New medications targeting triglyceride-rich lipoproteins: Can inhibition of ANGPTL3 or apoC-III reduce the residual cardiovascular risk?

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Abstract
Remarkably good results have been achieved in the treatment of atherosclerotic cardiovascular diseases (CVD) by using statin, ezetimibe, antihypertensive, antithrombotic, and PCSK9 inhibitor therapies and their proper combinations. However, despite this success, the remaining CVD risk is still high. To target this residual risk and to treat patients who are statin-intolerant or have an exceptionally high CVD risk for instance due to familial hypercholesterolemia (FH), new therapies are intensively sought. One pathway of drug development is targeting the circulating triglyceride-rich lipoproteins (TRL) and their lipolytic remnants, which, according to the current view, confer a major CVD risk. Angiopoietin-like protein 3 (ANGPTL3) and apolipoprotein C-III (apoC-III) are at present the central molecular targets for therapies designed to reduce TRL, and there are new drugs emerging that suppress their expression or inhibit the function of these two key proteins. The medications targeting these components are biological, either human monoclonal antibodies or antisense oligonucleotides. In this article, we briefly review the mechanisms of action of ANGPTL3 and apoC-III, the reasons why they have been considered promising targets of novel therapies for CVD, as well as the current status and the most important results of their clinical trials.

1. Introduction
The development of atherosclerosis requires both the accumulation of lipids, mainly cholesterol, in the arterial intima, and a concomitant chronic inflammation of the arterial wall [1]. In addition to LDL-C and other established conventional risk factors, the role of TRL as a causal CVD risk factor is increasingly recognized [2]. Variants of a number of key genes of TRL metabolism display strong associations with CVD risk, and the strength of these variants' impacts on plasma TG concentration correlates with the magnitude of their effects on the risk of CVD events [3]. TRLs are envisioned to contribute to the progression of CVD via multiple mechanisms: They directly mediate cholesterol deposition within the arterial intima and facilitate the activation of pro-inflammatory, pro-coagulant, and pro-apoptotic pathways [4]. Chylomicron and VLDL remnants have average diameters <70 nm and therefore penetrate into the arterial intima, and are there retained by the connective tissue matrix [5,6]. Besides TGs, these remnant particles also contain enriched amounts of cholesterol esters and can be taken up by arterial macrophages resulting in elevated cholesterol loading and accelerated foam cell formation in coronary arteries [7]. The crucial importance of TRL and their lipolytic remnants in CVD progression is supported by the fact that non-HDL cholesterol

Abbreviations: ANGPTL3, angiopoietin-like protein 3; apoB, apolipoprotein B; apoC-III, apolipoprotein C-III; ASO, antisense oligonucleotide; C, cholesterol; CAD, coronary artery disease; CVD, cardiovascular disease; FH, familial hypercholesterolemia; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LOF, loss-of-function; Lp(a), lipoprotein (a); LPL, lipoprotein lipase; MI, myocardial infarction; PCSK9, proprotein convertase subtilisin/kexin type 9; TG, triglyceride; TRL, triglyceride-rich lipoprotein; VLDL, very-low-density lipoprotein.
equalizing the sum of cholesterol carried in atherogenic apoB-containing particles: LDL, TRL, Lp(a) and TRL remnants, provides a better prediction of CVD risk than LDL-C [8]. Despite the statin, ezetimibe, antihypertensive and antithrombotic therapies routinely used to treat atherosclerotic CVD, as well as the PCSK9 antibody therapy which is currently being devised, these diseases are still the leading cause of death in the industrialized societies, and their role is constantly increasing also in the developing market economies [9]. Much of the progress in treating CVD with associated dyslipidemia can be attributed to the development and application of therapeutic approaches that reduce plasma low-density-lipoprotein cholesterol (LDL-C). However, even when applying aggressive LDL-C reducing tools, a significant residual risk remains, and during the last years the decline in CVD deaths has reached a plateau [10]. The current epidemic increase in obesity, metabolic syndrome and type 2 diabetes (T2D), which are associated with so called atherogenic dyslipidemia characterized by elevated plasma triglyceride (TG) and TRL levels, low HDL-C, and frequently with small dense LDL and small-sized HDL particles, critically contributes to the observed halt of the progress in risk reduction [11,12]. An intensive search for new molecular targets of CVD therapy is therefore ongoing. In addition, new medications are particularly important considering the treatment of patients with familial hypercholesterolemia (especially homozygous FH patients, HoFH), since the efficacy of both statin and PCSK9 inhibitor therapies largely depends on functional LDL receptors. Therefore, the functionality of the above widely used medications is poor in patients with FH mutations affecting the LDL receptor [3]. Several of the new CVD therapy targets impact the quality and/or concentration of serum lipoproteins that transport cholesterol and TG: Angiopoietin-like protein 3, ANGPTL3 [13], apolipoprotein C-III, apoC-III [14], cholesterol ester transfer protein, CETP [15], and lipoprotein (a), Lp(a) [16] all affect specific processes within cholesterol or TG metabolism. Of these, ANGPTL3 and apoC-III represent central regulators of TG and TRL metabolism. In the present article we briefly review the reasons why functional inhibition of these two molecular targets with specific effects on TLR and their metabolism has been anticipated to be beneficial and what we have thus far learned from their clinical trials.

2. ANGPTL3 as a therapy target

ANGPTL3 belongs to the ANGPTL-protein family comprising 8 members (ANGPTL1-8) and is composed of an N-terminal coiled-coil domain and a fibrinogen-like C-terminal domain [13,17]. ANGPTL3 is almost exclusively synthesized in the liver, acts in concert with the related protein ANGPTL8 [18,19], and inhibits the activity of LPL and thus the hydrolysis of TGs in capillaries of adipose tissue and muscles (Fig. 1). Apart from inhibiting LPL, ANGPTL3 can also inhibit endothelial lipase (EL) activity and thereby affect serum HDL-C levels [20]. ANGPTL3 expression in the liver of diabetic mice and in cultured human hepatocytes is dampened by insulin, suggesting that its down-regulation allows upon feeding a high LPL activity in extrahepatic tissues in order to facilitate efficient fatty acid release from TG and uptake by tissues for energy production or storage [21,22]. Progress in elucidating the role of ANGPTL3 in human lipid metabolism has been made through the identification of individuals carrying inactivating mutations in the ANGPTL3 gene [23]. Genetic loss-of-function (LOF) of ANGPTL3 causes familial combined hyperlipidemia (FHBL2, OMIM#605019) characterized by very low plasma TG, LDL-C and HDL-C concentrations. Particularly interesting is the ANGPTL3 S17X mutation detected in three generations in the small town of Campodimele in Italy [24,25]. This town has drawn the attention of researchers and was chosen as a site of WHO’s CVD research in the 1990s. The life expectancy of both females and males in Campodimele is 95 years, which is approximately 20 years more than elsewhere in Italy. The inbred inheritance of ANGPTL3 LOF mutations is considered one of the central factors underlying this longevity. Homozygous carriers of the S17X mutation had significantly higher LPL activity and mass in post-heparin plasma than non-carriers. Moreover, plasma free fatty acids, insulin, glucose, and homeostatic model assessment of insulin resistance (HOMA-IR) were significantly lower in homozygous S17X subjects than in heterozygotes or non-carriers. Complete ANGPTL3 deficiency was also associated with significantly blunted postprandial lipemia after a high fat, high calory-containing meal. Since the discovery of the ANGPTL3 S17X mutation, several additional LOF mutations have been identified in ANGPTL3, including G400VfsX5, 119fsX22, N147X,N121fsX3, N121fsX9, R332Q, S122KfsX3, E119fsX8, G56V, F295L, E96del, E95del [25–27]. When these variants exist in a homozygous or compound heterozygous state, they all lead to FHBL2. To conclude, ANGPTL3 seems to play important roles in both lipid and glucose metabolism [28,29]. The mechanism of LDL reduction upon loss of ANGPTL3 function was earlier not known; however, using a human hepatocyte model with CRISPR-Cas9 mediated knock-out of ANGPTL3 evidence was presented that the LDL reduction resulted from reduced apoB secretion and increased uptake of apoB-100 containing lipoproteins. The increased hepatic particle uptake was apparently a result of elevated LDL receptor and LRP-1 expression [30]. Consistent with an intracellular function of ANGPTL3 in hepatic lipid metabolism, knockdown of ANGPTL3 in cultured human hepatocytes reduced the lipidation of VLDL upon insulin stimulation and induced distinct changes in the expression of genes regulating lipid metabolism [31]. Meta-analysis of 19 studies (a total of 21,980 CVD patients and 158,200 controls) demonstrated that carriers of ANGPTL3 LOF mutations have 34% lower CVD risk than the control individuals [32]. Subjects in the lowest tertile of ANGPTL3 concentration displayed 29% lower myocardial infarction (MI) risk than the highest tertile. Consistently, a reduction of CVD risk in ANGPTL3 LOF mutation carriers was replicated when the exons of ANGPTL3 were sequenced among 58,335 participants of the DiscovEHR human genetics study [33]. Participants with heterozygous LOF variants in ANGPTL3 had significantly lower serum TG, HDL-C and LDL-C concentrations than participants lacking these variants. Association of ANGPTL3 LOF variants with coronary artery disease (CAD) in 13,102 CAD patients and 40,430 controls from the DiscovEHR study showed that the LOF variants were found in 0.33% of the cases and in 0.45% of the controls (adjusted odds ratio, 0.59; 95% confidence interval, 0.41 to 0.85; p = 0.004). These results were further confirmed in follow-up studies from four large population cohorts, showing that genetic antagonism of ANGPTL3 in humans is associated with decreased levels of all three major lipid fractions (VLDL, LDL, HDL) and a decreased risk of atherosclerotic CVD. The above epidemiological and preclinical studies suggest that the plasma TG lowering facilitated by reduced ANGPTL3 outweighs the potential negative consequences associated with the HDL-C lowering. Clinical trials on the inhibition of ANGPTL3 are ongoing at phases I and II. The therapeutic agents in these studies are antisense oligonucleotides (ASO, IONIS-ANGPTL3-L Rx), which dampen hepatic ANGPTL3 expression by targeting its mRNA, or a human monoclonal antibody against ANGPTL3 (Evinacumab). Both the ASO and antibody therapies significantly reduced the plasma concentrations of serum ANGPTL3 and all major lipoprotein classes. After 6 weeks of treatment, subjects who received ASO had reduced levels of ANGPTL3 protein (reductions of 46.6–84.5% from baseline, p < 0.01 for all doses vs. placebo) and of triglycerides (reductions of 33.2–63.1%), LDL cholesterol (1.3–32.9%), LDL-C (27.9–60.0%), non-HDL-C (10.0–36.6%), apoB (3.4–25.7%), and apoC-III
In the study of Dewey et al. \[33\], Evinacumab caused a dose-dependent placebo-adjusted reduction in fasting TG of up to 76% and LDL-C of up to 23%. Since the results of these clinical trials are very promising, the study of the ANGPTL3 inhibitors will most likely progress into phase III. In addition, it is noteworthy that Evinacumab administered on top of intense statin therapy for 4 weeks resulted in a further reduction of LDL-C (by 50%), TG (by 47%) and HDL-C (by 36%) in a group of 9 homozygotic FH patients, suggesting that ANGPTL3 inhibition could be employed as a strategy to reduce the high residual risk apparent in subjects with FH \[35\]. While Evinacumab antibody therapy mainly targets the ANGPTL3 protein in the circulation, ASO targets the synthesis of ANGPTL3 in hepatocytes and thus also its putative intracellular functions \[30,31\]. Therefore, these two types of biological drugs could, despite similar effects on plasma TG and lipoproteins, differ in their effects on liver physiology. This prompts further, detailed research of the intrahepatocellular functions of ANGPTL3.

### 3. Inhibition of apoC-III as a therapeutic strategy

Apolipoprotein C-III is a small, 79-amino acid glycosylated protein expressed by hepatocytes and enterocytes. It is primarily a component of the TRL, but also, to a lesser extent, detectable in LDL and HDL. The classic function of apoC-III is the inhibition of LPL and hepatic lipase, resulting in marked effects on the concentrations of circulating TRL \[36,37\]. Additionally, apoC-III is shown to facilitate the synthesis and secretion of VLDL by hepatocytes \[38\]. Consistently, two naturally occurring point mutations in APOC3, A23T and K58E, have been shown to abolish the intracellular assembly and secretion of large TG-rich VLDL particles from the liver \[39,40\]. Furthermore, apoC-III is shown to enhance the atherogenicity of LDL by increasing its affinity for arterial wall proteoglycans \[41\], to confer proatherogenic properties to HDL \[42\], as well as to promote the activation of endothelial cells and monocyte adhesion \[43,44\]. Importantly, apoC-III also interferes with the binding of apoB or apoE to hepatic LDL-receptor (LDLR) and LRP-1, thus resulting in a delayed catabolism of the highly atherogenic VLDL and chylomicron remnants \[45,46\]. Of note, the study of Gaudet et al. \[47\] on patients with familial chylomicronemia syndrome, characterized by the lack of LPL activity, showed a significant reduction of TGs upon apoC-III ASO treatment, consistent with an important, LPL-independent function of apoC-III. This effect was recently explained by a murine model study demonstrating that apoC-III ASO treatment failed to lower TGs in mice lacking both

![Fig. 1. The functions of angiopoietin-like protein 3 (ANGPTL3) and apolipoprotein C-III (apoC-III) in lipoprotein metabolism and their impacts on atherogenesis. The dashed blue lines depict the inhibition of LPL activity, the hepatic uptake of VLDL and chylomicron remnants or LDLR/LRP-1 expression, and the red arrows the resulting accumulation of atherogenic lipoproteins in atherosclerotic plaques within the arterial wall. The suggested intrahepatocellular functions of ANGPTL3 and apoC-III as facilitators of VLDL assembly and secretion are indicated with a green arrow. The role of apoC-III in intestinal TRL metabolism is thus far unknown. CM, chylomicron; HL, hepatic lipase; LDL, low-density-lipoprotein; LDLR, LDL receptor; LPL, lipoprotein lipase; LRP-1, LDL receptor-related protein 1; R, remnant; VLDL, very-low-density-lipoprotein. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)](image-url)
LDLR and LRP-1 [48]. Thus, according to the latest view, the role of apoC-III in lipoprotein clearance seems to be a key factor underlying its impact on plasma TG levels. Epidemiological studies have shown that the concentration of apoC-III in plasma, VLDL, and LDL predicts CVD [49–51], and a 15-year prospective study demonstrated that it also predicts mortality [52]. Moreover, apoC-III levels in patients with T2D were found to associate with increased plasma TGs and elevated coronary artery calcification [53]. Genome-wide association studies (GWAS) revealed an association of plasma TG concentrations and risk of CVD with the apoA-V/A-IV/C-III/A-I gene cluster [54]. This association is now believed to reflect a causal role of apoC-III in regulating the concentration of plasma TG, revealed upon the discovery of LOF variants in the APOC3 gene. Consistently, LOF variants that inactivate apoC-III not only reduce the serum TG concentration but also protect from CAD [55–57], and a recent meta-analysis of published studies confirmed a positive association of plasma apoC-III and CVD [58].

Previously, homozygous APOC3 LOF variant carriers were not found despite the fact that approximately 200,000 US and European subjects have been studied, possibly suggesting that a complete deficiency of apoC-III could be harmful. However, this seems not to be the case since four subjects homozygous for an APOC3 LOF variant (p.Arg19Ter) were recently discovered [59]. Compared with non-carriers, p. Arg19Ter homozygote subjects presented with an extremely low plasma apoC-III concentration (−89%, 𝑝 = 5 × 10⁻²¹), reduced TG concentration (−60%, 𝑝 = 7 × 10⁻⁶), elevated HDL-C (+0.69 mmol/L, 𝑝 = 3 × 10⁻⁸) and very similar LDL-C levels. Interestingly, when these human APOC3 deficient subjects were challenged with an oral fat load they displayed a markedly blunted post-prandial response in plasma TG as compared with control family members. Due to these observations, apoC-III has evoked marked interest as a target of CVD drug development.

Statins have been reported to moderately reduce plasma apoC-III concentration [60,61]. Moreover, there is evidence from both animal and human studies that peroxisome proliferator receptor α (PPARα) agonists (fibrates) reduce the hepatic expression of apoC-III and its concentration in plasma [62]. However, there is no data available on a putative connection of the CVD outcomes in clinical trials of fibrate with the fibrate effects on circulating apoC-III levels [14]. The major strategy for inactivation of apoC-III is dampening its expression with ASO (Volanesorsen = IONIS-APOCIIIRx). In phase I trials the ASO robustly reduced the plasma apoC-III and TG levels in multiple animal models and in human volunteers [63]. In phase II clinical trials, Volanesorsen reduced serum apoC-III in a dose-dependent manner by 40–80%, TG by 31–71%, and elevated HDL-C by 37–46% without significant side effects [47,63,64]. Importantly, the ASO treatment also strongly reduced the highly atherogenic TRL remnants [64]. At the moment, there are three phase III clinical trials ongoing in subjects with various forms of severe hypertriglyceridemia: APPROACH on patients with familial chylomicronemia (https://clinicaltrials.gov/ct2/show/NCT02211209), COMPASS on hyper-TG patients (https://clinicaltrials.gov/ct2/show/NCT02300233), and BROADEN on patients with familial partial lipodystrophy (https://clinicaltrials.gov/ct2/show/NCT02527343) [65]. These studies will allow us to evaluate the efficacy of apoC-III inhibition in reducing CVD events in extreme hyper-TG cases, hopefully paving the way for apoC-III-targeting therapies to reduce the residual risk also in other groups of patients.

4. Conclusion

To reduce the residual risk remaining after conventional CVD therapies, it is necessary to find new drug modalities. Furthermore, the efficacy of statins and PCSK9 inhibitors is largely based on LDL receptor function. Therefore, these therapies typically have a poor efficacy in carriers of FH mutations, and new treatment regimes are needed. The current epidemic of obesity, metabolic syndrome and T2D frequently results in a dyslipidemia characterized by elevated levels of TRLs and their remnants, which form a major source of CVD risk. Of the new biological medications that specifically affect the metabolism of TRLs, the most promising are those targeting ANGPTL3 and apoC-III. Phase III trials are currently underway with an apoC-III ASO (IONIS-APOCIII-Rx = Volanesorsen). The biological ANGPTL3 inhibitors (IONIS-ANGPTL3-LRx and Evinacumab) are subject to great expectations, and will most likely soon proceed to phase III trials. As the choice of available medications will in the future expand, the treatment of atherosclerotic CVD will become more complex. On top of statin therapy, which forms the basis of the therapeutic regimes for atherosclerotic CVD, additional treatments will be tailored individually for each patient. Targeting the metabolism of TRLR with the presently developed biological drugs will most likely constitute a fundamental tool in overcoming the residual CVD risk remaining after conventional therapies, as well the risk in specific groups of subjects with whom satisfactory results are not achieved with the present therapeutic approaches. Although human genetics has identified a number of novel genes with key roles in lipid metabolism, which can be specifically targeted to attenuate the risk for CVD, drugs targeting these genes/ mRNAs or their protein end-products typically require frequent administration through injections for decades to facilitate maximal benefit. Thus, albeit the new TRL-targeting biological medications hold a lot of promise, the administration methods, combined with the high financial cost of the medications, will limit their use. The recently introduced genome editing technologies, such as CRISPR-Cas9, have the advantage of permanently modifying genes and may thereby enable long-term, even lifelong protection against atherosclerotic CVD. Even though a number of challenging technical problems need to be solved before this technology can be safely applied to cardiovascular medicine [66], we believe that the genome editing approaches will in the long run emerge as major therapeutic strategies.

Conflicts of interest

The authors declare that they have nothing to disclose regarding conflict of interest with this manuscript.

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