ESC European Society of Cardiology

European Heart Journal (2023) **00**, 1–3
https://doi.org/10.1093/eurheartj/ehad294

CardioPulse

Global Spotlights

Reducing reductionism: addressing risk for atherosclerotic cardiovascular disease by apolipoprotein proteomics

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For decades, strategies aimed at predicting and preventing atherosclerotic cardiovascular disease (ASCVD) have rightly focused on low-density lipoprotein cholesterol (LDL-C) due to its significant role in the development and progression of ASCVD. Despite considerable advances in lowering LDL-C levels for the prevention of ASCVD, studies such as DA VINCI[1] have highlighted discrepancies between LDL-C targets and clinical outcomes. While the LDL-C-related paradigm 'the lower, the better' remains undisputed at levels even as low as 1.25 mmol/L (50 mg/dL), the question is as follows: when will we reach the limits of what lowering LDL-C can achieve in the prevention of ASCVD? The utilization of combination therapies involving statins plus bempedoic acid, ezetimibe, or proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors is increasing but remains low,[1] and effects on cardiovascular mortality have been difficult to demonstrate in the short time frame of the randomized periods of the trials. For example, a recent meta-analysis of 14 trials concluded that ezetimibe or PCSK9 inhibitors may reduce non-fatal myocardial infarction and stroke in adults at very high or high cardiovascular risk who are receiving maximally tolerated statin therapy or are statin-intolerant but not in those with moderate and low cardiovascular risk.[2] Despite the ability to lower LDL-C to very low levels, a substantial residual risk remains in patients at very high risk for cardiovascular events. Therefore, it is necessary to explore new ways to capture this residual risk in ASCVD, which may arise from lipid-related factors beyond LDL-C as well as an increased inflammatory burden.

In addition to small molecules, new classes of LDL-C-lowering drugs are now on the market and offer less frequent administration.[3] Initial attempts to inhibit apolipoprotein B (ApoB)-100 synthesis with the antisense oligonucleotide (ASO) mipomersen, or ApoB lipidation with the microsomal triglyceride transfer protein (MTTP) inhibitor lomitapide, were limited by hepatic liver enzyme elevation and steatosis that may have been on target due to the liver not being able to transport triglycerides. The identification of PCSK9 as an integral protein in down-regulating hepatocyte LDL receptor density has allowed the novel approach of using PCSK9 monoclonal antibodies, such as alirocumab and evolocumab, to reduce LDL-C levels by up to 60% on top of existing statin therapy.[3] These drugs were approved by the European Medicines Agency (EMA) in 2015 and require injections every 2–4 weeks. In a further advance, inclisiran, a small interfering ribonucleic acid (siRNA), received approval from the EMA in 2020 for the treatment of dyslipidaemias including hypercholesterolaemia and has a bi-annual dosing regimen. Inclisiran reduces LDL-C by 50% in patients who are on statin therapy.[3]

In addition to targeting LDL-C, ApoB-100, and PCSK9, recent human genetic studies have highlighted the contribution of other ApoB-containing lipoproteins, such as lipoprotein(a) [Lp(a)] and triglyceride-rich lipoproteins, to a higher risk for ASCVD.[4,5] As a result, drugs with novel mechanisms have been developed to address this residual lipid risk (*Figure 1*). For example, pelacarsen is an ASO that reduces the hepatic synthesis of apolipoprotein(a), the pathognomonic apolipoprotein of Lp(a). Similarly, siRNAs, such as olpasiran and SLN360,[6] also target apolipoprotein(a). Two Phase 3 trials are underway to test if Lp(a) lowering can reduce ASCVD [Lp(a) HORIZON and OCEAN(a)]. Volanesorsen and olezarsen are ASOs that target *APOC3* mRNA, an inhibitor of lipoprotein lipase (LPL). Lipoprotein lipase is a key enzyme of triglyceride metabolism, and ApoC3 delays the clearance of triglyceride-rich lipoproteins. Volanesorsen was recently shown to significantly reduce hepatic steatosis in three randomized trials.[7] Other modulators of LPL include angiopoietin-related proteins 3 and 4 and ApoA5.[3] ApoL1 is another apolipoprotein that is being targeted with ASOs for applications in patients with kidney disease.[8]

As lipid-lowering options for ASCVD continue to expand, conventional lipid measurements may not provide sufficient information to identify the patients who would benefit most from these new

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Lipoprotein	Related apolipoprotein	Novel apolipoprotein-lowering medicine	Drug type
Lp(a)	Apo(a)	Olpasiran (phase 3)	siRNA
		Pelacarsen (phase 3)	ASO
		SLN360 (phase 2)	siRNA
LDL	ApoB	Mipomersen* (FDA 2013)	ASO
VLDL	ApoC1 ApoC2 ApoC3	ARO-APOC3 (phase 3)	siRNA
		Olezarsen (phase 3)	ASO
		Volanesorsen (EMA 2019)	ASO
	ApoE	ApoE ε4 ASO (preclinical)	ASO
HDL	ApoA1 ApoA2 ApoA4 ApoD ApoH ApoL1 ApoM	ION532/AZD2373 (phase 1)	ASO

Figure 1 Overview of novel apolipoprotein-lowering medicines already approved or under development. *Mipomersen was withdrawn from the market in 2019. Apo, apolipoprotein; ASO, antisense oligonucleotide; EMA, European Medicines Agency; FDA, Food and Drug Administration; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Lp(a), lipoprotein(a); RISC, ribonucleic acid-induced silencing complex; siRNA, small interfering ribonucleic acid; VLDL, very low-density lipoprotein. Created with BioRender.com.

treatments. Measuring a wider panel of apolipoproteins may offer a more comprehensive understanding of ASCVD risk and may enable personalized treatment decisions. Recent studies have shown that assessing individual apolipoprotein levels, in addition to conventional lipid measurements, can provide information on residual lipid risk for ASCVD that might otherwise be overlooked. In the PROCARDIS (Precocious Coronary Artery Disease) study and the community-based Bruneck study, levels of 13 apolipoproteins were measured simultaneously in a single mass spectrometry (MS) assay.[9] Mass spectrometry allows for the determination of multiple apolipoproteins at once compared with conventional immunoassays. In agreement with the results in the prospective Bruneck study, the levels of various apolipoproteins, including Apo(a), ApoB, ApoC1, ApoC3, and ApoE, were shown to associate with coronary heart disease in the PROCARDIS case–control study. High-density lipoprotein (HDL)-related ApoL1 was also positively associated with coronary heart disease, whereas ApoA4 and ApoM were inversely associated with coronary heart disease. The associations between apolipoproteins and ASCVD in PROCARDIS were remarkably concordant with the findings from the Bruneck prospective study, except for ApoM.

With the development of novel lipid-modifying drugs that directly target various apolipoproteins, monitoring apolipoprotein levels is becoming increasingly important to assess treatment effects. For example, MS measurements of apolipoproteins revealed that volanesorsen reduced not only its immediate target ApoC3 but also ApoC2 and ApoE.[10] Therefore, it is advisable to expand our oversimplified view of lipoprotein particles based on lipid content and recognize the intricate and dynamic nature of lipoprotein metabolism by also assessing apolipoprotein levels. Advances in MS technology enable a more nuanced understanding of lipid metabolism. The current classification of lipoproteins into discrete subfractions is historically based on ultracentrifugation and has its limitations. It does not capture the dynamic nature of lipoprotein metabolism and the complexity of lipoprotein particle composition, resulting in a reductionist approach to lipid metabolism and a potential underestimation of the risk associated with certain lipoprotein subfractions. To gain a better understanding of residual lipid risk, future research could focus on exploring the distribution of apolipoproteins across lipoprotein particles, investigating the interactions of apolipoproteins with other accessory proteins and with each other, and examining their exchange processes between lipoprotein particles. These insights could provide a more comprehensive picture of lipoprotein metabolism and inform the development of personalized approaches to managing ASCVD risk, moving healthcare professionals closer towards personalized treatment for their patients.

Pre-registered clinical trial number

None supplied.

Ethical approval

Ethical approval was not required.

Data availability

No data were generated or analysed for this manuscript.

Conflict of interest

M.M. reports support for attending meetings and/or travel from Biognosys. W.S. reports support for attending meetings and/or travel from Amgen and consulting fees and payment or honoraria for lectures, presentations, speaker bureaus, manuscript writing, or educational events from Amgen, Daiichi, Novartis, and Sanofi. S.T. reports personal fees from Ionis Pharmaceuticals, other from Oxitope Inc. and Kleanthi Dx, and research grants from Novartis and NHLBI, outside the submitted work. In addition, S.T. has a patent 'Methods of identifying a subject having or at risk of having or developing coronary artery disease' with royalties paid to Kleanthi Diagnostics, a patent 'Methods for assessing atherogenesis by determining oxidized phospholipid to apolipoprotein B ratios' with royalties paid to Kleanthi Diagnostics, a patent 'Methods for assessing atherogenesis by determining oxidized phospholipid to apolipoprotein B ratios' with royalties paid to Kleanthi Diagnostics, and a patent 'Oxidative biomarkers in predicting risk of stroke, transient ischemic attack (TIA) and peripheral arterial disease (PAD)' with royalties paid to Kleanthi Diagnostics.

Funding

M.M. is a British Heart Foundation (BHF) Chair Holder (CH/16/3/32406) with BHF Programme (RG/F/21/110053) Grant support. M.M. also received support from the VASCage—Research Centre on Clinical Stroke Research. VASCage is a COMET Centre within the Competence Centers for Excellent Technologies (COMET) programme and funded by the Federal Ministry for Climate Action, Environment, Energy, Mobility, Innovation and Technology, the Federal Ministry of Labour and Economy, and the federal states of Tyrol, Salzburg, and Vienna. COMET is managed by the Austrian Research Promotion Agency (Österreichische Forschungsförderungsgesellschaft, project number: 898252).

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