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# Inhibition of Secretory Phospholipase $A_2$ in Patients with Acute Coronary Syndromes: Rationale and Design of the Vascular Inflammation Suppression to Treat Acute Coronary Syndrome for 16 Weeks (VISTA-16) Trial

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**Abstract**
*Background* The action of secretory phospholipase $A_2$ ($sPLA_2$) on lipoproteins may render them more susceptible to oxidation, thereby promoting vascular inflammation and increasing cardiovascular risk. Patients with acute coronary syndrome face a high risk of early, recurrent cardiovascular events that is associated with biomarkers of inflammation, including $sPLA_2$. The Vascular Inflammation Suppression to Treat Acute Coronary Syndrome for 16 Weeks (VISTA-16, NCT01130246) tests the hypothesis that varespladib methyl, an inhibitor of several $sPLA_2$ isoforms with a causal role in atherosclerosis, reduces cardiovascular risk among patients with acute coronary syndromes.
*Methods* Up to 6,500 patients with acute coronary syndrome will be randomized to receive treatment with varespladib methyl 500 mg daily or placebo for 16 weeks, in addition to background treatment with atorvastatin and other evidence-based therapies. The primary efficacy parameter is the combination of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke or hospitalization for unstable angina with objective evidence of myocardial ischemia. Effects of varespladib methyl on lipid and inflammatory markers, in addition to safety and tolerability, will also be evaluated.
*Conclusion* $sPLA_2$ inhibition has the potential to exert a favorable effect on the artery wall. The VISTA-16 study will determine whether varespladib methyl has a beneficial impact on cardiovascular events in patients with an acute coronary syndrome.

**Key words** Inflammation · Acute coronary syndromes · Secretory phospholipase · Risk factors

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## Introduction

Despite advances in the treatment of acute coronary syndrome (ACS) including percutaneous coronary intervention, dual anti-platelet agents, and intensive statin therapy, the residual short-term risk of adverse cardiovascular outcomes remains high, with one in five patients experiencing a recurrent

coronary event, despite the use of established medical therapies [1]. Accordingly, there is a need to develop additional therapeutic strategies to further reduce risk particularly in the ensuing period after an ACS.

## Inflammation as a target for novel cardiovascular therapies

A substantial body of evidence indicates that activation of inflammatory pathways is important in the pathogenesis and progression of atherosclerosis. Cellular and animal studies have demonstrated that inflammatory mediators participate in all stages of the disease process, from its early formation through to plaque rupture. These findings are supported by observations that elevated circulating biomarkers of inflammation, inclusive of $sPLA_2$, independently predict the likelihood of adverse cardiovascular outcomes in patients across a broad range of risk. In parallel, mounting evidence suggests that anti-inflammatory properties contribute to the clinical benefits of established medical therapies, such as statins. For example, clinical trials demonstrating the benefit of statins in both primary and secondary prevention report lower cardiovascular event rates and slower disease progression among patients achieving lower levels of both low-density lipoprotein cholesterol (LDL-C) and high sensitivity C-reactive protein (hsCRP). While these studies suggest that modulating inflammatory pathways is important, they leave open the question whether the observations are simply a consequence of lowering LDL-C levels. Accordingly, there is an ongoing search to identify therapeutic strategies that specifically target the inflammatory mediators involved in atherosclerosis.

## Phospholipases and cardiovascular disease

The phospholipases are a family of enzymes that share the ability to hydrolyze the sn-2 ester bond in phospholipids, located in both cell membranes and circulating lipoprotein particles. The action of phospholipases include generating fatty acid, arachidonic acid, prostaglandin and leukotriene metabolites with potential pro-inflammatory effects. While phospholipase $A_2$ ($PLA_2$) has been found in many species, its physiological role in humans is unknown. Lipoprotein associated and secretory phospholipase $A_2$ (Lp-$PLA_2$ and $sPLA_2$) are the forms most closely linked to cardiovascular disease [2–4].

Lp-$PLA_2$ is bound to apolipoprotein B-containing lipoproteins and localizes within animal and human atherosclerotic plaques [5–7]. Most, but not all, case–control studies have demonstrated that systemic Lp-$PLA_2$ levels independently predict cardiovascular events. Early clinical studies of a pharmacological inhibitor of Lp-$PLA_2$ have demonstrated favorable effects on the necrotic core of atherosclerotic plaques in porcine models and on intravascular imaging in clinical trials [8]. Accordingly, the impact of this therapeutic approach on cardiovascular morbidity and mortality is currently being evaluated in large-scale phase III trials [9, 10].

$sPLA_2$ is a small molecular weight (15 KDa) protein with multiple subtypes [11]. It has shown to be a factor in numerous inflammatory disease processes including rheumatoid arthritis, acute respiratory distress syndrome, pancreatitis, inflammatory bowel disease and sepsis [12–14]. $sPLA_2$ has been also been found to be involved in a variety of pathways related to the development of atherosclerosis and cardiovascular disease. In contrast to $LpPLA_2$, relatively little $sPLA_2$ is associated with LDL particles. Nonetheless, $sPLA_2$ has been similarly shown to colocalize with macrophages and smooth muscle cells found in atherosclerotic plaques [15]. The identification of $sPLA_2$ within atherosclerotic plaques complements data from in vitro, animal, and clinical studies that suggest mechanisms for the promotion of atherosclerosis by $sPLA_2$ [16]. The action of $sPLA_2$ on LDL results in smaller particles that are more susceptible to oxidative modification and to uptake by macrophages, the seminal events in foam cell formation [17]. Indeed, clinical data associate increasing levels of $sPLA_2$ with higher levels of oxidized LDL and smaller circulating LDL particles [18]. Moreover, the hydrolysis of lipoprotein phospholipid by $sPLA_2$ generates free fatty acids and lysophospholipid products that may independently promote inflammation and oxidative stress within the arterial wall [16, 19]. These observations are supported by reports that $sPLA_2$ plays an important role in animal models of atherosclerosis and ischemia-reperfusion injury.

The potential role of $sPLA_2$ in cardiovascular disease is also supported by consistent demonstration of the direct relationship between $sPLA_2$ mass or activity and prospective cardiovascular risk in a broad range of clinical settings. Case–control studies have demonstrated that $sPLA_2$ levels predict cardiovascular risk in asymptomatic subjects and patients with stable or unstable coronary heart disease. $sPLA_2$ is an acute phase reactant, with levels rising in the early period after both ACS and percutaneous coronary intervention [3, 20]. Measures of $sPLA_2$ mass and activity in these settings have been reported to predict adverse cardiovascular outcomes, independent of markers of cardiac myonecrosis. Accordingly, the role of $sPLA_2$ in both atherosclerotic plaque rupture and consequent ischemic tissue damage highlights the potential for clinical benefit of $sPLA_2$ inhibition in patients with an ACS.

### $sPLA_2$ inhibition with varespladib methyl

Given the body of evidence implicating $sPLA_2$ in the evolution of atherosclerotic disease, there has been considerable interest in development of pharmacological inhibitors as a potential therapeutic strategy to reduce cardiovascular risk. Varespladib is a pro-drug, converted to its active form via carboxylic acid hydrolysis by plasma esterases. In animal studies, administration of varespladib sodium was demonstrated to attenuate development of atherosclerotic lesions and result in plaques that contained greater fibrous cap area. A greater protective effect was observed when varespladib sodium was administered in combination with pravastatin [21]. These findings suggest a potential role in plaque stabilization, in addition to long-term anti-atherosclerotic effects.

Early phase II evaluation of varespladib in clinical trials have provided further support for ongoing development as a potential protective agent. The $sPLA_2$ Inhibition to Decrease Enzyme Release after Percutaneous Coronary Intervention (SPIDER-PCI) study randomized 144 patients undergoing elective PCI to treatment with either varespladib 500 mg or placebo twice daily, commenced 3–5 days prior to and continued for 5 days following the procedure [22]. While there was no difference in biomarker evidence of periprocedural myocardial infarction between the groups, varespladib methyl administration attenuated the post-procedural rises in $sPLA_2$ activity. Given the previous demonstration of an association between the magnitude of $sPLA_2$ increase after PCI and adverse cardiovascular outcomes [20], this study provides biomarker data suggesting a potentially favorable effect of varespladib methyl in patients undergoing revascularization.

The Phospholipase Levels and Serological Markers of Atherosclerosis (PLASMA) study compared varespladib methyl 50–500 mg twice daily with placebo for eight weeks in patients with stable coronary artery disease [23]. Varespladib methyl treatment resulted in dose-dependent increases in $sPLA_2$ inhibition by up to 96%, in association with a 10% reduction in low-density lipoprotein cholesterol (LDL-C), driven predominantly by a reduction in small, dense LDL particles. The PLASMA-2 study subsequently compared the biomarker efficacy and safety of varespladib methyl 250 mg or 500 mg daily with placebo in statin-treated patients with stable coronary artery disease. After 8 weeks of treatment, a high degree of $sPLA_2$ inhibition was observed in varespladib-treated patients, in association with 15% lower LDL-C levels, 15% lower non-HDL-C levels and 11% reduction in apoB.

The Fewer Recurrent Acute Coronary Events With Near-Term Cardiovascular Inflammation Suppression (FRANCIS) trial compared varespladib 500 mg daily or placebo, added to atorvastatin 80 mg, in 625 patients with ACS and additional high-risk characteristics. Treatment began within 96 h of hospitalization and continued for 24 weeks [24]. In the varespladib arm, greater reductions in $sPLA_2$, LDL-C and CRP were observed, resulting in a greater proportion of patients achieving the dual treatment goals of LDL-C <70 mg/dL and CRP <1 mg/L. These biomarker findings were observed without adverse safety signals. The number of patients with cardiovascular events was similar in both groups. The combination of biomarker efficacy, safety and tolerability of varespladib methyl in Phase II trials provided the impetus to evaluate its impact on cardiovascular events in a large Phase III study.

### Study design

The Vascular Inflammation Suppression to Treat Acute Coronary Syndrome for 16 Weeks (VISTA-16) is a randomized, double-blind, placebo-controlled multinational clinical trial designed to demonstrate the efficacy and safety of varespladib methyl in patients with ACS receiving standard medical therapy, including atorvastatin. The primary objective is to determine whether varespladib methyl, compared with placebo, reduces major adverse cardiac events during 16 weeks of treatment. In addition to typical symptoms, ECG changes, or elevation of troponin or CK-MB consistent with ACS, qualifying patients are required to have at least one additional high-risk characteristic including (i) diabetes, (ii) metabolic syndrome, (iii) history of stroke or transient ischemic attack, (iv) established peripheral vascular disease and/or (v) prior coronary revascularization. Key inclusion and exclusion criteria are listed in Table 1.

After providing written informed consent, up to 6,500 eligible patients will be randomized in a 1:1 ratio to treatment with varespladib methyl 500 mg daily or placebo with treatment beginning within 96 h of hospitalization for ACS and continuing for 16 weeks. Co-treatment with atorvastatin, at a minimum dose of 20 mg daily, is required. All other lipid-modifying therapies must be withdrawn prior to study drug randomization. Randomization is stratified according to the use of lipid-modifying therapy prior to hospitalization and the type of index event (unstable angina, non-ST elevation MI, or ST elevation MI). Any clinically indicated coronary revascularization in the setting of the acute coronary syndrome must occur prior to randomization. In a subgroup of patients, blood sampling for early biomarker evaluation is performed at 24, 48, 72 and 96 h following randomization. For all patients, follow-up visits occur at 1, 2, 4, 8 and 16 weeks following randomization. If LDL-C levels remain greater 100 mg/dL at the 8-week visit, investigators are instructed to intensify statin therapy. Six months following cessation of study

**Table 1** Inclusion and exclusion criteria

| Inclusion Criteria | Exclusion Criteria |
|---|---|
| Men and women ≥40 years of age | Treatment for cancer in the last 5 years |
| Written informed consent from the subject | Severe liver disease, active hepatitis, ALT/AST >3XULN, active cholecystitis, biliary obstruction with total bilirubin >2XULN |
| Diagnosis of unstable angina, NSTEMI or STEMI | Severe renal impairment (creatinine clearance <30 mL/min, creatinine >3XULN), nephrotic syndrome, or subjects undergoing dialysis |
| One additional risk factor for recurrent ischemic events | Uncontrolled diabetes mellitus (HbA1c >11%) |
| Type 2 diabetes mellitus | History of alcohol or drug abuse |
| Metabolic syndrome | Known HIV, HBV, HCV or tuberculosis infection |
| Previous stroke or transient ischemic attack | Acute bacterial, fungal or viral infection |
| Previous myocardial infarction | Concomitant use of potent cytochrome P450 inhibitors |
| Prior coronary revascularization | NYHA Class III/IV heart failure or left ventricular ejection fraction <30% |
| Established peripheral vascular disease | Moderate or severe aortic or mitral valvular disease |
| | Ventricular arrhythmias requiring chronic drug treatment or implantable cardioverter-defibrillator |
| | No stenosis >50% on coronary angiography, if known |
| | Permanent pacemaker or persistent left bundle branch block |
| | Fasting triglyceride levels of ≥400 mg/dL (4.5 mmol/L) |
| | History of statin intolerance, significant myopathy/rhabdomyolysis with any lipid- modifying drugs |
| | Subjects currently treated with the maximum labeled dose of a statin and not at LDL-C target for their level of risk as defined by NCEP ATP III |

*ALT* alanine transaminase; *AST* aspartate transaminase; *HbA1c* glycosylated hemoglobin; *HBV* hepatitis B virus; *HCV* hepatitis C virus; *HIV* human immunodeficiency virus; *LDL-C* low-density lipoprotein cholesterol; *NCEP ATP III* National Cholesterol Education Program Adult Treatment Panel III; *NSTEMI* non-ST segment elevation myocardial infarction; *NYHA* New York Heart Association; *STEMI* ST-segment elevation myocardial infarction; *ULN* upper limit of normal

drug, vital status is ascertained as an additional safety evaluation.

The primary efficacy measure is the time to first occurrence of cardiovascular death, non-fatal myocardial infarction, non-fatal stroke or documented unstable angina with objective evidence of ischemia requiring hospitalization. Secondary efficacy measures include (i) the composite of all-cause mortality, non-fatal myocardial infarction, non-fatal stroke and unstable angina requiring hospitalization, (ii) incidence of the primary endpoint in the first 60 days following randomization, (iii) all-cause mortality and (iv) incidence of each of the components of the primary endpoint. All endpoints will be adjudicated centrally by a clinical endpoint committee blinded to treatment allocation.

Changes in levels of $sPLA_2$, LDL-C, CRP and IL-6 will be evaluated. The incidence of the primary endpoint will also be evaluated in a number of pre-specified subgroups, including use of lipid modifying therapy at baseline, type of index event (STEMI, NSTEMI, unstable angina), biomarker positive ACS at the time of index event, requirement for percutaneous coronary intervention for the index event, diabetes, hypertension, metabolic syndrome, heart failure, smoking status, age (less than or greater than 65 years), gender and baseline $sPLA_2$ mass (less than or greater than median). Safety will be assessed via monitoring of adverse events and laboratory parameters.

Recruitment will continue until at least 385 primary endpoint events have occurred or are projected to occur based on evolving aggregate data, up to a maximum sample size of 6,500 subjects. The sample size was determined on the basis of an assumed primary endpoint rate of 8.5% over 16 weeks in placebo patients. A total of 2,396 patients per group would be required to provide 80% power to demonstrate a 25% reduction in events with varespladib methyl. An interim analysis will be performed when 50% of primary endpoint events are observed, with O'Brien-Fleming stopping rules employed and a nominal $p<0.001$ required for significance. If the study continues to completion, the last patient randomized subject will complete the 16-week treatment period. The final efficacy analysis will require a $p<0.0498$ to maintain overall significance.

## Summary

A substantial and growing body of evidence links $sPLA_2$ to atherosclerosis and associated cardiovascular risk, providing a strong rationale to test whether pharmacological inhibition

of $sPLA_2$ reduces cardiovascular risk. The proof-of-concept VISTA-16 trial evaluates the strategy of $sPLA_2$ inhibition with varespladib methyl in patients at highest risk for recurrent events, those with recent ACS with additional high-risk features.

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