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CARDIOMETABOLIC DISEASE AND TREATMENT (E. BRINTON, SECTION EDITOR)

ANGPTL3 and Apolipoprotein C-III as Novel Lipid-Lowering Targets

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Abstract

Purpose of Review Despite significant progress in plasma lipid lowering strategies, recent clinical trials highlight the existence of residual cardiovascular risk. Angiopoietin-like protein 3 (ANGPTL3) and apolipoprotein C-III (Apo C-III) have been identified as novel lipid-lowering targets.

Recent Findings Apo C-III and ANGPTL3 have emerged as novel regulators of triglyceride (TG) and low-density lipoprotein-cholesterol (LDL-C) levels. ANGPTL3 is an inhibitor of lipoprotein lipase (LPL), reducing lipolysis of Apo B-containing lipoproteins. Loss-of-function *ANGPLT3* mutations are associated with reduced plasma cholesterol and TG, while novel ANGPLT3 inhibition strategies, including monoclonal antibodies (evinacumab), *ANGPLT3* antisense oligonucleotides (IONIS-ANGPTL3-L_{Rx}), and small interfering RNA (siRNA) silencing techniques (ARO-ANG3), result in increased lipolysis and significant reductions of LDL-C and TG levels in phase I and II clinical trials. Similarly, Apo C-III inhibits LPL while promoting the hepatic secretion of TG-rich lipoproteins and preventing their clearance. Loss-of-function *APOC3* mutations have been associated with reduced TG levels. Targeting of Apo C-III with volanesorsen, an *APOC3* siRNA, results in significant reduction in plasma TG levels but possibly also increased risk for thrombocytopenia, as recently demonstrated in phase I, II, and III clinical trials. ARO-APOC3 is a novel siRNA-based agent targeting Apo C-III which is currently under investigation with regard to its lipid-lowering efficiency.

Summary ANGPTL3 and Apo C-III targeting agents have demonstrated striking lipid-lowering effects in recent clinical trials; however, more thorough safety and efficacy data are required. Here, we evaluate the role of ANGPLT3 and Apo C-III in lipid metabolism, present the latest clinical advances targeting those molecules, and outline the remaining scientific challenges on residual lipid-associated cardiovascular risk.

Keywords Angiopoietin-like protein 3 · Apolipoprotein C-III · Evinacumab · Volanesorsen · Gene silencing · Cardiovascular risk · Triglycerides · Cholesterol

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Introduction

Dysregulation of lipid metabolism, characterized by increased plasma levels of low density lipoprotein cholesterol (LDL-C) and triglycerides (TG), has long been identified as a core pathogenic aetiology of atherosclerosis [1, 2]. However, despite the use of statins and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, recent clinical studies revealed the presence of residual cardiovascular risk associated with increased morbidity and mortality [3, 4]. Interestingly, accumulating evidence suggests that this residual risk exists even at very low levels of circulating LDL-C [5] and can be better quantified by non-high-density lipoprotein cholesterol (non-HDL-C) than by LDL-C [6]. Optimal lipid reduction thus remains an elusive therapeutic target in cardiovascular disease (CVD) prevention, warranting further investigation [7].

Angiopoietin-like protein 3 (ANGPLT3), a member of the angiopoietin-like proteins that exert angiogenic properties, participates in the regulation of lipoprotein metabolism [8]. Under normal conditions, it is primarily important for the maintenance of physiological levels of plasma TG, though it also regulates plasma LDL cholesterol levels. Due to its latter property, it has attracted a lot of attention as a potential target for the efficient management of dyslipidaemia [9]. The main mechanism underlying the effect of ANGPTL3 on lipid levels involves the inhibition of lipoprotein lipase (LPL), hepatic lipase (HL), and endothelial lipase, thereby inhibiting the lipolysis of plasma lipoprotein TG and phospholipids [9, 10]. Importantly, pharmacological strategies for efficient inhibition of ANGPTL3 function and expression have been developed with promising preliminary results [11, 12].

Apolipoprotein (Apo) C-III is found in TG-rich lipoproteins and high-density lipoprotein (HDL) [13, 14] and genetic studies have linked reduced Apo C-III levels with low TG levels [11, 15]. Apo C-III increases the hepatic synthesis of TG-rich lipoproteins while reducing their clearance by inhibiting LPL and hepatic lipoprotein receptor uptake [11, 15, 16]. As such, Apo C-III is a promising target in hypertriglyceridaemia; thus, gene silencing techniques such as antisense oligonucleotides (ASO) and small interfering RNA (siRNA) have been applied in human phase III trials, demonstrating significant reductions in plasma TG levels [11, 15]. Ongoing research is focusing on optimizing the in vivo treatment characteristics in order for Apo C-III inhibition to find its path towards clinical practice [11, 17].

The aim of this review is the comprehensive evaluation of the current role of ANGPTL3 and Apo C-III in lipid reduction strategies. At first, we provide a summary of lipid pathophysiology in atherosclerosis, the underlying role of ANGPTL3 and Apo C-III as well as the mechanistic evidence for their pharmacological targeting. We next summarize the clinical studies investigating the efficacy of ANGPTL3 and Apo C-III as targets for lipid reduction, providing insights into their potential integration in clinical practice.

Lipids and Atherosclerosis: an Ongoing Challenge

Lipids in the circulation form macromolecular assemblies with plasma apolipoproteins such as Apo B-48, B-100, E, C-II, C-III, A-I, and Lp(a) which are collectively termed as lipoproteins [12, 18–20]. TG are mainly circulated with the TG-rich lipoproteins that are very low-density lipoprotein (VLDL) particles, chylomicrons, and their remnants. VLDL particles are produced by the liver and contain Apo B-100 as their main apolipoprotein. Chylomicrons are assembled postprandially by the intestine and contain Apo B-48 [12, 19]. TG-rich lipoproteins are circulated to the adipose tissue, cardiac and skeletal muscle and the liver, where TG are sequentially hydrolyzed by the endothelium-bound LPL to form free fatty acids (FFA) that are taken up by the tissues [12, 21]. Sequential LPL-mediated hydrolysis leads to the formation of lipoprotein particles of decreasing size, decreasing TG content and increasing cholesterol content [12, 21]. These include chylomicron remnants, intermediate density lipoproteins (IDL) and low density lipoproteins (LDL) that are atherogenic lipid particles (Fig. 1) [12, 21]. Dysregulated lipid levels, particularly elevated LDL-C and TG levels as well reduced HDL levels, have consistently been linked with the pathogenesis of atherosclerosis [2, 22, 23].

ANGPTL3 and Atherosclerosis

Role and Disease Mechanisms

ANGPTL3 belongs to the protein family of ANGPTLs, angiopoietin-like proteins involved in various processes such as lipid metabolism and angiogenesis. ANGPTL3 is exclusively expressed in the liver (hence being considered a hepatokine [9]), where undergoes a variety of post-translational modification such as protein glycosylation and cleavage, which prompt its activation [24]. Its C-terminal fibrinogen-like domain interacts with integrin $\alpha v \beta 3$ receptor regulating angiogenesis [24], while its N-terminal end inhibits LPL, HL and EL activities [8, 10].

The ability of ANGPTL3 to inhibit LPL and HL activities which are primarily responsible for the hydrolysis of lipoprotein TG has attracted significant interest. ANGLPTL8 promotes ANGPTL3 cleavage, while it has been demonstrated that interaction of the N-terminal ANGPTL3 fragment with ANGPTL8 significantly increases the ability of the former to allosterically inhibit lipoprotein lipases at physiological concentrations [25]. Inhibition of ANGPTL3 would be expected to reduce lipid levels via enhanced LPL activity facilitating lipoprotein lysis and clearance. In line with this hypothesis, gain-of-function and loss-of-function transgenic mouse models have confirmed that ANGPTL3 regulates lipid levels

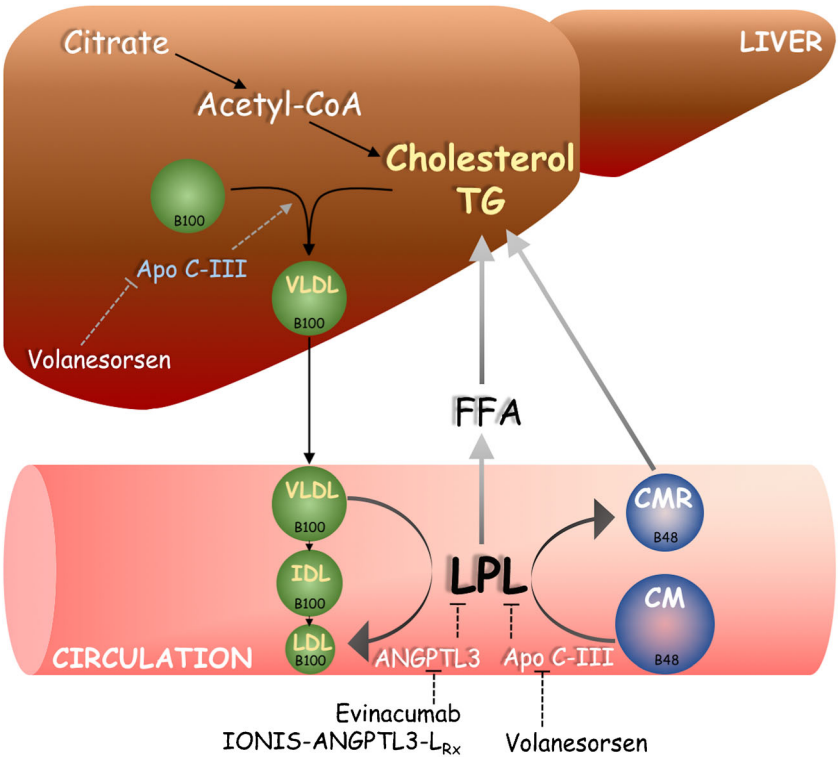

Fig. 1 Overview of lipid turnover and effects of angiopoietin-like protein 3 (ANGPTL3) and apolipoprotein C-III (Apo C-III) inhibition. Chylomicrons (CM) are formed by triglycerides (TG) and Apo B-48 in the intestine following dietary lipid absorption. Endothelial lipoprotein lipase (LPL) converts CM to chylomicron remnants (CMR), which are internalized by the liver to contribute to the hepatic lipid pool. Hepatic cholesterol and TG, partly synthesized de novo from citrate and acetyl co-enzyme A, interact with Apo B-100 to form very low-density lipoprotein (VLDL) particles, which are secreted in the circulation. Triglycerides are hydrolyzed by LPL converting VLDL particles to intermediate density lipoproteins (IDL) and subsequently to low density lipoproteins (LDL), the main carrier of cholesterol in the bloodstream. Triglyceride lysis by LPL forms free fatty acids (FFA) which are taken up by peripheral tissues and the liver. ANGPTL3 inhibits LPL activity; therefore, its inhibition by the monoclonal antibody evinacumab or by the gene-silencing IONIS-ANGPTL3-L_{Rx} facilitates TG-rich particle lipolysis by LPL, reducing most Apo B-containing lipoprotein particles. Apo C-III enhances hepatic VLDL synthesis and also inhibits LPL; therefore, its inhibition by gene-silencing agents such as volanesorsen reduces TG-rich lipoprotein levels. The aforementioned agents have demonstrated their lipid-lowering abilities in vivo and are being further investigated in large randomized clinical trials

in vivo, with loss of ANGPTL3 resulting in global hypolipidaemia while overexpression of ANGPTL3 in increased blood lipids concentration [26•]. This has also been described in human genome-wide association and Mendelian randomization studies causally linking homozygous loss of ANGPTL3 with reduced levels of LDL-C, VLDL-CHOLESTEROL (VLDL-C), TG-rich lipoproteins and HDL CHOLESTEROL (HDL-C) (but not Lp(a)), such as in the case of homozygous hypobetalipoproteinaemia-2 [11, 27••, 28]. Additionally, in vitro, ANGPLT3 silencing reduces the biosynthesis, lipidation, and secretion of VLDL by human hepatocytes in response to various stimuli [29, 30], which may be a complementary mechanism, beyond LPL regulation, via which ANGPTL3 inhibition reduces serum VLDL levels. The exact mechanism of LDL-C reduction following ANGPTL3 inhibition is not well-known. It has been proposed that it involves increased uptake [31] as well as decreased hepatic secretion of Apo B-containing lipoproteins (in a way similar to other lipid-lowering drugs blocking ApoB-containing lipoprotein synthesis, namely niacin [32], mipomersen [33], and lomitapide [34]). However, other experimental studies showed that ANGPTL3 inhibition did not affect the number of ApoB-containing lipoproteins secreted by the liver [35]. The effect of ANGPTL3 inhibition on LDL-C levels was attenuated in LDL RECEPTOR (LDLR)-deficient mice, suggesting dependence on LDLR-mediated clearance of LDL particles [31]. Furthermore, other investigators showed that ANGPTL3 inhibition reduced the lipidation of VLDL and LDL particles in mice [36], and these altered particles are possibly cleared more rapidly from the circulation through noncanonical pathways [35].

Besides research directly linking ANGPTL3 with lipid levels, several studies have also associated ANGPTL3 function with disease phenotypes such as obesity, diabetes and atherosclerosis at an observational level [37–39]. Hepatic ANGPTL3 was increased in insulin-deficient and insulin-resistant experimental models of diabetic mice, while genetic variants of *ANGPTL3* were associated with future risk of incident diabetes in humans [39]. Loss-of-function ANGPTL3 mutations have been associated with reduced risk for diabetes and obesity [30],

as well as with reduced plaque burden and overall atherosclerosis risk in humans [40]. Despite the fact that HDL-C has been previously linked with decreased cardiovascular risk [41], ANGPLT3 loss is associated with decreased cardiovascular risk despite reducing HDL-C, apparently due to the overarching effect on LDL-C and TG reduction [27••].

Targeting of ANGPTL3

ANGPTL3 is a promising therapeutic target considering its ability to modulate lipid levels including LDL-C, VLDL-C and TG. To date, two strategies have been developed to target ANGPTL3, namely monoclonal antibody targeting and transcriptional modulation by ASO [11].

i) Monoclonal antibody targeting

Evinacumab is a fully human monoclonal antibody specifically binding to ANGPTL3. It has displayed its efficacy in inhibiting ANGPTL3 and lipoprotein lipases in several animal in vivo studies and human clinical studies. In a representative study using an APOE*3Leiden/CETP mouse model, evinacumab reduced plasma cholesterol and TG levels, as well as atherosclerotic lesion size, compared to controls [27••]. Human studies have shown a dose-dependent reduction of TG levels up to 76% and LDL-C up to 23% in response to evinacumab treatment, administered as weekly doses ranging from 75 to 450 mg subcutaneously and 5 to 20 mg/kg intravenously [27, 42••]. Other studies have further confirmed the efficacy of evinacumab in patients with homozygous familiar hypercholesterolaemia in open-label phase 2 studies [43, 44••], which was found to be independent to LDL receptor (LDLR) activity [43], resulting in marked LDL-C reductions, even in the context of homozygous familial hypercholesterolemia (Table 1).

Importantly, evinacumab appears to have additive benefit in reducing TG and LDL-C levels on top of statin and PCSK9 inhibitor treatment as evidenced in both recent animal studies in APOE*3Leiden/CETP mice [47, 48] and in humans with homozygous hypercholesterolaemia [49]. This triple combination therapy was accompanied by striking reduction in atherosclerosis burden in mice [47]. Preliminary data indicate that evinacumab is well-tolerated, however, large outcome trials assessing the safety of evinacumab and its relationship with cardiovascular events are lacking.

ii) Antisense oligonucleotide targeting

The use of ASO comprises another approach that has been used to inhibit ANGPTL3 [11, 46••] by inhibiting its translation [50]. N-acetylgalactosamine (GalNAc)–conjugated mouse *Angptl3* ASOs (IONIS-ANGPTL3-L_{Rx}) have been developed and tested in a variety of mouse models, resulting in reduced levels of all ApoB-containing lipoproteins and attenuated systemic insulin resistance [46••].

Importantly, the safety and efficacy of IONIS-ANGPTL3-L_{Rx} have been investigated in a phase 1 trial of 44 human participants, using single (subcutaneous 20, 40, or 80 mg injections) versus multiple (subcutaneous 10, 20, 40, or 60 mg weekly injections for 6 weeks) administration. In the single-dose groups, a non-significant, dose-dependent trend for lower VLDL-C, TG and LDL-C levels was reported [46••]. Clearer, statistically significant lipid reductions were achieved in the multiple-administrations groups, in a dose-dependent manner, inducing a maximum of 63% TG reduction and 36.6% reduction in non-HDL-C levels [46••]. No serious side effects or significant alterations of blood cell counts, renal or liver function were observed, while headache and dizziness were reported by few participants [46••]. These observations suggest that IONIS-ANGPTL3-L_{Rx} may be a viable option for reducing ApoB-containing lipoproteins in humans [46••] (Table 1). However, larger clinical trials linking ANGPTL3 ASOs with cardiovascular outcomes are required.

iii) siRNA silencing

Recently, another gene silencing agent has been developed (ARO-ANG3), which is able to target ANGPTL3 expression via siRNA [51]. ARO-ANG3 is currently being tested in phase I/II trials, where single drug doses caused TG reduction of up to 66%, accompanied by dose-dependent reductions in LDL-C, albeit not significant probably due to small effect size [51]. It remains to be seen whether this siRNA technique could be a similarly efficient means of ANGPTL3 targeting in humans.

Apo C-III and Atherosclerosis

Role and Disease Mechanisms

Apo C-III, encoded by the gene *APOC3* located on chromosome 11q23, is an apolipoprotein which primarily comprises part of VLDL and chylomicrons [11], while it can also be found in LDL and HDL particles [13, 14, 16, 52, 53]. It is mainly expressed in the intestine, where it helps form chylomicrons postprandially, and in the liver, where it modulates central lipid metabolism and turnover [16].

The in vivo relationship of Apo C-III with hyperlipidaemia, particularly TG levels, has been confirmed in genome wide association studies (GWAS) [48, 54–56]. In addition, Mendelian randomization studies have demonstrated reduced TG levels and coronary calcification in humans with null mutations in *APOC3*, characterized by markedly decreased plasma Apo C-III levels [57, 58]. Loss-of-function mutations of *APOC3* have since been linked with both low TG levels and

Table 1 Clinical studies assessing the lipid-lowering effects of angiopoietin-like protein 3 (ANGPTL3) targeting

Study	Study type and population	Drug tested	Design	Main findings
FE Dewey et al. 2017 [27••]	Phase 1, healthy volunteers	Evinacumab (monoclonal antibody)	Evinacumab SC 75 mg (n = 11), 150 mg (n = 12), 250 mg (n = 9) vs placebo (n = 9) Evinacumab IV 5 mg/kg (n = 10), 10 mg/kg (n = 9), 20 mg/kg (n = 11) vs placebo (n = 12)	Dose-dependent reduction of LDL-C up to 23% and TG up to 76%
Z Ahmad et al. 2019 [42]	Phase 1, healthy volunteers	Evinacumab (monoclonal antibody)	Single dose arm Evinacumab SC 75 mg (n = 11), 150 mg (n = 12), 250 mg (n = 9) vs placebo (n = 9) Evinacumab IV 5 mg/kg (n = 10), 10 mg/kg (n = 9), 20 mg/kg (n = 11) vs placebo (n = 12) Multiple doses arm Evinacumab SC 150 mg QW (n = 6), 300 mg QW (n = 6), 300 mg Q2W (n = 6), 450 mg QW (n = 6), 450 mg Q2W (n = 7) vs placebo (n = 12) Evinacumab IV 20 mg/kg Q4W (n = 7) vs placebo (n = 2)	Dose-dependent drop in LDL-C up to 22.8% for single SC and IV doses and up to 25.1% for 300 mg QW SC and 20 mg/kg Q4W IV doses Dose-dependent drop in TG up to 55.5% for all single SC doses, up to 80.3% for single IV doses, up to 88.2% for multiple IV doses and up to 51.9% for multiple SC doses
D Gaudet et al. 2017 [45•]	Phase 2 open label single-group, homozygous FH adults	Evinacumab (monoclonal antibody)	Evinacumab SC 250 mg at baseline (single dose, n = 9) followed by evinacumab SC 450 mg (QW, n = 2) or evinacumab IV 15 mg/kg at week 2 (single dose, n = 7)	Mean LDL-C reduction of 49% Mean Apo B reduction of46% Median TG reduction of 47% Median HDL-C reduction of 36%
MJ Graham et al. 2017 [46••]	Phase I, healthy participants with TG >150 mg/dL	IONIS-ANGPTL3-L_{Rx} (antisense oligonucleotide)	IONIS-ANGPTL3-L_{Rx} single SC doses of 20 mg (n = 3), 40 mg (n = 3) or 80 mg (n = 3) vs placebo (n = 3) IONIS-ANGPTL3-L_{Rx} weekly SC doses of 10 mg (n = 6), 20 mg (n = 6), 40 mg (n = 6), or 60 mg (n = 6) vs placebo (n = 6)	Non-significant trend for lipid reduction in the single dose groups Dose-dependent reduction of TG by up to 63.1% and of non-HDL-C by up to 36.6% for the multiple doses groups

SC, subcutaneous; *IV*, intravenous; *LDL-C*, low density lipoprotein cholesterol; *TG*, triglycerides; *QW*, once a week; *Q2W*, once every 2 weeks; *Q4W*, once every 4 weeks; *Apo*, apolipoprotein; *HDL-C*, high density lipoprotein cholesterol

decreased cardiovascular risk in large studies [59••, 60••]. Furthermore, association studies have independently associated circulating Apo C-III with the presence of atherosclerosis as well as adverse cardiovascular outcomes [61–63].

At a cellular level, Apo C-III inhibits lipoprotein lipases and prevents the interaction of ApoB and ApoE apolipoproteins with their hepatic receptors, thereby increasing the bioavailability of circulating TG-rich lipoproteins via reduced lipolysis and reduced hepatic uptake [64]. This is further augmented by the ability of Apo C-III to stimulate hepatic synthesis and secretion of VLDL [65], while it has also been revealed that Apo C-III also decreases the hepatic clearance of TG-containing lipoproteins via regulating the LDLR/low density lipoprotein receptor-related protein 1 (LRP1) pathway [66]. Furthermore, a higher Apo C-III content has been noticed in HDL particles from patients with obesity and atherosclerosis [64]. In addition to TG-rich lipoproteins, Apo CIII may also be capable of promoting the de novo biogenesis of HDL (Apo C-III-HDL) in the absence of Apo A-I, which is the essential component of classical HDL [13]. Interestingly, the extent of Apo CIII accumulation on TG-rich lipoproteins, and thus the extent of inhibition of their LPL-mediated lipolysis, appears dependent on the activity of the lipid transporter ABCA1 which is key to free cholesterol uptake by very small, nascent HDL, and which also regulates the relative partitioning of Apo C-III on HDL vs other lipoproteins [13]. In experimental mice, Apo C-III-HDL was found to be a poorer acceptor of free [14C]-cholesterol in RAW 264.7 cells than was Apo A-I-HDL, indicating reduced cholesterol efflux, which, in turn, appears to be a key property for the effective unloading of free cholesterol from peripheral tissues and its shuttling back to the liver for catabolism [14]. Moreover, Apo C-III potentiated the effect of lipopolysaccharides on tumor necrosis factor (TNF)-α release in RAW 264.7 macrophage cells suggesting a proinflammatory role in circulation [14]. Apo C-III-HDL had a much higher antioxidant capacity than classical HDL, suggesting a positive role in reducing oxidative stress [14]. In case-control studies Apo C-III-HDL appeared to be associated with increased cardiovascular risk as opposed to Apo A-I-HDL, implying that Apo C-III may be related to HDL dysfunction in humans [64].

Despite having attracted scientific attention mainly due to its direct relationship with TG levels, Apo C-III appears to have pleiotropic biological effects beyond lipid metabolism [64, 67]. Apo C-III promotes proinflammatory nuclear factor kappa beta (NFkB) signaling, which in turns activates monocytes and increases their integrin-mediated interaction with ECs [68], while it also upregulates vascular cell adhesion molecule-1 (VCAM1) expression in ECs [69], thus facilitating local macrophage recruitment and inflammation [64]. Apo C-III also induces reactive oxygen species-mediated proliferation of VSMCs, which is also involved in early atherogenesis [64]. In addition, Apo C-III has been associated with increased thrombin production which could promote a hyper-coagulant state [70]. It should be noted however that Apo C-III-HDL stimulates brown adipose tissue (BAT) metabolic activation in mice [14], which explains the sensitivity of *apoc3*-deficient (*apoc3*$^{-/-}$) mice towards diet-induced obesity that has been previously reported in literature [71].

Targeting of Apo C-III

Modulation of Apo C-III has been attempted by lifestyle and dietary means, while several approved medications have shown moderate Apo C-III-lowering effects [15]. On the other hand, more recent techniques specifically targeting Apo C-III involve gene silencing via siRNA, which is currently being tested in large clinical trials [15].

i) Lifestyle and dietary interventions

Experimental studies have revealed opposing direct effects of glucose and insulin on hepatocellular *APOC3* expression [15]. Glucose stimulates hepatic *APOC3* expression via transcriptional regulation involving hepatocyte nuclear factor 4α [72]. On the other hand, insulin induces Foxo1-mediated transcriptional pathways downregulating *APOC3* expression in hepatocytes [73]. Therefore, glucose intolerance in the context of systemic insulin resistance, metabolic syndrome or diabetes could dually increase *APOC3* expression in the liver by the direct effect of hyperglycaemia and the loss of the inhibitory insulin effect (due to hepatic insulin resistance) [15]. Consistently, several human observational studies have indicated that high-carbohydrate diet is associated with increased serum Apo C-III levels [74, 75], while low-carbohydrate diets may reduce Apo C-III [76].

Beyond carbohydrate consumption, Apo C-III levels have also been linked with the type of dietary lipids. Consumption of saturated fats has been associated with elevated Apo C-III levels [77], while unsaturated fats may reduce Apo C-III levels, with 3-poly-unsaturated fats (3-PUFA) demonstrating a robust ability in that regard [78].

Recently, aerobic exercise was demonstrated to decrease TG levels via reducing Apo C-III in patients with coronary artery disease. The underlying mechanism is not fully understood, but it may involve favorable changes in skeletal muscle and hepatic insulin sensitivity as well other metabolic adjustments owing to aerobic exercise [79].

ii) Clinically used drugs with pleiotropic effects on Apo C-III

Statins are anti-hyperlipidaemic drugs mainly inhibiting de novo cholesterol biosynthesis, however human studies have revealed a dose-dependent role of statins such as rosuvastatin in reducing Apo C-III [80, 81], while this has been confirmed in meta-analyses of large randomized clinical trials with statins [82].

Fibrates are drugs mainly used to reduce TG levels. Fibrates exert their main actions via peroxisome proliferator-activated receptor alpha (PPARα), and part of their actions may be due to Apo C-III reduction as evidenced by mechanistic studies on the effects of PPARα [83] and demonstrated in human studies [84–86]. Interestingly, Apo C-III data from large clinical trials with fibrates are quite limited, raising the question as to the clinically relevant contribution of Apo C-III reduction to the overall TG-decreasing effects of fibrates [15].

In a randomized study of hypercholesterolaemic, obese individuals, circulating Apo C-III levels were reduced in the subgroups treated with ezetimibe, a drug targeting enteric cholesterol absorption, and orlistat, an intestinal lipase inhibitor used to treat obesity [87]. Therefore, Apo C-III may mediate the minor to modest TG-lowering effects of ezetimibe and orlistat respectively, which however require more definite investigation.

iii) APOC3 silencing

Gene silencing by ASOs is a molecular technique which has also been used to target *APOC3*, volanesorsen (IONIS-APO-CIII$_{Rx}$) being an ASO that inhibits Apo C-III expression [15]. Volanesorsen is a second-generation 2′-O-methoxyethyl (2′-MOE) chimeric ASO targeting the *APOC3* mRNA, preventing its translation [88, 89•].

Volanesorsen has been validated in several animal models with regard to its TG-lowering abilities [89•]. Importantly, subcutaneous injection doses ranging from 50 mg to 400 mg of volanesorsen markedly and dose-dependently reduced TG levels in apparently healthy volunteers [89•]. Human studies in patients with hypertriglyceridaemia have demonstrated significant reductions of TG levels with volanesorsen (weekly 300 mg injections) [88], while, in a 15-week trial in patients with diabetes, volanesorsen, besides reduction of TG levels by ~69%, also ameliorated glucose intolerance [90•] (Table 2). In phase II clinical trials, volanesorsen, administered weekly at a dose range from 100 mg to 300 mg, was able to reduce TG levels dose-dependently by up to ~90% in patients with

Table 2 Clinical studies assessing the lipid-lowering effects of apolipoprotein C-III (Apo C-III) targeting

Study	Study type and population	Drug tested	Design	Main findings
MJ Graham et al. 2013 [89•]	Double-blind, placebo-controlled phase 1 study of healthy volunteers	Volanesorsen (antisense oligonucleotide)	SC doses of volanesorsen at 50 mg (n = 3), 100 mg (n = 3), 200 mg (n = 3), 400 mg (n = 3) vs placebo (n = 4) weekly	Adjusted drop of TG up to 72.3% Adjusted drop of LDL-C up to 9.4% Non-significant effect on HDL-C
D Gaudet et al. 2015 [91••]	Double-blind, placebo-controlled, phase 2 study of healthy patients with TG > 350 mg/dL	Volanesorsen (antisense oligonucleotide)	Weekly SC injections of volanesorsen at 100 mg (n = 11), 200 mg (n = 13), 300 mg (n = 11) vs placebo (n = 16) Weekly SC injections of volanesorsen at 200 mg (n = 8), 300 mg (n = 10) vs placebo (n = 8) on baseline fibrate treatment	Adjusted TG drop up to 91% as monotherapy and up to 56.3% as add-on to fibrates Adjusted HDL-C increase up to 45% as monotherapy and up to 55.7% as add-on to fibrates
X Yang et al. 2016 [92•]	Phase 2 study of FCS patients and volunteers with hypertriglyceridaemia	Volanesorsen (antisense oligonucleotide)	Volanesorsen weekly SC 300 mg in FCS patients (n = 3) and follow-up without placebo Volanesorsen weekly SC 100 mg (n = 11), 200 mg (n = 13), 300 mg (n = 11) vs placebo (n = 16) Volanesorsen weekly SC 200 mg (n = 8), 300 mg (n = 10) vs placebo (n = 8) on baseline fibrate therapy	TG reduction of up to 86% in FCS patients on volanesorsen treatment ApoCIII-ApoB, ApoCIII-AI, and ApoCIII-Lp(a) reduction by ~80% for volanesorsen monotherapy ApoCIII-ApoB, ApoCIII-AI, and ApoCIII-Lp(a) reduction by ~75% for volanesorsen add-on therapy
JL Witztum et al. 2019 [93•]	Phase 3 double-blind study in FCS patients (APPROACH)	Volanesorsen (antisense oligonucleotide)	Weekly SC volanesorsen 300 mg (n = 33) vs placebo (n = 33)	TG reduction of 76.5% Chylomicron reduction of 82.7% VLDL-C reduction of 58.3% LDL-C increase of 135.6% HDL-C increase of 46.1%
I Gouni-Berthold et al. 2017 [94••]	Phase 3 double-blind study in patients with hypertriglyceridaemia (COMPASS)	Volanesorsen (antisense oligonucleotide)	Weekly SC volanesorsen 300 mg (n = 75) vs placebo (n = 38)	Mean reduction of TG by 71.2% in the volanesorsen group TG < 500 mg/dL in 82% of the volanesorsen group vs 14% in the placebo group

SC, subcutaneous; *TG*, triglycerides; *LDL-C*, low density lipoprotein cholesterol; *HDL-C*, high density lipoprotein cholesterol; *VLDL*, very low density lipoprotein cholesterol; *FCS*, familiar chylomicronaemia syndrome; *Apo*, apolipoprotein

hypertriglyceridaemia, both as a monotherapy and as an add-on therapy on top of standard fibrate therapy [91••, 92]. Volanesorsen was associated with decreased familial chylomicronemia syndrome (FCS) symptom burden in 22 FCS patients according to ReFOCUS, a retrospective web-based study [95].

Two phase III randomized clinical trials have further explored the safety and efficacy of volanesorsen in reducing TG levels (Table 2). The APPROACH trial (NCT02211209) evaluated the effect of volanesorsen (300 mg administered subcutaneously on a weekly basis) vs placebo on TG levels in 66 FCS patients over a period of 52 weeks [93•, 96•]. Volanesorsen reduced TG levels by 77% as opposed to the 18% increase observed in the placebo group [96•]. On the other hand, the COMPASS trial (NCT02300233) evaluated the effect of volanesorsen (300 mg delivered as a weekly subcutaneous injection) on TG levels in 113 patients with fasting triglycerides greater than 500 mg/dL [94••]. Volanesorsen significantly reduced TG levels by 71.8% vs 0.8% in the placebo group [94••]. Clinical trials directly comparing volanesorsen with fibrates or n-3 fatty acids as a means of reducing TG levels are still lacking; however, the striking TG reductions achieved with volanesorsen appear to be far greater than those achieved with the other agents [92•]. Interestingly, volanesorsen may also reduce hypertriglyceridaemia-associated acute pancreatitis risk [97]. Finally, clinical trials exploring the potential effect of volanesorsen on cardiovascular events are needed.

A major side effect of volanesorsen revealed by the APPROACH study comprised thrombocytopenia, which was in rare cases severe, but not associated with bleeding events and was corrected after stopping the drug [15, 96•]. Although the COMPASS trial did not show an increase in thrombocytopenia and in bleeding events [11, 94], the FDA did not approve volanesorsen for use in FCS patients [11]. However, there is a lot of positivity with regard to the clinical utility of *APOC3* silencing, while the side effect on platelet count is proposed to be a consequence of off-target properties of the specific agent rather than being a general result of APOC3 silencing [15]. Indeed, the 2′-MOE modification

found in volanesorsen has previously been associated with dose-dependent thrombocytopenia in both humans and animal models [98]. *APOC3* silencing with a GalNAc-conjugated ASO (IONIS-APO-$CIII_{LRx}$) instead of 2′-MOE has recently been used in healthy volunteers, resulting in significant TG reduction without inducing thrombocytopenia [17]. These considerations might pave the way for clinical approval of *APOC3*-targeting ASOs to reduce TG both efficiently and without severe side effects, even in FCS.

Conclusion

Despite significant progress in the field of hyperlipidaemia management, recent clinical trials indicate an unmet need for more aggressive and efficient lipid-lowering therapy in high-risk patient subgroups. Apo C-III has long been known to be a key regulator of TG and HDL metabolism. Recently, ANGPTL3 has been revealed as a novel regulator of lipid metabolism in genetic studies. They have both been validated in a multitude of animal and human studies, including in relationship to obesity and diabetes (Fig. 1). In vivo targeting of ANGPTL3 and Apo C-III has recently been possible with novel monoclonal antibodies and gene silencing techniques, yielding marked reductions of LDL-C, VLDL-C and TG in phase I, II and III trials.

It should be kept in mind that to this date we do not have long-term safety and efficacy data for inhibition of these targets like we do for other drugs such as statins. ANGPTL3 and Apo CIII may likely also have beneficial long-term properties and their extreme inhibition might be harmful, as suggested by the ancient Greek saying "μέτρον άριστον," or "everything in moderation." Regarding atherosclerotic CVD risk reduction, the ability of ANGPTL3 inhibition to reduce LDL-C levels (the main lipid CVD risk factor), along with TG concentration, seems more promising. Both ANGPTL3 and Apo C-III inhibition may be beneficial in patients with mixed dyslipidaemia and increased residual CVD risk. The relatively stronger TG-lowering ability of Apo-CIII inhibition may benefit patients with severe hypertriglyceridaemia and increased risk of pancreatitis, while combination treatment may confer additive effects. Additionally, a combination of these drug classes could result in significant improvement of overall lipid profile and confer CVD protection. Long-term clinical trials will be required to resolve all these emerging issues and proof-of-concept hypotheses.

Declarations

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of Interest Theodosios D. Filippatos reports personal fees from Boehringer Ingelheim, personal fees from Mylan, personal fees from Astra Zeneca, personal fees from Lilly, personal fees from Recordati, personal fees from Bausch Health and personal fees from Servier outside the submitted work. Ioannis Akoumianakis, Evangelia Zvintzou and Kyriakos Kypreos declare no conflicts of interest relevant to this manuscript.

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- • Of importance
- •• Of major importance

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