

On the present and future role of Lp-PLA₂ in atherosclerosis-related cardiovascular risk prediction and management

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Abstract

Circulating concentration and activity of secretory phospholipase A₂ (sPLA₂) and lipoprotein-associated phospholipase A₂ (Lp-PLA₂) have been proven as biomarkers of increased risk of atherosclerosis-related cardiovascular disease (ASCVD). Lp-PLA₂ might be part of the atherosclerotic process and may contribute to plaque destabilisation through inflammatory activity within atherosclerotic lesions. However, all attempts to translate the inhibition of phospholipase into clinically beneficial ASCVD risk reduction, including in randomised studies, by either non-specific inhibition of sPLA₂ (by varespladib) or specific Lp-PLA₂ inhibition by darapladib, unexpectedly failed. This gives us a strong imperative to continue research aimed at a better understanding of how Lp-PLA₂ and sPLA₂ regulate vascular inflammation and atherosclerotic plaque development. From the clinical viewpoint there is a need to establish and validate the existing and emerging novel anti-inflammatory therapeutic strategies to fight against ASCVD development, by using potentially better animal models and differently designed clinical trials in humans.

Key words: atherogenesis, phospholipases, biomarker, secretory phospholipases A₂, lipoprotein-associated phospholipase A₂ (Lp-PLA₂), prognosis, anti-inflammatory agents.

Introduction – the A₂ group phospholipases (PLA₂s)

The phospholipases are enzymes that hydrolyse phospholipids. They are classified into different groups by their molecular weight, their catalytic residues, and their dependence (or lack thereof) on calcium [1]. The A₂ group of phospholipases specifically hydrolyse the ester bond of the fatty acid at the sn-2 position of the glycerophospholipids and, by doing so, release both fatty acids and lysophospholipids [1, 2].

Secretory PLA₂ (sPLA₂) are calcium-dependent, low-molecular-weight enzymes that include different groups, named I-III, V, and IX-XIV. Also cytosolic PLA₂ (cPLA₂, GIV) are calcium dependent. On the other hand, calcium independent groups are GV PLA₂ (iPLA₂) and lipoprotein-associated phospholipase A₂ (Lp-PLA₂ or, as it is also called, platelet-activating fac-

tor acetylhydrolases; PAF-AH, GVII/GVIII). We also know of lysosomal PLA₂ (GXV) and adipose-specific phospholipase A₂ (AdPLA₂, GXVI) [1, 3–6].

sPLA₂ family includes 12 isoforms, and despite sharing some common features they are functionally distinct proteins with specific tissue distributions [7–9] and enzymatic properties [9]. They hydrolyse phospholipids from the surface of cell membranes, native lipoproteins, and oxidatively-modified lipoproteins to produce many different bioactive lipids that include arachidonic acid (and consequently also eicosanoids – prostaglandins, thromboxanes, leukotrienes), non-esterified fatty acids, lyso-phospholipids, lyso-platelet acting factor, and oxidised non-esterified fatty acids [3, 8]. In contrast, Lp-PLA₂ requires oxidised phospholipids as a substrate (platelet-activating factor (PAF), PAF-like substances and oxidised phospholipids) [3, 10].

Circulating concentration and enzymatic activity of secretory phospholipase A₂ (sPLA₂) and lipoprotein-associated phospholipase A₂ (Lp-PLA₂) have been evaluated as biomarkers of cardiovascular risk in populations of apparently healthy individuals, as well as in patients with established coronary heart disease (CHD) [1, 3–6].

On the role of PLA₂s in atherogenesis

Secretory phospholipase A₂ (sPLA₂) and atherosclerosis

Six isoforms of the sPLA₂ family are described to be present in atherosclerotic lesions: IIA, IID, IIE, III, V, and X, and they have been reported to have a potential causal role in atherogenesis [3, 5] (Figure 1).

Phosphatidylcholine hydrolysis by sPLA₂ results in very-low density lipoprotein (VLDL) and low density lipoprotein (LDL) particles with altered conformation of apolipoprotein B (apoB). These processes result in smaller, denser, and more electronegative lipoprotein particles [1, 3, 5] that are less avidly internalised by the hepatic apoB/E (LDL) receptor [3, 11], with a prolonged residence time in the circulation and further susceptibility to oxidation [3]. GV and GX sPLA₂ enzymes hydrolyse phosphatidylcholine on the surface of VLDL and LDL at least 20-fold more efficiently than GIIA sPLA₂ and as such have a potential to act extracellularly [1, 3, 12–14]. On the other hand, GIIA sPLA₂ shows enhanced ability to hydrolyse oxidised LDL and acts within intima and macrophages [1, 3]. Hydrolysis of phospholipids on high-density lipoprotein (HDL) results in impaired cholesterol-efflux capacity – the ability of HDL to accept cholesterol from macrophages [3, 5, 15]. The conformational changes in apoB also increase intimal proteoglycan binding [1, 3, 16–18] and therefore promote retention of these atherogenic lipoproteins and cholesterol crystal precipitation [19]. sPLA₂-mediated phospholipid hydrolysis further increases its vasoactive, chemotactic, and proinflammatory role [3, 5] because it increases oxidative stress through generation of arachidonic acid (including eicosanoids), lyso-phospholipids, and non-esterified fatty acids [3, 5, 20].

Lipoprotein-associated phospholipase A₂ and atherosclerosis

Lp-PLA₂ is secreted primarily by macrophages and by some other inflammatory and non-inflam-

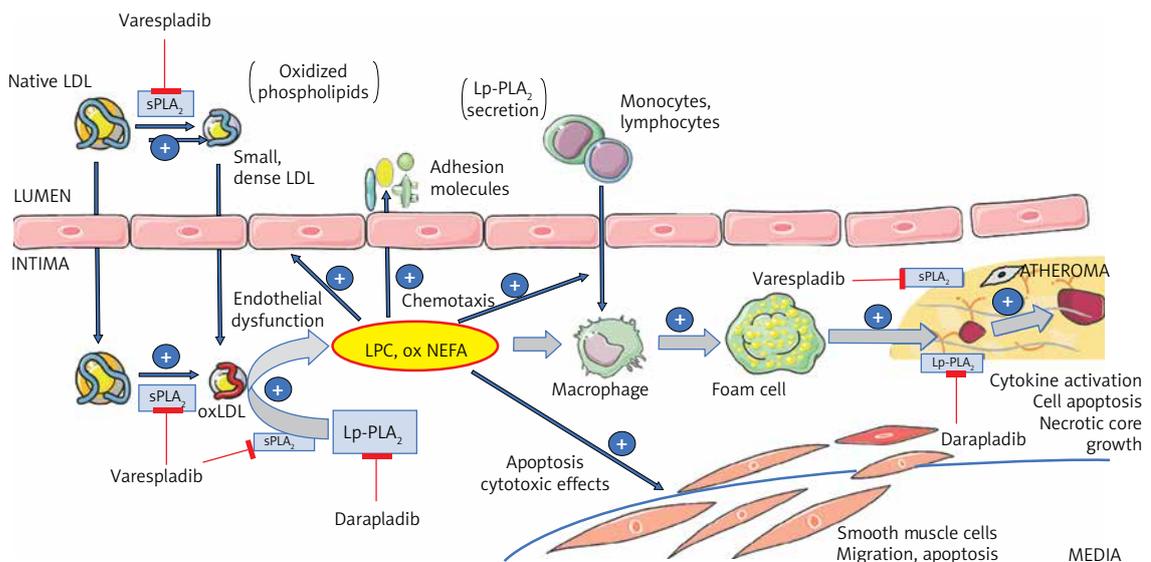


Figure 1. Schematic presentation of the roles of sPLA₂ and Lp-PLA₂ in atherogenesis, as well as the potential sites for therapeutic inhibition, by using either sPLA₂ non-specific (e.g. varespladib), or a specific Lp-PLA₂ inhibitor (darapladib)

sPLA₂ – secretory phospholipase A₂, Lp-PLA₂ – lipoprotein-associated phospholipase A₂, LPC – lysophosphatidylcholine, oxNEFA – oxidised non-esterified fatty acids, oxLDL – oxidised low-density lipoprotein particle.

matory cells involved in atherogenesis [21] (Figure 1). In plasma, Lp-PLA₂ circulates in active form as a complex with LDL (80–85%), HDL (15–20%), and, to a lesser extent, Lp(a) [10, 21].

Endothelial dysfunction is a well-established vascular response to cardiovascular risk factors, which precedes the development of atherosclerosis and is involved in the promotion of both the early and late mechanisms of progression [22]. It is characterised by the expression of more adhesion molecules and increased endothelial permeability; hence, LDL particles can transmigrate more easily to arterial intima [10, 22]. Because of the reduced content of antioxidants in arterial intima LDL particles are exposed to further oxidation. Consequently, the Lp-PLA₂ is activated by an abundance of oxidised phospholipids present in OxLDL [10]. As such, Lp-PLA₂-driven hydrolysis of the oxidized phospholipids to some extent confines the modifications of OxLDL and may be interpreted as a protective function [2, 10]. Some studies have provided evidence that loss-of-function (V279F) mutations possibly increase the incidence and severity of cardiovascular conditions compared with non-carriers [2, 4]. However, most of the studies support the concept that Lp-PLA₂ contributes to the development and progression of atherosclerosis. The underlying explanation is that, following the process of Lp-PLA₂-enhanced phospholipid hydrolysis, high contents of oxidised non-esterified fatty acids (OxNEFA) and lysophospholipids are produced, which promote expression of adhesion molecules, stimulate cytokines production (TNF- α , IL-6), and attract macrophages to the arterial intima [3, 10]. A deleterious feed-forward mechanism may also be associated because recruitment of additional inflammatory cells in activated plaques may result in further Lp-PLA₂ production and activity [2]. Activated macrophages and foam cells produce even more Lp-PLA₂ [23]. The presence of OxLDL, as well as lysophospholipids and OxNEFA produced by Lp-PLA₂, stimulate the growth of the plaque and eventually lead to the formation of a necrotic core and can be decisive regarding plaque rupture susceptibility that can culminate in a cardiovascular event [10]. High levels of Lp-PLA₂ and lysophospholipids are found in thin-cap fibroatheromas and ruptured plaques but are almost absent in stable lesions [2, 24].

Circulating Lp-PLA₂ determination

To detect Lp-PLA₂ in blood we can measure either its mass concentration (ng/ml) or its enzymatic activity (nmol/min/ml). Lp-PLA₂ mass concentration measured by the standard ELISA immunoassays has been proven to be less accurate than spectrophotometric assay of enzymatic activity assessment for risk stratification, presum-

ably because it only detects a reduced percentage of total Lp-PLA₂ that is not in interaction with lipoprotein [25, 26]. The Food and Drug Administration (FDA) approved the PLAC[®] Test for measuring the Lp-PLA₂ mass concentration (2003) and PLAC[®] Test Activity (2014) for enzymatic activity in order to improve diagnostics and prediction of ASCVD in clinical practice [26, 27]. The performance of the PLAC[®] Test is superior to other alternative commercially available tests [27]. It is a standard indirect ELISA immunoassay that uses two monoclonal antibodies: a primary antibody to bind to Lp-PLA₂ enzyme from the blood sample and an enzyme-conjugated secondary antibody to detect it. The PLAC[®] Test Activity uses the Lp-PLA₂ to hydrolyse the sn-2 position of the substrate, 1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine, producing a coloured reaction product 4-nitrophenol. The rate of 4-nitrophenol production is monitored spectrophotometrically, and the Lp-PLA₂ activity is calculated by the rate of change of absorbance.

To be clinically useful, a biomarker must have a precisely established decision value. AACE/ACE Guidelines for Management of Dyslipidaemia and Prevention of Cardiovascular Disease (2017) state that an Lp-PLA₂ mass concentration less than 200 ng/ml is normal, ≥ 200 and < 223 ng/ml is intermediate, and ≥ 223 ng/ml is high [28]. Lp-PLA₂ mass concentration of 400 ng/ml does not seem to impart much more risk than 250 ng/ml [23]. The manufacturer of the PLAC[®] Test Activity declares an enzyme activity cut-off point at 225 nmol/min/ml that identifies high cardiovascular risk patients.

Lp-PLA₂ and cardiovascular risk

Epidemiological studies

Lp-PLA₂ has continuously been confirmed as a cardiovascular risk marker independent of and additive to traditional risk factors [28–30]. The presence and activity of Lp-PLA₂ within a plaque was associated with vulnerable, rupture-prone plaques [31–33], and it appears that Lp-PLA₂ is released from these plaques into the circulation [34, 35]. Contrary to hs-CRP, Lp-PLA₂ is a specific vascular inflammation marker [35, 36] that potentially fills an important vacancy of having a non-invasive marker indicating the existence of atherosclerotic plaques prone to rupture. When both Lp-PLA₂ and hs-CRP are increased together, they provide an even greater predictive capability [28, 37–39].

When adjusting for cardiac, inflammatory, and renal function biomarkers, the independent associations between Lp-PLA₂ activity in stable patients at high risk for, or with established CHD remained a significant marker of increased risk

for MI, ischaemic stroke, and other composite cardiovascular events [21, 23, 40], hospitalisation for heart failure, and cardiovascular and total mortality, whereas the associations with recurrent ischaemic events such as MI and stroke were attenuated [21, 23, 40]. 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 [21, 23, 40, 41]. Many studies support Lp-PLA₂ activity as a risk indicator of adverse outcomes both in patients with stable CHD and in the general population [6, 40, 42–51]. Although most of the studies confirm that Lp-PLA₂ activity is significant in ASCVD risk assessment in low-risk populations, the magnitude of this improvement is relatively modest in comparison to improvement in the moderate or higher-risk population [23].

Lp-PLA₂ should be considered as a clinically useful cardiovascular risk marker that in conjunction to other major risk factors and markers improves the identification of individuals whose ASCVD risk is greater than clinically apparent. Therefore, we are potentially able to adjust absolute ASCVD risk status and modify the intensity of risk-reducing interventions [23]. The low biologic fluctuation and high vascular specificity of Lp-PLA₂ makes it possible to use a single measurement in clinical decision making, and it also permits clinicians to follow the Lp-PLA₂ marker serially [23].

Genetic studies

The Lp-PLA₂ mass concentration or activity are heritable traits [52]. The gene encoding Lp-PLA₂ (PLA2G7), (6p12-21.1) is organised in 12 exons and encodes 441 amino acids [2]. Over the years several polymorphisms of PLA2G7 have been identified. On the foundation that elevated Lp-PLA₂ levels were correlated with certain diseases, including atherosclerosis, most studies so far have focused on gene variants that weaken Lp-PLA₂ mass concentration or activity (loss-of-function alleles) [2].

Some of the most commonly researched polymorphisms of PLA2G7 gene are Val279Phe mutation (rs16874954/rs76863441), Val379Ala mutation (rs1051931), Arg92His mutation (rs1805017), and Ile198Thr mutation (rs1805018) [2, 53, 54]. A recent study also identified significant association of rs13218408 mutation with the level of Lp-PLA₂ mass concentration and activity, which also has a significant joint effect with widely validated coding polymorphism rs16874954 on the level of Lp-PLA₂ [54]. Huang *et al.* reported that the heterozygous carriers of the rs16874954 minor allele had a significant reduction of 32.4% in Lp-PLA₂ activity and 34.4% in mass concentration, and

almost no detectable enzyme activity and mass were found in the homozygous carriers [54]. Similarly, Gregson *et al.* showed the reduction of Lp-PLA₂ activity by 45% for every rs16874954 minor allele inherited [53].

The Genome-Wide Association Study of Lp-PLA₂ Activity and Mass in the Framingham Heart Study identified four distinct gene regions showing highly significant associations with Lp-PLA₂ activity, all of which are known to include genes involved in cholesterol metabolism – APOE/APOC1 region on chromosome 19, CELSR2/PSRC1 on chromosome 1, SCARB1 on chromosome 12, and ZNF259/BUD13 in the APOA5/APOA1 gene region on chromosome 11 [52]. All of these loci remained significantly associated with Lp-PLA₂ activity after accounting for their association with serum lipid and lipoprotein levels [52].

Many genetic studies have established that PLA2G7 variants that reduce Lp-PLA₂ activity to levels comparable to darapladib have no effect on the risk of CHD and outcomes. These results oppose, to a certain extent, a causal role of Lp-PLA₂ during atherosclerotic progression [2, 53, 55, 56].

Reflections on evidence-based ASCVD prevention guidelines

Different studies have shown that circulating Lp-PLA₂ activity levels could be an index of systemic inflammation and serve as an independent risk factor for CAD [57, 58]. A meta-analysis that included all prospective studies conducted on Lp-PLA₂ showed a relationship between Lp-PLA₂ activity and mass and incidence of CAD, stroke, and cardiovascular mortality [6]. This evidence means that nearly a decade ago the guidelines of major international societies, including the European Society of Cardiology, the American College of Cardiology, the American Heart Association, and the American Society of Endocrinology, included Lp-PLA₂ activity measurement among the biomarkers deemed useful for ASCVD risk stratification of asymptomatic adult patients (in most cases by Class IIb recommendation). The use of this marker was declared to be particularly advantageous as part of a refined risk assessment in patients at moderate cardiovascular risk (> 2 risk factors) and in those at high-risk of a recurrent acute atherothrombotic event in whom an increase of Lp-PLA₂ activity levels was supposed to guide the lipid-lowering treatment to reach LDL-cholesterol levels lower than the primarily recommended target [59].

The updated, contemporary guidelines abandoned such recommendations because there is a lack of studies that examine the degree to which Lp-PLA₂ improves on existing traditional risk prediction models in terms of clinically important

magnitudes of reclassification, which can then be translated into differences in treatment approach that would potentially improve patient outcomes [60–63]. This could in principle be demonstrated with clinical trials, but the expected difference in outcomes would probably be so small that the sample size of the trial would be impractically large. Decision modelling could be used as another approach to estimate differences in patient outcomes due to improved reclassification of risk [64, 65]. A robust validated model using Lp-PLA₂ levels to predict CHD outcomes is necessary in order to use the test to manage patients. No studies identified whether a testing strategy that uses Lp-PLA₂ levels improves health outcomes. Although Lp-PLA₂ is associated with ASCVD risk, changes in patient management that would occur as a result of obtaining its levels in practice are not well defined. Lp-PLA₂ decreased substantially after treatment with different lipid lowering medications, including statins, ezetimibe, fibrates, and omega-3-fatty acids [66–70]. However, in treated patients, Lp-PLA₂ levels may no longer be associated with risk of CAD, and thus may not be useful as a measure of treatment response.

Therapeutic modalities specifically for reduction of sPLA₂/Lp-PLA₂ that have been tested

In addition to numerous laboratory observations as well as animal studies, which confirmed the role of sPLA₂ in the inflammatory milieu of atherosclerosis, clinical research also supported its value as a prognostic marker among patients with (both stable and unstable) ASCVD. Significantly higher sPLA₂ levels were observed among patients immediately after undergoing PCI, and elevated sPLA₂ was shown as an independent predictor of recurrent events in the longer term [71]. Increased sPLA₂ was highly predictive of recurrent CV events, revascularisation, as well as death also in patients with acute coronary syndromes (unstable AP, NSTEMI, and STEMI) [72, 73].

On this basis, sPLA₂ was identified as an attractive therapeutic target to improve cardiovascular outcomes (Figure 1). By using varespladib methyl, a non-specific inhibitor of sPLA₂ activity (inhibiting sPLA₂-GIIA, GV and GX), in phase 2 testing trials, the sPLA₂ levels were decreased by approximately 80% in a dose-dependent manner, in patients with stable CHD, as well as in those with acute coronary syndrome (ACS) (Table I), while there were no differences in rates of major adverse CV events between the actively treated and placebo groups [74–76]. An interventional study on patients with ACS followed (VISTA-16 (Vascular Inflammation Suppression to Treat Acute Coronary Syndrome

for 16 weeks)), in which, despite lowering plasma levels of sPLA₂-IIA by 78%, as well as LDL-C and hs-CRP, varespladib surprisingly failed to express its clinical efficacy; even more, the composite primary outcome (CV mortality, nonfatal MI and stroke, and unstable AP) increased by 25% [77]. At least partly this failure can be explained from the perspective of the so-called sPLA₂ pan-inhibition, which influences not only its atherogenic, but also its athero-protective roles (e.g. of the sPLA₂-GX) [78].

As described above, Lp-PLA₂ also mediates the formation of some important bioactive mediators (lysophosphatidylcholine and oxNEFA), known to be proinflammatory and directly implicated into the ASCVD process. Lysophosphatidylcholine serves as a potent chemoattractant for monocytes, resulting in foam cell accumulation within the arterial wall, and Lp-PLA₂ was shown to be highly expressed in the necrotic cores as well as thin-cap fibroatheromas and ruptured plaques. These data suggest that inhibition of Lp-PLA₂ may stabilise atherosclerotic plaque and is potentially beneficial in patients with both stable and unstable ASCVD. Using darapladib, a direct selective inhibitor of Lp-PLA₂, its activity can be reduced by over 60% [79] (Table I, Figure 1). In preclinical studies on animal models it was indeed shown that besides decreasing Lp-PLA₂ in plasma, darapladib also inhibits the development of atherosclerotic lesions and reduces the macrophage content and the necrotic core area in plaques [80]. In an early clinical study, IBIS-2 (Integrated Biomarker and Imaging Study 2), a significant difference was found in the reduction of the atheroma necrotic core volume in comparison to the group treated with placebo [79]. Highly surprisingly and unexpectedly, in the clinical intervention trials which followed, both in the STABILITY (Stabilisation of Atherosclerotic Plaque by Initiation of Darapladib Therapy), studying patients with chronic stable CAD, and SOLID-TIMI 52 (Stabilisation Of plaques using Darapladib-Thrombolysis In Myocardial Infarction 52), encompassing patients with either NSTEMI or STEMI within 30 days after the event, no significant beneficial clinical effects (on incidence rates of death, MI, stroke, or urgent revascularisation) were demonstrated by using selective inhibition of Lp-PLA₂ with darapladib [41, 81]. The effect of Lp-PLA₂ inhibition on arterial wall inflammation in carotid arterial disease was studied using rilapladib. Analyses performed on the basis of MRI/PET imaging of the carotid plaques failed to demonstrate a significant effect of short-term rilapladib (compared with that of placebo) in individuals with stable atherosclerotic disease concurrently on statin therapy [82]. It is worth noting that in plaque-based analysis, the nominal reduction from baseline seen in the rilapladib group

(ca. 3% to 6%) approaches that typically observed with low-dose statins [83].

The failure of translation of specific (in case of Lp-PLA₂) or nonspecific (in case of sPLA₂) inhibition into the clinically effective therapeutic modality can be discussed using several possible explanations. First of all, and probably the most reliable one, is that it is in principle not necessary that there is a true causal relationship between

the biomolecule identified as a biomarker of increased ASCVD risk and the clinical endpoints. Because it is not necessary for the targeted biomarker to actually be lying in the pathway from disease to clinical endpoint, the elevated plasma levels of phospholipases may not represent the optimal surrogates for clinical events despite being proven as clearly correlated with advanced atherosclerotic lesions [84]. Because there are

Table I. Summary presentation of clinical studies (phases II and III) using either non-specific sPLA₂ inhibitor varespladib or specific Lp-PLA₂ inhibitor darapladib, in various clinical settings

Variable	Patients	Duration	Effect(s)	Reference
sPLA ₂ inhibition with varespladib methyl:				
Phase II clinical trials:				
PLASMA	N = 396; stable CHD	8 weeks	↓ of sPLA ₂ -GIIA by 69–96%	Rosenson RS, <i>et al.</i> (2009, 2011) [74]
PLASMA II	N = 135; stable CHD	8 weeks	↓ of sPLA ₂ -GIIA by 73–84%	Rosenson RS, <i>et al.</i> (2011) [75]
FRANCIS	N = 625; up to 96 hrs after the ACS	24 weeks	↓ of sPLA ₂ -GIIA by 82.4%; ↓ of hs-CRP and LDL-C; ↓ of major adverse CVD events (NS)	Rosenson RS, <i>et al.</i> (2010) [76]
Rosenson RS, <i>et al.</i>	N = 624; pts with ACS, comparison of pts with and without DM	8 weeks	↓ of sPLA ₂ -GIIA by 83.6% in DM pts, and by 82.4% in nonDM	Rosenson RS, <i>et al.</i> (2011) [86]
SPIDER-PCI	N = 144; stable CHD, before & after PCI	3–5 days before & 5 days after PCI	↓ of sPLA ₂ -GIIA by up to 95%	Dzavik V, <i>et al.</i> (2010) [87]
Phase III clinical trials:				
VISTA-16	N = 5,145; recent ACS	16 weeks, survival at 6 months	↑ of 1° outcome (CV death, non-fatal MI, UAP) – HR 1.25; ↓ of sPLA ₂ by 78%, ↓ of hs-CRP and LDL-C	Nicholls SJ, <i>et al.</i> (2014) [77]
Lp-PLA ₂ inhibition with darapladib:				
Phase II clinical trials:				
Johnson A, <i>et al.</i>	N = 59; pts before elect. endarterectomy	2 weeks	↓ of Lp-PLA ₂ by 80%	Johnson A, <i>et al.</i> (2004) [88]
Mohler ER, <i>et al.</i>	N = 959; stable CHD	12 weeks	↓ of Lp-PLA ₂ by 43–66%; no significant effect on plasma lipids or hsCRP; ↓ of IL-6	Mohler ER, <i>et al.</i> (2008) [89]
IBIS-2	N = 330; pts with documented coronary disease, after PCI	12 months	↓ of Lp-PLA ₂ by 59%, ↓ plaque necrotic core volume; no change of atheroma deformability	Serruys PW, <i>et al.</i> (2008) [79]
Phase III clinical trials:				
STABILITY	N = 15,828; stable CHD	3.7 years	No difference in 1° outcome (CV death, MI, stroke) – HR 0.94; ↓ of total and major coronary events	White HD, <i>et al.</i> (2014) [81]
SOLID-TIMI 52	N = 13,026; up to 30 days after MI	2.5 years	No difference in 1° outcome (CHD death, MI, urg.revasc.) – HR 0.99; ↓ of Lp-PLA ₂ by 65%	O'Donoghue ML, <i>et al.</i> (2014) [41]

CHD – coronary heart disease, ACS – acute coronary syndrome, UAP – unstable angina pectoris, MI – myocardial infarction, PCI – percutaneous coronary intervention, LDL-C – low-density lipoprotein cholesterol, hs-CRP – high-sensitivity C-reactive protein, DM – diabetes mellitus.

multiple pathways involved in the inflammatory atherosclerotic disease process, affecting only one of them could not be sufficient to translate clinically. In addition, in both the STABILITY and SOLID-TIMI 52 trials the great majority of patients (95% and 94%, respectively; despite only 6.2–7.4% of patients being on high-intensity statins) were already treated with statins, already shown as potent multifaceted anti-inflammatory agents. Second, the estimation of the effect of the treatment could be over- or underestimated due to the fact that out of many existing atherogenesis-related inflammatory molecules and pathways, targeting only one surrogate cannot exclude the possibility that the others remain active in their influence on the clinical outcome of interest. If the targeted surrogate lies in several pathways, and its treatment may affect them differentially, it may be beneficial on one, but with undesirable or harmful (or unpredicted) consequences on the other. Third, medications targeted at one pathway or molecule may also have substantial off-target effects, e.g. as in the case of statins, which besides their potent LDL-C lowering effects may also have multiple beneficial effects on inflammation (by reducing oxidised LDL, macrophages, and T cells in atherosclerotic plaques as well as levels of hs-CRP and adhesion molecules, such as E-selectin, P-selectin, and ICAM-1) [85]. Direct targeting of any of these pathways individually would probably have been less successful.

Concluding remarks – in summary

Circulating concentrations and enzymatic activities of both sPLA₂ and Lp-PLA₂ have been proven as biomarkers of increased risk of ASCVD, independently and in addition to traditional risk factors. In the recent past, many international guidelines have supported the measurement of Lp-PLA₂ in order to refine the overall cardiovascular risk assessment in subjects classified as being at intermediate or high ASCVD risk. Such recommendations were recently abandoned, due to the presumed limited supplementary value in absolute ASCVD risk determination, not unrelated also to the almost unknown cost-effectiveness.

Bearing in mind the abundance of data demonstrating the predictive value of Lp-PLA₂, as well as with contemporary orientation towards a more personalised approach, it could still be of use as one of the valid prognostic markers playing a role in everyday clinical practice. Probably the most appropriate groups of patients in whom its measurement is warranted, and for whom inclusion into an overall risk assessment/stratification could prove itself to be beneficial, are patients with metabolic syndrome, diabetes mellitus, and those with already manifested ASCVD. As for the

latter, the results of one of the earliest statin trials, LIPID (Long-term Intervention with Pravastatin in Ischaemic Disease), have to be highlighted, which showed not only the persistence of the remarkably high prognostic value of reduced Lp-PLA₂ during treatment even after adjustment for more than 20 risk factors at inclusion, but a predictive value at least equal to or higher than the decrease in LDL-C [50].

From the clinical viewpoint there is a need to establish and validate the existing and emerging novel anti-inflammatory therapeutic strategies to treat ASCVD. This can be achieved not only by the use of improved animal models in preclinical research, which would better resemble the whole complexity of the phospholipase involvement in the ASCVD pathogenesis, but also with somewhat differently designed future clinical trials in humans.

Failure of translation of the inhibition of phospholipases into clinically beneficial risk reduction in some of the randomised clinical studies so far somehow challenged also the pathogenetic role of sPLA₂/Lp-PLA₂ in ASCVD development and plaque destabilisation. However, it gives us a strong imperative to continue research aimed at better understanding how Lp-PLA₂ and sPLA₂ regulate vascular inflammation and atherosclerotic plaque development. It seems plausible to point towards some of the major intrinsic limitations of the completed phase III clinical trials. It is important to note that in all of them almost the entire study population were taking statins, which are known to decrease Lp-PLA₂ by up to 35%, and to point out the relatively high reported rate of drug discontinuation (up to 20% in both SOLID TIMI 52 and STABILITY trials) [41, 81]. Besides the true potential for the improvement of the overall compliance with the existing drugs calling at repeatedly, but without significant success, it could be that there is a room for another, possibly better tolerated Lp-PLA₂ inhibitor.

The five most important challenges for further research related to sPLA₂/Lp-PLA₂

1. Better understanding of the complex role of sPLA₂/Lp-PLA₂ in atherogenesis
2. Improved animal models to be used in pre-clinical phase studies
3. More precise quantification of the additive value of sPLA₂/Lp-PLA₂ in absolute ASCVD risk assessment
4. Cost-effectiveness studies related to the use of sPLA₂/Lp-PLA₂ both as a prognostic biomarker and/or as a therapeutic target
5. Better designed clinical trials with the use of either existing or newly developed sPLA₂/Lp-PLA₂ inhibitors

Conflict of interest

ZF reports grants, has given talks, acted as a consultant of Amgen, Astra Zeneca, Bayer, Boehringer Ingelheim, Krka Pharma, Novo Nordisk, Pfizer, Sanofi, and Servier, all outside the submitted work. JT – none. MB has received research grant(s)/support from Amgen, Mylan, Sanofi and Valeant, and has served as a consultant for Akcea, Amgen, Daiichi-Sankyo, Freia Pharmaceuticals, KRKA, MSD, Mylan, Novartis, Polfarmex, Polpharma, Sanofi-Aventis, Servier, Esperion, and Resverlogix.

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