**Introduction**

Randomized clinical trials have demonstrated that targeting established risk factors such as cholesterol and blood pressure reduce cardiovascular event rates. While this has led to a profound reduction in coronary mortality rates, there remains a substantial residual risk of clinical events [1]. This supports the need to develop more effective strategies to achieve more effective reductions in cardiovascular risk. The discovery of factors involved in the pathogenesis of cardiovascular disease provides potential targets for the development of novel cardioprotective agents.

**Role of inflammation in atherosclerosis**

Increasing evidence supports the concept that atherosclerosis is a chronic inflammatory process. Migration of leukocytes from the circulation into the artery wall represents one of the earliest features of plaque formation. Ongoing accumulation of inflammatory cells within plaque promotes formation of the mature atherosclerotic plaque and its rupture, the underlying pathological event leading to acute ischemia [2]. This is supported by reports that circulating inflammatory markers predict adverse cardiovascular outcomes in population studies [3] and that reductions in these levels are independently associated with the clinical benefit of established therapies [4, 5]. There remains considerable interest in the development of novel agents that primarily target inflammatory mediators of the disease process.

**Role of sPLA2 in atherosclerosis**

Phospholipases hydrolyze the sn-2 ester bond in phospholipids in cell membranes and circulating lipoprotein particles generating fatty acid, arachidonic acid, prostaglandin and leukotriene metabolites involved in the promotion of inflammatory pathways (see Figure 1). Five distinct groups of phospholipase A2 (PLA$_2$) enzymes have been described, with their true physiological role in humans is yet to be elucidated. The forms of secretory (sPLA$_2$) and lipoprotein-associated (Lp-
PLA$_{2}$ forms of the enzyme have been most extensively studied with regard to their potential role in atherosclerotic cardiovascular disease [6–8].

The various subtypes of sPLA$_{2}$ have numerous functional properties that activate immune and inflammatory pathways [9], with a well-documented role in a range of systemic inflammatory processes [10–12]. Increasing evidence has implicated sPLA$_{2}$ in the formation, progression and rupture of atherosclerotic plaque, including co-localization with macrophages and smooth muscle cells [13], and mechanistic studies demonstrating that sPLA$_{2}$ promotes the generation of atherogenic small, dense LDL particles and phospholipid products in the artery wall that upregulate the activity of inflammatory and oxidative pathways [14, 15]. The transgenic expression of sPLA$_{2}$ results in a greater extent of atherosclerotic plaque in mouse models [16], supporting the atherogenic properties from mechanistic studies. Beyond a potential role of sPLA$_{2}$ in the progression of atherosclerotic disease, additional reports implicate activity in ischemia–reperfusion tissue injury. The direct binding of sPLA$_{2}$ to ischemic cardiomyocytes focuses inflammatory and oxidative-induced cellular damage, providing a potential further role in the events that link atherosclerosis and its adverse clinical sequelae [17].

**Evidence implicating sPLA$_{2}$ in heart disease in humans**

Further evidence associating sPLA$_{2}$ with cardiovascular disease has been derived from numerous population studies that demonstrate a direct relationship between systemic levels of sPLA$_{2}$ mass and activity with adverse cardiovascular events. sPLA$_{2}$ levels have been demonstrated to be greater in the setting of obesity, and consistent with mechanistic studies, to correlate directly with systemic levels of small LDL particles and oxidized LDL [18]. This translates to findings from large cohort studies, spanning the full spectrum of cardiovascular risk, which demonstrate an association between elevated sPLA$_{2}$ levels and the prospective risk of cardiovascular events.

In studies of asymptomatic individuals with no overt evidence of cardiovascular disease, sPLA$_{2}$ mass predicted the subsequent likelihood of a cardiovascular event, independent of traditional risk measures [19]. The observation of independence of this relationship from measures of apolipoprotein B is in contrast to sim-
ilar associations reported for Lp-PLA$_2$, which cannot be completely dissociated from measures of atherogenic lipoproteins. Similar findings of a relationship between sPLA$_2$ levels and subsequent cardiovascular events have been reported in patients with either stable [19] or unstable [7] ischemic syndromes. In the coronary care setting, sPLA$_2$ levels have been demonstrated to increase in the first few days in association with the degree of myocardial damage [20]. Furthermore, increases in sPLA$_2$ levels have been observed following percutaneous coronary interventions, with the degree of increase associating with the subsequent risk of ischemic events due to either the progression of native atherosclerosis or the development of in-stent restenosis [21, 22].

**Early experience with sPLA$_2$ inhibition**

On the basis of preclinical studies and findings from population cohorts implicating a role for sPLA$_2$ in the pathogenesis of cardiovascular disease, there has been considerable interest in the development of pharmacological sPLA$_2$ inhibitors to reduce cardiovascular risk. Varespladib is the first sPLA$_2$ inhibitor to proceed to an advanced stage of clinical development. Initially developed as a potential therapeutic agent for asthma patients, subsequent studies focused primarily on its potential cardioprotective effects. In the form of a prodrug, varespladib is converted to its active form by plasma esterases that hydrolyze carboxylic acid. Preclinical studies in mouse models have demonstrated that varespladib had a favorable effect on both the size and composition of atherosclerotic lesions, when administered as monotherapy or in combination with a statin [23]. The potential ability to stabilize plaque, in addition to more chronic effects on the vasculature, suggested that sPLA$_2$ inhibition with varespladib might be beneficial in patients with an acute coronary syndrome.

A number of early clinical studies in patients with coronary artery disease provided further evidence to support the concept that varespladib might have a favorable effect on clinical event rates. In the sPLA$_2$ Inhibition to Decrease Enzyme Release after Percutaneous Coronary Intervention (SPIDER-PCI) study, 144 patients scheduled to undergo an elective, planned intervention were treated with either varespladib 500 mg or placebo twice daily, from 3 to 5 days before their procedure through to 5 days following successful intervention. Treatment with varespladib was associated with smaller postprocedural rises in sPLA$_2$ levels, suggesting a potential favorable effect in the setting of coronary revascularization [24].

The Phospholipase Levels and Serological Markers of Atherosclerosis (PLASMA) study evaluated the effect of treatment with varespladib 50–500 mg or placebo twice daily for 8 weeks in patients with stable coronary artery disease. Dose-dependent reductions in sPLA$_2$, associated with a 10% reduction in LDL-cholesterol, were observed with varespladib. The improvement in lipid profile was predominantly driven by a reduction in the concentration of small, dense LDL particles with the sPLA$_2$ inhibitor [25]. A follow-up study (PLASMA-2) confirmed LDL-cholesterol lowering with varespladib, when administered in statin-treated patients with established coronary artery disease, suggesting potential lipid-modifying effects in addition to its impact on inflammatory pathways [26].

These biomarker findings were subsequently investigated in patients with an acute coronary syndrome, treated with potent statin therapy. In the Fewer Recurrent Acute Coronary Events with Near-term Cardiovascular Inflammation Suppression (FRANCIS) study, patients within the first 96 hours of an acute coronary syndrome, with diabetes, metabolic syndrome or an elevated C-reactive protein (CRP) level, treated with atorvastatin 80 mg daily were treated with varespladib or placebo. A beneficial effect was observed in the varespladib group, with greater reductions in sPLA$_2$, LDL-cholesterol and CRP observed [27].

Each of these preliminary reports provided support for the concept that varespladib might have a favorable effect on cardiovascular event rates in patients at high vascular risk. The Vascular Inflammation Suppression to Treat Acute Coronary Syndrome for 16 Weeks (VISTA-16) study compared the effect of varespladib 500 mg or placebo daily for 16 weeks when administered to atorvastatin-treated patients, commenced within 96 hours of the acute event. Patients were required in addition to have: diabetes; metabolic syndrome; previous stroke or transient ischemic attack; peripheral vascular disease; or previous coronary revascularization [28]. In March 2012, the Data and Safety Monitoring Board recommended premature cessation of VISTA-16 due to futility [29]. The complete findings of the study have yet to be fully reported in the scientific literature, but will provide important information regarding the potential efficacy and safety of sPLA$_2$ inhibition in patients with coronary artery disease.
This has important implications not only for understanding the impact of varespladib on cardiovascular risk, but also for other phospholipase inhibitors. Darapladib, a pharmacological inhibitor of the lipoprotein-associated variant of the enzyme, is currently undergoing investigation in two large clinical outcome trials. Early studies suggest that darapladib may have a favorable effect on the necrotic core of atherosclerotic plaques in patients with coronary disease [30]. Whether a similar finding of no clinical benefit as observed with varespladib will be reported is unknown.

Conclusion
Despite a large body of evidence implicating a role for sPLA2 in the pathogenesis of atherosclerotic cardiovascular disease, the first pharmacological inhibitor to advance to a large clinical outcomes trial failed to show any cardiovascular benefit. Whether this reflects futility for the molecule itself or a broader failure for sPLA2 inhibition in terms of cardioprotection remains uncertain. Nevertheless, the search to identify anti-inflammatory therapies to reduce cardiovascular risk continues.

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