16 (36)</td>
<td>32 (56)</td>
<td>18 (38)</td>
</tr>
<tr>
<td>Statin (%)</td>
<td>40 (89)</td>
<td>41 (93)</td>
<td>81 (91)</td>
<td>40 (87)</td>
</tr>
</tbody>
</table>

Data are represented as means ± standard deviations. The number of subjects was stated for categorical variables with percentages in parentheses. There were no significant differences between groups for all comparisons. ASA, aspirin; ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker.

bBody mass index (BMI) is the weight in kilograms divided by the square of the height in metres.
Table 2  Plasma levels of lipids and inflammatory markers at baseline and the end of treatment phase

<table>
<thead>
<tr>
<th>Variable and study phase</th>
<th>Varespladib methyl 250 mg (n = 45)</th>
<th>Varespladib methyl 500 mg (n = 44)</th>
<th>Placebo (n = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sPLA₂ concentration (pmol/L)†‡</td>
<td>179 (14, 436)</td>
<td>161 (21, 429)</td>
<td>150 (14, 407)</td>
</tr>
<tr>
<td>Pre</td>
<td>179 (14, 436)</td>
<td>161 (21, 429)</td>
<td>150 (14, 407)</td>
</tr>
<tr>
<td>Post</td>
<td>57 (4, 186)†‡</td>
<td>29 (4, 314)†‡</td>
<td>171 (21, 429)</td>
</tr>
<tr>
<td>Per cent change</td>
<td>−73 (−97, −7)†‡</td>
<td>−84 (−99, 83)†‡</td>
<td>8 (−41, 175)†‡</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>4.10 ± 0.15</td>
<td>3.91 ± 0.12</td>
<td>4.14 ± 0.16</td>
</tr>
<tr>
<td>Post</td>
<td>3.85 ± 0.13</td>
<td>3.54 ± 0.13†‡</td>
<td>4.03 ± 0.17</td>
</tr>
<tr>
<td>Per cent change</td>
<td>−3 ± 2</td>
<td>−11 ± 25†‡</td>
<td>−2 ± 2</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>2.01 ± 0.11</td>
<td>1.89 ± 0.10</td>
<td>2.12 ± 0.13</td>
</tr>
<tr>
<td>Post</td>
<td>1.88 ± 0.10</td>
<td>1.63 ± 0.09†‡</td>
<td>2.01 ± 0.13</td>
</tr>
<tr>
<td>Per cent change</td>
<td>−2 ± 4</td>
<td>−15 ± 25†‡</td>
<td>−1 ± 3</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>2.83 ± 0.13</td>
<td>2.64 ± 0.11</td>
<td>2.91 ± 0.16</td>
</tr>
<tr>
<td>Post</td>
<td>2.60 ± 0.11</td>
<td>2.29 ± 0.12†‡</td>
<td>2.82 ± 0.17</td>
</tr>
<tr>
<td>Per cent change</td>
<td>−4 ± 3</td>
<td>−15 ± 25†‡</td>
<td>−1 ± 2</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1.27 ± 0.06</td>
<td>1.27 ± 0.04</td>
<td>1.23 ± 0.04</td>
</tr>
<tr>
<td>Post</td>
<td>1.25 ± 0.05</td>
<td>1.24 ± 0.04†‡</td>
<td>1.21 ± 0.04</td>
</tr>
<tr>
<td>Per cent change</td>
<td>−1 ± 1</td>
<td>−3 ± 2†</td>
<td>−2 ± 2</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1.83 ± 0.14</td>
<td>1.65 ± 0.11</td>
<td>1.72 ± 0.12</td>
</tr>
<tr>
<td>Post</td>
<td>1.57 ± 0.11†‡</td>
<td>1.46 ± 0.14†‡</td>
<td>1.68 ± 0.14</td>
</tr>
<tr>
<td>Per cent change</td>
<td>−7 ± 4.14†‡</td>
<td>−11 ± 4.30†‡</td>
<td>3 ± 4.41‡</td>
</tr>
<tr>
<td>Apolipoprotein B (mg/L)†‡</td>
<td>6.63 (4.21, 15.8)</td>
<td>6.96 (4.26, 13.9)</td>
<td>6.28 (4.07, 20.2)</td>
</tr>
<tr>
<td>Pre</td>
<td>6.63 (4.21, 15.8)</td>
<td>6.96 (4.26, 13.9)</td>
<td>6.28 (4.07, 20.2)</td>
</tr>
<tr>
<td>Post</td>
<td>6.17 (3.94, 15.0)</td>
<td>6.07 (2.65, 11.8)†‡</td>
<td>6.96 (4.14, 17.7)</td>
</tr>
<tr>
<td>Per cent change</td>
<td>−10 (−60, 49)</td>
<td>−15 (−58, 86)†‡</td>
<td>4 (−88, 97)</td>
</tr>
<tr>
<td>ox-LDL (U/L)†‡</td>
<td>40.7 (27.2, 72.6)</td>
<td>41.6 (23.8, 73.5)</td>
<td>43.3 (25.4, 110.5)</td>
</tr>
<tr>
<td>Pre</td>
<td>40.7 (26.4, 63.3)</td>
<td>39.2 (21.9, 71.1)</td>
<td>43.8 (28.1, 110.7)</td>
</tr>
<tr>
<td>Post</td>
<td>−3 (−45, 51)</td>
<td>−7 (−42, 32)</td>
<td>0.4 (−40, 110)</td>
</tr>
<tr>
<td>High-sensitivity C-reactive protein (mg/L)†‡</td>
<td>1.2 (0.2, 7.1)</td>
<td>1.1 (0.2, 5.7)</td>
<td>1.3 (0.2, 8.2)</td>
</tr>
<tr>
<td>Pre</td>
<td>1.2 (0.2, 8.0)</td>
<td>0.9 (0.3, 9.9)</td>
<td>1.5 (0.2, 8.6)</td>
</tr>
<tr>
<td>Post</td>
<td>3 (−75, 1300)†‡</td>
<td>6 (−93, 900)</td>
<td>22 (−82, 580)†‡</td>
</tr>
</tbody>
</table>

Data are expressed as least square means ± standard errors unless otherwise indicated as geometric means ± standard errors (†‡) or medians (inter-quartile ranges) (‡‡). sPLA₂, secretory phospholipase A₂; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; ApoB, apolipoprotein B; ox-LDL, oxidized low-density lipoprotein.  
*P < 0.05 vs. baseline.  
†P < 0.01 vs. baseline.  
‡P < 0.001 vs. baseline.  
§P < 0.0001 vs. baseline.  
¶P < 0.05 vs. placebo.  
*P < 0.01 vs. placebo.  
**P < 0.001 vs. placebo.  
***P < 0.0001 vs. placebo.
once-daily-treated subjects ($P < 0.05$), a 6% increase in varespladib methyl 500 mg once-daily-treated subjects ($P = \text{NS}$), and a 22% increase in placebo-treated subjects ($P < 0.005$) (Table 2).

Treatment emergent adverse events that were ascribed to the study medication and resulted in study discontinuation were reported in four subjects in the varespladib methyl 250 mg treatment group (diarrhea, syncope, arthralgia with chest tightness and fatigue, and elevated C-reactive protein), and three subjects in the varespladib methyl 500 mg treatment group [diarrhea, acute coronary syndrome (ACS), and elevated C-reactive protein]. No deaths were reported.

**Discussion**

Elevated sPLA₂-IIA levels predict cardiovascular morbidity and mortality in a variety of CHD patient populations\(^{16–19}\) as well as among apparently healthy men and women.\(^{20,21}\) PLASMA II evaluated the effect of once-daily dosing of the specific sPLA₂ inhibitor varespladib methyl in patients with stable CHD. The study included patients with similar characteristics to those enrolled in the first study of varespladib methyl,\(^{13}\) and extends the understanding of the dose-related effects of varespladib methyl on lipid and lipoprotein profiles reported from that trial.

The rationale for using once-daily dosing was primarily based on patient convenience since varespladib methyl is envisioned for use in conjunction with a statin that is also given once daily.

Historically, it was demonstrated in rheumatoid arthritis and ulcerative colitis patients that varespladib methyl dosed twice daily was associated with reductions in LDL cholesterol and triglycerides (data on file; Eli Lilly and Company, Indianapolis, IN, USA). Although we did not measure plasma drug concentrations in PLASMA I, it is known that varespladib methyl dosing of 50 mg or higher twice daily results in a plasma drug concentration that is sufficient to inhibit more than 90% of the enzyme activity over a 12 h period (data on file). At higher dosages of varespladib methyl (250–500 mg twice daily), the plasma drug concentrations are several orders of magnitude above the IC\(_{50}\) for the enzyme. Indeed, plasma drug concentrations in PLASMA II confirmed our hypothesis. At the end of the dosing interval, plasma drug levels were 10- to 20-fold higher than the \textit{in vivo} IC\(_{50}\) (data not shown).

Among CHD patients in PLASMA II, treatment with varespladib methyl 250 or 500 mg administered once daily was accompanied by a dose-dependent reduction in sPLA₂ concentration, and concentrations of VLDL and LDL particles (Table 2; see Supplementary material online, Table S1). In particular, treatment with varespladib methyl 500 mg daily reduced LDL cholesterol by 15% ($P = 0.0007$). In comparison, in PLASMA I, treatment with varespladib methyl 500 mg daily given as two divided doses reduced LDL cholesterol by 8% ($P = 0.003$).\(^{13}\)

The larger magnitude of LDL cholesterol-lowering effect of varespladib methyl 500 mg once daily in PLASMA II vs. PLASMA I (placebo-subtracted difference of 14 vs. 8.5%) may derive from
enhanced LDL receptor-mediated clearance of larger-size LDL particles that are formed when sPLA2-induced LDL remodelling is inhibited. A shift in the distribution of LDL particles contributes to enhanced LDL cholesterol lowering seen in statin-treated patients who are known to have increased LDL receptor activity. In addition, sPLA2 modification of LDL particles results in conformational changes in ApoB that increases binding of these modified particles to intimai proteoglycans. Through inhibition of sPLA2, it is possible that varespladib methyl may not only facilitate LDL clearance, but it may also reduce LDL retention in the vessel wall.

Another possible explanation for the enhanced LDL cholesterol lowering of varespladib methyl in statin-treated subjects is a pharmacokinetic interaction that increases blood levels of statins. However, we have demonstrated that there are no changes in either varespladib methyl or statin levels (data on file, Anthera Pharmaceuticals).

Low-density lipoprotein particles modified by sPLA2 are more susceptible to in vitro oxidative modification and result in the formation of multiple bioactive lipids that include lysophospholipids, oxidized non-esterified fatty acids, and eicosanoids. These bioactive lipids contribute to further LDL oxidation and activation of inflammatory responses in arterial cells. However, in contrast to PLASMA I, we did not observe a statistically significant reduction in ox-LDL levels or high-sensitivity C-reactive protein in PLASMA II. This may be in part due to much lower median baseline levels of these biomarkers in PLASMA II (40.7–43.3 U/L and 1.1–1.3 mg/L, respectively) as well as reflecting the higher use of statin therapy and smaller sample size in the current trial.

Consistent with PLASMA I, we observed non-significant reductions in HDL cholesterol between varespladib methyl- and placebo-treated subjects. However, in PLASMA II, we report a reduction in HDL particle concentration, in particular the small HDL particle fraction. On the basis of the effects of sPLA2 inhibition on LDL remodeling, we might have anticipated similar directional changes in HDL particles. The significance of the dose-dependent reduction in HDL particles is unknown particularly since varespladib methyl reduced concentrations of atherogenic particles (VLDL and LDL).

In the PLASMA trials, we did not observe differences in high-sensitivity C-reactive protein levels, nor was this trial designed to investigate group differences in high-sensitivity C-reactive protein values. It is worth noting that there were large ranges in high-sensitivity C-reactive protein values in the treatment groups, and two subjects treated with varespladib methyl had high sensitivity C-reactive protein concentrations (local laboratory values) that were considered an adverse event by the local investigator. The effect of varespladib methyl 500 mg daily on high-sensitivity C-reactive protein concentrations was a primary objective of the FRANCIS (Fewer Recurrent Acute Coronary events with Near-term Cardiovascular Inflammation Suppression) trial, NCT00455546. In this trial of 24 weeks duration, varespladib methyl plus atorvastatin 80 mg daily was more effective than placebo plus atorvastatin 80 mg daily in reducing median high sensitivity C-reactive protein levels, and these changes were statistically significant at week 16 (81.6% vs. 71.8%, P = 0.002) and week 24 (79.8% vs. 77.0%, P = 0.02).

This trial investigated the effects of a novel and selective inhibitor of sPLA2 on several biomarkers in contemporary treated CHD patients. Our study also has limitations. First, serum sPLA2 activity was not measured as we previously reported in PLASMA I that the low sPLA2-IIA concentration in the samples from varespladib methyl treated subjects was below the limit required for the activity assay. When sPLA2 inhibitors bind to the active site of sPLA2, the enzyme—inhibitor complex binds tighter to membranes than does the enzyme without inhibitor. Thus, it is possible that in the presence of the inhibitor, total sPLA2 activity may be more accurately measured on whole blood samples. Secondly, we were unable to measure sPLA2-V or sPLA2-X concentration as there are no specific assays for these isoenzymes, and further, these enzymes are primarily localized in the vessel wall. Last, although the biomarkers reduced by varespladib methyl have been shown to predict atherosclerosis progression and identify CHD patients at increased risk of cardiovascular events, the effects of varespladib methyl on atherosclerosis and cardiovascular events require further study.

In summary, this study extends the understanding of varespladib methyl and demonstrates further the potential clinical utility of this pharmacology to reduce concentrations of atherogenic lipoprotein particles. On the basis of the lipid and lipoprotein changes in varespladib methyl-treated subjects, we anticipate that more individuals will achieve minimal acceptable LDL cholesterol targets. Other potential benefits of sPLA2 inhibition that cannot be addressed in this biomarker trial include suppression of vascular inflammation. From these results, we hypothesize that treatment with varespladib methyl 500 mg daily should be considered for further investigation as an anti-atherosclerotic agent in statin-treated patients. Currently, we are investigating the effects of varespladib methyl 500 mg daily on cardiovascular events in 6500 ACS patients (NCT01130246).

The safety and efficacy of varespladib methyl in reducing cardiovascular events will require evaluation in a large, prospective, double-blind, randomized clinical trial.

Supplementary material
Supplementary material is available at European Heart Journal online.

Acknowledgments
The principal investigator (R.S.R.) had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. C.H. was a member of the Steering Committee and together with the principal investigator (R.S.R.) provided feedback on study design, analysis, interpretation, and writing of the final report. Study design and conduction of the study were performed by M.E. and Y.S. Statistical analysis was performed by an independent organization (Pharm-Olam International, Houston, TX, USA). The authors would like to thank Heather Fraser for her review of the manuscript.

Funding
Anthera Pharmaceuticals provided financial and material support including the design, conduct, collection, and management of the study.
Conflicts of interest: R.S.R. has received honoraria and acted as a consultant to Anthera Pharmaceuticals for activities unrelated to the PLASMA trials. He has served as a consultant to LipoScience, Inc., and he has stock ownership in LipoScience. C.H. is an employee, and M.E. and Y.S. were employees of Anthera Pharmaceuticals and have ownership interest in Anthera Pharmaceuticals.

Appendix

The investigators who participated in the PLASMA II study are as follows: M. Imburgia, Louisville Cardiovascular Medical Group, Louisville, KY, USA; R. Weiss, Maine Research Associates, Auburn, ME, USA; P. Underwood, North Phoenix Heart Center, Phoenix, AZ, USA; D. Ende, Wisconsin Heart Center, Madison, WI, USA; C. Brown, Mobile Heart Specialists, PC, Mobile, AL, USA; V. Nadar, Heritage Cardiology Associates, Camp Hill, PA, USA; R. Carlson, Cardiology, PC, Syracuse, NY, USA; T. Carlson, Austin Heart, PA Austin, TX, USA; D. Wombolt, Clinical Research Associates of Tidewater Norfolk, VA, USA; F. Matar, Florida Cardiovascular Institute Tampa, FL, USA; P. Rossi, Pasco Cardiology Center Hudson, FL, USA; F. Eder, United Medical Associates Binghamton, NY, USA; D. Hotchkiss, Charlotte Cardiovascular Institute Port Charlotte, FL, USA.

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