**REVIEW ARTICLE**

# A Tale of Two New Targets for Hypertriglyceridaemia: Which Choice of Therapy?

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## Abstract

Angiopoietin-like protein 3 (ANGPTL3) and apolipoprotein C-III (apoC-III) are novel metabolic targets for correcting hypertriglyceridaemia (HTG). As a background to their potential clinical use, we review the metabolic aetiology of HTG, particular abnormalities in triglyceride-rich lipoproteins (TRLs) and their role in atherosclerotic cardiovascular disease (ASCVD) and acute pancreatitis. Molecular and cardiometabolic aspects of ANGPTL3 and apoC-III, as well as inhibition of these targets with monoclonal antibody and nucleic acid therapies, are summarized as background information to descriptions and analyses of recent clinical trials. These studies suggest that ANGPTL3 and apoC-III inhibitors are equally potent in lowering elevated plasma triglycerides and TRLs across a wide range of concentrations, with possibly greater efficacy with inhibition of apoC-III. ANGPTL3 inhibition may, however, have the advantage of greater lowering of plasma LDL cholesterol and could specifically address elevated LDL cholesterol in familial hypercholesterolaemia refractory to standard drug therapies. Large clinical outcome trials in relevant populations are still required to confirm the long-term efficacy, safety and cost effectiveness of these potent agents for mitigating the complications of HTG. Beyond targeting severe chylomicronaemia in the prevention of acute pancreatitis, both agents could be useful in addressing residual risk of ASCVD due to TRLs in patients receiving best standard of care, including behavioural modifications, statins, ezetimibe, fibrates and proprotein convertase subtilisin/kexin type 9 inhibitors.

## 1 Introduction

Beyond the significant causal role of elevated low-density lipoprotein (LDL) cholesterol, epidemiological and Mendelian randomization studies have demonstrated that elevated plasma concentrations of triglycerides, which reflect the accumulation of triglyceride-rich lipoproteins (TRLs), are predictive of atherosclerotic cardiovascular disease (ASCVD) [1, 2]. However, treatments that lower plasma triglycerides have not been consistently shown to reduce cardiovascular outcomes. Angiopoietin-like protein 3 (ANGPTL3) and apolipoprotein C-III (apoC-III) are two new metabolic targets

| **Key Points** |
| --- |
| Hypertriglyceridaemia, specifically due to elevated triglyceride-rich lipoproteins (TRLs), is associated with increased risk of atherosclerotic cardiovascular disease; severe chylomicronaemia can also cause acute pancreatitis. |
| Treatments that target the production and clearance of TRLs mitigate these complications. |
| Nucleic acid-based therapies that target ANGPTL3 or apoC-III offer a new approach to lowering elevated TRLs, with preliminary studies testifying to their utility in clinical practice. |
| ANGPTL3 inhibition also lowers LDL particle concentration and may therefore have an overall advantage as a lipid-regulating agent. |
| Large clinical outcome trials are needed to confirm the efficacy, safety, cost effectiveness and clinical value of these new agents. |

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for the specific treatment of hypertriglyceridaemia (HTG). This article summarizes the aetiology and current treatment of HTG and reviews the recent clinical trials that may elucidate the therapeutic role of inhibitors of ANGPTL3 and apoC-III.

## 2 Metabolism of Triglyceride-Rich Lipoproteins (TRLs)

TRLs are composed of both exogenous and endogenous triglycerides. Triglycerides that originate from dietary fat are absorbed following a meal by enterocytes, where they combine with apolipoprotein (apo) B-48 to form chylomicrons, entering the circulation and picking up apoC-II, apoC-III and apoE [3, 4]. Within the circulation, chylomicrons are hydrolysed by lipoprotein lipase (LPL) to produce free fatty acids and chylomicron remnants; LPL activity is highly regulated by various proteins including apoC-II, apoC-III, apoA-V, ANGPTL3 and ANGPTL4 [5, 6]. The released free fatty acids are then used by various cell types as a fuel source or resynthesized with glycerol into triglycerides and stored. Chylomicron remnants, now rich in cholesterol esters and apoE, are removed from the circulation via the LDL receptor or LDL receptor-related proteins in the liver [7].

Triglycerides synthesized in the liver from free fatty acids and glycerol can form very low-density lipoprotein (VLDL) particles in association with apoB-100. During secretion, apoC-I, apoC-II, apoC-III and apoE are added to the surface of VLDL and the particles are then hydrolysed in the circulation by LPL, producing progressively smaller VLDL particles and then intermediate-density lipoproteins (IDLs); IDLs are taken up by the liver and catabolized or further catabolized by LPL and triglyceride lipase to form LDL particles (Fig. 1) [3, 4]. Significantly, ANGPTL3 and apoC-III are negative regulators of the clearance of all TRLs from the circulation at the level of LPL and hepatic receptors.

## 3 Hypertriglyceridaemia

Elevated plasma triglycerides or HTG may be defined as borderline (mild), moderately elevated, severe and extreme, according to progressive gradations in plasma triglyceride concentrations (Table 1). A commonly agreed global definition of HTG is a plasma triglyceride concentration persistently ≥ 1.7 mmol/L (≥ 150 mg/dL) [8]. Over 95% of people susceptible to HTG carry multiple genes that interact with non-genetic factors and perturb the metabolism of TRLs [1, 9–12].

A subset of severe HTG is a rare form (1–10 per million) of monogenic HTG, called familial chylomicronaemia syndrome (FCS) due to mutations in one of at least six genes (*LPL*, *APOC2*, *LMF1*, *GPHIHBP1*, *APOA5*, *G3PDH1*) [9, 10]. Homozygous or compound heterozygous mutations in these genes markedly impair chylomicron clearance, leading to severe HTG that can manifest in youth as eruptive xanthomata, lipaemia retinalis and acute pancreatitis. Multifactorial chylomicronaemia syndrome (MCS) has a prevalence of 1 in 600 of the population, resulting from a combination of heterozygous variants in the six FCS genes and/or accumulated common small-effect triglyceride-raising polymorphisms identified in genome-wide association studies (GWAS), such as *APOA1-C3-A4-A5*, *TRIB1*, *LPL*, *MLXIPL*, *GCKR*, *FADS1-2-3*, *NCAN*, *APOB*, *PLTP* and *ANGPTL3* [9, 10].

*E2E2* homozygosity (prevalence 1%) is a necessary but not sufficient cause of type III dysbetalipoproteinaemia, which is rare (1 in 10,000) and causes HTG and premature atherosclerosis due to accumulation of TRL remnants that are not cleared by the liver [13].

Mild-to-moderate HTG is usually a result of multiple common allelic gene variants interacting with an unhealthy diet and lifestyle that impair TRL metabolism [12]. The most common forms of genetic HTG are familial combined hyperlipidaemia (FCHL) and familial hypertriglyceridaemia [14, 15]. FCHL can present as elevated total and LDL cholesterol levels with mild triglycerides, elevated triglycerides alone, or predominately elevated triglycerides and slightly elevated LD. FCHL is characterized by an increase in apoB and small dense LDL particles and is often seen in patients with metabolic syndrome, central obesity, type 2 diabetes mellitus (T2DM) and a family history of premature coronary artery disease (CAD). FCHL results from hepatic overproduction of VLDL apoB particles that may be variably enriched in triglycerides and impair clearance of apoB-containing particles from the circulation [16]. Familial hypertriglyceridaemia (type IV hyperlipoproteinaemia), also a multigenic condition [15], is due to an increase in triglyceride content in VLDL particles with no concomitant elevation of apo B concentration. The precise kinetic defect remains unclear but may be caused by increased triglyceride synthesis and secretion of very large triglyceride-enriched VLDL particles, and/or impaired clearance of VLDL-TGs [17, 18].

Secondary causes of HTG can also include T2DM, hypothyroidism, pregnancy, hepatosteatosis, significant weight re-gain after weight loss, nephrotic syndrome and some medications [1, 4, 12]. As reviewed by others [3, 4, 6, 12, 19], increased availability of free fatty acids (FFAs) from de novo lipogenesis (DNL) from glucose and the adipose tissue through increased lipolysis increases hepatic triglyceride synthesis, leading to overproduction
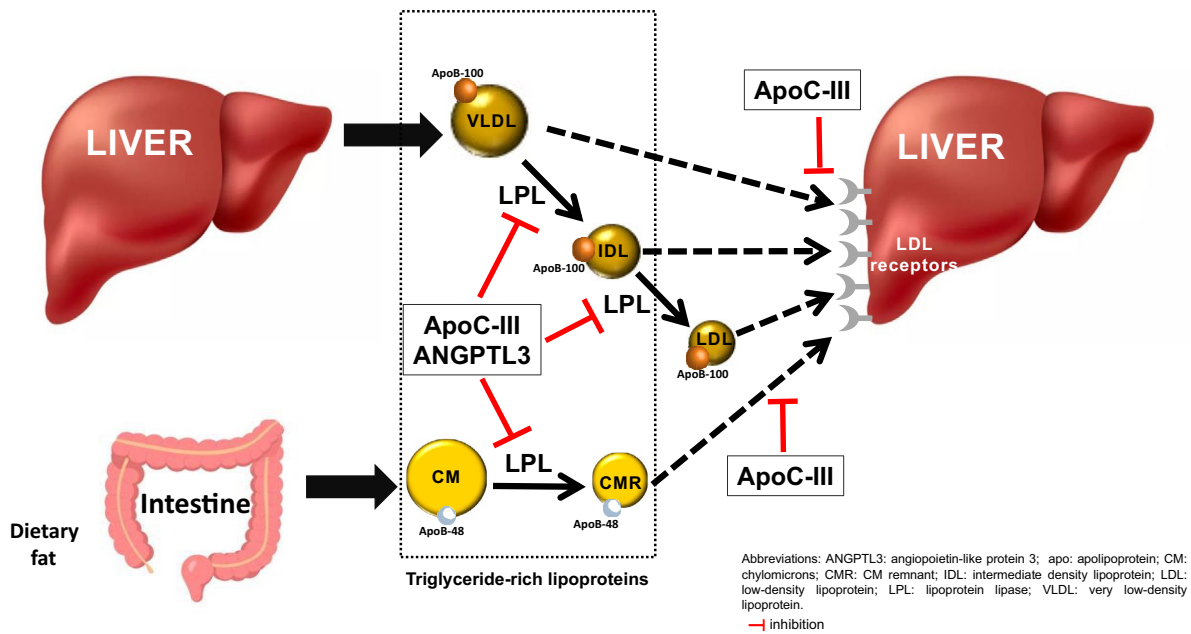
**Fig. 1** Schematic representation of the putative mode of action of angiopoietin-like protein 3 (ANGPTL3) and apolipoprotein C-III (apoC-III) inhibition on lipoprotein transport in the circulation in humans

**Table 1** Definitions of hypertriglyceridaemia according to recent guidelines

| Category | European Atherosclerosis Society | American College of Cardiology* |
|---|---|---|
| Optimal | < 1.2 (<100) | |
| Borderline | 1.2–1.7 (100–150) | |
| Moderately elevated | 1.7–5.7 (150–500) | ≥ 1.7 (≥150) |
| Severe | 5.7–10.0 (500–880) | ≥5.7 (≥500) |
| Extreme | > 10 (>880) | |

Values expressed as mmol/L (mg/dL)

* Persistent over 4 weeks

of large triglyceride-rich VLDL particles in patients with T2DM. Furthermore, insulin resistance increases chylomicron particle concentrations by stimulating DNL, increasing microsomal triglyceride transfer protein activity and enhancing intracellular chylomicron stability in the intestine. It also increases FFAs delivery to the enterocytes, impairs insulin signalling and increases intestinal lipid absorption during the postprandial period. Collectively, these effects could increase the enterocytic secretion of apoB-48-containing chylomicrons. Increased competition between chylomicron and VLDL remnants for hepatic receptors also delays the uptake of TRL remnants by this pathway. Insulin resistance also increases

apoC-III synthesis, decreases LPL production and down-regulates LDL receptor expression, limiting TRL lipolysis and removal [3, 6]. The role of ANGPTL3 and apoC-III in the pathogenesis of HTG is reviewed later.

## 4 Hypertriglyceridaemia, Atherosclerotic Cardiovascular Disease and Acute Pancreatitis

Several Mendelian randomization studies have consistently demonstrated that genetically elevated remnant cholesterol and apoB-containing TRLs increase the risk of ASCVD events [20–22]. An observational analysis from the Copenhagen General Population Study and the Copenhagen City Heart Study has demonstrated that non-fasting plasma triglycerides (≥5 mmol/L) or remnant cholesterol (>2.3 mmol/L) increases risk for aortic stenosis, ischaemic stroke and myocardial infarction by 1.5-fold, 3-fold and 5-fold, respectively [22–24]. In a meta-regression analysis of randomized controlled statin and non-statin trials, triglyceride lowering (per 1 mmol/L reduction) is associated with 16% lower risk of major vascular events after adjusting for LDL cholesterol [25]. In a Canadian cohort, approximately 25% of patients with ASCVD had HTG despite controlled LDL cholesterol in the general population [26].

Experimental evidence suggest that small TRL remnants readily infiltrate the subendothelial space, where they are trapped by a connective tissue matrix. These particles are rapidly phagocytosed by arterial wall macrophages, which are then transformed into 'foam cells' [27]. Furthermore, TRL remnants impair endothelial function, inhibit fibrinolysis, enhance coagulation and activate monocytes and inflammation. TRL lipolysis also releases toxic products, such as oxidized fatty acids and lysolecithin, that induce endothelial cell inflammation and coagulation [28–32].

Severe HTG is known to increase risk of acute pancreatitis, which accounts for 1–10% of episodes of acute pancreatitis. Mild-to-moderate HTG (non-fasting plasma triglyceride levels ≥ 5 mmol/L) is associated with a 10-fold increase in risk of acute pancreatitis. The mechanism for how HTG contributes to the development of acute pancreatitis is unclear, but may principally involve lipotoxicity and low-grade inflammation [33, 34].

## 5 Treatment Guidelines for Hypertriglyceridaemia and the REDUCE-IT Trial

TRLs are well recognized as a risk-enhancing factor and target for therapy in recent international lipid guidelines and position statements [4, 8, 35, 36]. The general consensus is to optimize lifestyle and behavioural interventions as a first step, after ruling out secondary causes, followed by statin therapy in those with moderate HTG and elevated 10-year risk of ASCVD. In patients with severe HTG, dietary therapy followed by use of a fibrate is recommended to mitigate risk of acute pancreatitis. Importantly, the use of high pure eicosapentaenoic acid (EPA), as in the REDUCE-IT (Reduction of Cardiovascular Events with Icosapent Ethyl—Intervention Trial) [37], has been incorporated in the most recent American Heart Association/ American College of Cardiology (AHA/ACC) guideline for persistent HTG in high-risk patients receiving statin treatment [8]. The use of fibrates, in particular fenofibrate, has been recommended as an add-on to statin therapy for ASCVD prevention in high-risk patients only in the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS) guidelines [35].

REDUCE-IT investigated the effect of icosapent ethyl on ischaemic events in statin-treated patients with elevated triglycerides and cardiovascular disease (CVD) or diabetes [37]. In 8179 patients who were followed for a median of 4.9 years, high-dose icosapent ethyl (4 g/day) significantly reduced the burden of first, subsequent and total ischaemic events [37]. Although icosapent ethyl effectively lowers triglycerides, it also has anti-inflammatory and plaque stabilizing properties, which might have contributed to the benefits seen in the trial. It was also recently suggested that the beneficial effects seen in REDUCE-IT are largely attributable to the negative effect of the placebo corn oil on events [38]. The STRENGTH trial investigated a carboxylic formulation of omega-3 fatty acids on major cardiovascular events in 13,078 statin-treated patients with HTG and high ASCVD risk [39]. However, this study was terminated prematurely owing to futility. A particular complication of high-dose EPA is an attendant risk of atrial fibrillation [40]. An outcome trial is currently underway to investigate the effect of EPA on the incidence of cardiovascular events in patients with CAD (RESPECT-EPA; UMIN000012069).

An international consensus statement testifying to the potential value of pemafibrate, a new selective peroxisome proliferator-activated receptor alpha modulator (SPPARMα), supports the PROMINENT study [41]. The study is currently underway and due for completion in 2022, and is addressing the effect of pemafibrate on cardiovascular outcomes in statin-treated patients with T2DM and mild-to-moderate HTG [42]. Beyond pure EPA and SPPARMα, inhibition of ANGPTL3 and apoC-III could pave the way for more effective and sustained reductions in plasma triglycerides and TRLs and the inclusion of these agents in future clinical practice.

## 6 Angiopoietin-Like Protein 3 (ANGPTL3): Molecular and Cardiometabolic Aspects

Angiopoietin-like protein 3 (ANGPTL3) is a 460 amino acid glycoprotein that is secreted predominately by the liver, where its expression is activated by the oxysterol-stimulated liver X receptor [5]. It appears to be active predominately after feeding [43], where it works with ANGPTL8 to inhibit LPL, the enzyme responsible for hydrolysis of circulating triglycerides (Fig. 1). ANGPTL3 also inhibits endothelial lipase (EL) in vitro [44]. Composed of an N-terminal signal peptide, an N-terminal coiled-coil domain, a linker region and a C-terminal fibrinogen-like domain, it shares high sequence homology with both ANGPTL4 and ANGPTL8 [5]. ANGPTL3 undergoes cleavage and glycosylation to give an N-terminal fragment containing the coiled-coil region and a C-terminal fragment containing the fibrinogen-like domain. Intracellular cleavage is mediated mainly via furin (or proprotein convertase subtilisin/kexin type 3, PCSK3) in hepatocytes, while extracellular cleavage is mediated via paired basic amino acid-cleaving enzyme 4 (PACE4 or PCSK6) [45]. The N-terminal coiled-coil domain is involved in binding and inhibiting both LPL and EL, while the C-terminal fibrinogen domain mediates the angiogenic effects [5]. This inhibitory effect appears to be via a mechanism that involves unfolding and

dimeric disassociation of the LPL protein, which is enabled by ANGPTL8 [43]. The inhibitory actions of ANGPTL3 on EL are mechanistically unclear, but appear to result in low plasma HDL levels [44]. ANGPTL3 also has secondary and possibly direct effects on glucose metabolism, with bi-allelic loss of function mutations in ANGPTL3 giving rise to increased insulin sensitivity and low plasma glucose levels [46].

Animal experiments utilising ANGPTL3 loss-of-function (LOF) mutations and large-scale genomic studies in humans provide significant evidence for the inhibition of ANGPTL3 as a therapeutic target in ASCVD [5, 47–52]. Carriers of LOF mutations in *ANGPTL3* have on average a 35% reduction in the risk of CAD events, with smaller studies demonstrating a direct association between plasma ANGPTL3 and imaging evidence of atherosclerosis [50–53]. Furthermore, the positive association between plasma ANGPTL3 levels and incident CAD events appears to be independent of plasma lipid levels [51], suggesting that inhibiting ANGPTL3 may have a direct effect on atherosclerosis [47].

## 7 Apolipoprotein C-III (ApoC-III): Molecular and Cardiometabolic Aspects

Apolipoprotein C-III (apoC-III) is a 79 amino acid glycoprotein that is synthesized predominately in the liver, transported freely among plasma lipoproteins, and inhibits both lipolytic activity and hepatic uptake of TRLs (Fig. 1) [3, 6, 54]. Structurally, it contains two amphipathic helices and aromatic tryptophan residues in the carboxyl-terminal, which appear to be important for interaction with TRLs. After synthesis, apoC-III undergoes post-translational modification that results in at least three different glycoforms—unsialylated apoC-III$_o$, monosialylated apoC-III$_1$ and disialylated apoC-III$_2$—which pertains to hepatic clearance [55]. The rate of transcription of apoC-III is stimulated by glucose and decreased by insulin, PPARα and farnesoid X receptor [3, 6, 56]. Accordingly, apoC-III expression is upregulated in insulin resistance, with glycaemic control a major determinant of apoC-III secretion [56]. In the circulation, apoC-III is mainly present in TRLs and HDL, and to a lesser extent on LDL. The distribution of apoC-III among lipoproteins is dependent on the metabolic status of the patient, varying with fasting and feeding, and triglyceride levels [6].

ApoC-III has pro-atherogenic properties and is accordingly predictive of cardiovascular mortality [6, 57, 58]. ApoC-III increases the affinity of LDL for artery wall proteoglycans, increasing subendothelial accumulation of lipoprotein, and increases the enrichment of LDL particles with apoC-III [59, 60]. Animal apoC-III knockout models have been shown to have reduced triglyceride levels and increased protection against atherosclerosis [61]. In humans, LOF mutations in *APOC3* are associated with reduced plasma triglycerides and reduced risk for ASCVD [57, 58]. Patients with heterozygous *APOC3* LOF mutation (R19X) had 35% lower plasma triglyceride levels due to markedly higher fractional clearance rates of VLDL-TG and VLDL-apoB100 but increased production of LDL-apoB [62]. As reviewed later, this supports the effect of apoC-III inhibitors on LDL cholesterol in patients with severe HTG.

## 8 Pharmacological Approaches to Inhibit ANGPTL3 and ApoC-III

Several promising therapies, using antibodies or nucleic acid inhibition, targeting ANGPTL3 and apoC-III protein or mRNA, respectively, have been developed and trialled [63–67]. Monoclonal antibodies (mAb) work by binding to and inactivating the target protein of interest. These therapies are complex and expensive to produce and can result in the activation of innate immunity and the development of neutralising auto-antibodies [64]. As critically reviewed by others [63–67], antisense oligonucleotides (ASOs) and small interfering RNA (siRNA) therapies work by inhibiting the mRNA transcripts of a selected protein, halting its translation and resulting in the protein mRNA's degradation [63–67]. ASOs utilize short, single strands of RNA that bind to the specific mRNA, leading to competitive inhibition of translation or to degradation of the resulting complex. By contrast to ASOs, siRNA therapy utilizes double-stranded RNA, which consists of a guide (or anti-sense) strand and a passenger (or sense) strand, and interacts with an endogenous, multi-enzyme complex in the cytoplasm, called the RNA-induced silencing complex (RISC). The passenger strand loads the RNA duplex onto RISC, which contains a key endonuclease called Argonaute 2 (AGO2) that efficiently effectuates mRNA cleavage (see review by Macchi et al. [63]). Inclusion of a triantennary *N*-acetylgalactosamine (GalNAc$_3$) complex conjugated to the oligonucleotide or siRNA, enhances liver specificity resulting in greater potency at lower doses as well as reduced systemic toxicity (Table 2) [68].

### 8.1 Monoclonal Antibody-Based Therapies

Evinacumab is a fully human IgG4 mAb that specifically inhibits ANGPTL3 in the circulation [50, 69] inducing ANGPTL3 deficiency and activating both LPL and EL activity [70, 71]. Bioavailability and lipid-lowering potency is greater with intravenous compared with subcutaneous administration and as it is not metabolized by cytochrome

**Table 2** Newly developed therapies for hypertriglyceridaemia

| Therapy | Target | Mode of action | Benefits | Risks |
|---|---|---|---|---|
| Monoclonal antibodies (mAbs) | Protein | Binds to and inactivates the target protein | Effective at lowering triglyceride levels<br>High specificity and favourable safety profile | Complex<br>Expensive<br>Susceptible to development of neutralizing auto-antibodies |
| Antisense oligonucleotides (ASO) | mRNA | Inhibits mRNA transcription of target protein, resulting in mRNA degradation | Longer administration intervals<br>Effective at lowering triglyceride levels<br>High specificity and favourable safety profile | Addition of GalNAc$_3$ increases specificity and lowers dose required |
| Small interfering RNA (siRNA) | mRNA | Inhibits mRNA transcription of target protein, facilitating cleavage and degradation and halt to protein production | Longer administration intervals<br>Effective at lowering triglyceride levels<br>High specificity and favourable safety profile | Addition of GalNAc$_3$ increases specificity and lowers dose required |

P450, evinacumab does not interact with other drugs [72]. However, as with all other mAb therapies, evinacumab is potentially susceptible to the development of an adaptive immune response and autoantibodies could neutralize its therapeutic effects over time [73].

Monoclonal antibodies targeting apoC-III are difficult to produce owing to the relatively high abundance of the lipoprotein and its association with lipoproteins [74]. STT505 is a humanized mAb that targets lipoprotein-bound human apoC-III and promotes its dissociation from lipoproteins. Although only tested in mice expressing human apoC-III, it has been shown to reduce circulating apoC-III levels by 40–60%, predominately due to increased clearance [75].

## 8.2 Nucleic Acid Based Therapies

### 8.2.1 Antisense Oligonucleotide (ASO) Therapy

Vupanorsen (AKCEA-ANGPTL3-L$_{Rx}$) is a ligand-conjugated ASO targeted at *ANGPTL3* [76]. Mouse models demonstrated significant reductions in plasma triglyceride concentrations that were associated with reduced hepatic triglyceride content, increased insulin sensitivity and reduced atherosclerosis progression [76]. ASO-mediated inhibition of ANGPTL3 has also recently been shown to increase reverse cholesterol transport in mice, as measured by an in vivo macrophage-to-faeces assay utilizing an injection of $^3$H-cholesterol-labelled macrophage foam cells [77].

Volanesorsen is a 2'-O-methoxyl-modified single-stranded ASO that decreases the mRNA of *APOC3* in animal models and can lower plasma triglycerides in humans by > 70% [78–80]. Although not yet approved by the US Federal Drug Administration (FDA), volanesorsen has been granted conditional marketing authorization by the European

Medicines Agency as an adjunct to diet and triglyceride-lowering therapy for mitigating acute pancreatitis in genetically defined patients with FCS. Olezarsen (formerly AKCEA-APOCIII-L$_{Rx}$) is a next-generation, ligand-conjugated apoC-III ASO that has similar metabolic effects (reductions in triglycerides and apoC-III) to volanesorsen and a better overall safety profile, particularly an absence of significant thrombocytopaenia [81].

### 8.2.2 Small Interfering RNA (siRNA) Therapy

The most advanced siRNA to date for therapeutic inhibition of ANGPTL3 is the Targeted RNAi Molecule (TRIM™, Arrowhead Pharmaceuticals). In ARO-ANG3, each RNA strand is 2'-methoxy (or 2'-F) and phosphorothioate modified to induce resistance to endonucleases and offset immune activation [63, 82–84]. Furthermore, the sense strand is ligand-conjugated to ensure specific delivery to the liver, with dose-dependent, potent and sustained reductions in serum ANGPTL3 and triglycerides.

*APOC3* mRNA may also be targeted with a ligand-bound, double-stranded siRNA platform [63, 66, 67]. As reviewed earlier, this approach leverages RISC and can thereby potently and durably lower plasma apoC-III and triglyceride concentrations in humans [85], with no significant adverse effects, particularly thrombocytopaenia.

## 9 Clinical Trials

The following section reviews the clinical trials targeting ANGPTL3 and apoC-III, focusing on triglyceride-lowering effects in different populations, including those with HTG and FCS. The findings are summarized in Tables 3 and 4.

### 9.1 ANGPTL3 mAbs

In a phase I, double-blind, single ascending dose study in 83 healthy volunteers with plasma triglyceride levels between 150 and 450 mg/dL, receiving different subcutaneous (SC) or intravenous (IV) doses of evinacumab, resulted in reductions in fasting triglycerides ($-63\%$) and LDL cholesterol ($-28\%$) with the highest IV treatment regimen (20 mg/ kg body weight) at day 15 [50]. The results of this single ascending dose study were subsequently combined with a multiple ascending dose study in 56 healthy volunteers [69]. Dose-dependent reductions in triglycerides were observed, with a mean reduction of 78% at 8 weeks with the 20-mg/ kg IV dose administered every 4 weeks; a significant reduction in plasma LDL-cholesterol concentration ($-35\%$) was also observed at this dose, with no serious treatment-related adverse events reported [69]. Kinetic data suggest that evinacumab can decrease VLD-apoB-100 production and increase IDL-apoB-100 clearance in patients with homozygous familial hypercholesterolaemia (FH) [70]. Whether a similar effect of evinacumab also applied to patients with HTG remains to be demonstrated.

### 9.2 ANGPTL3 Nucleic Acid Therapies

In a phase I dose-finding study in 44 healthy volunteers, vupanorsen resulted in dose-dependent lowering of plasma triglyceride concentrations, with mean reductions after 6 weeks of 60 mg SC weekly being 50%, associated with a decrease in LDL cholesterol ($-33\%$) and apoB ($-22\%$) concentrations; no serious adverse events were reported [76]. In a placebo-controlled phase II study in patients with fasting triglycerides > 150 mg/dL, T2DM and hepatic steatosis [86], significant mean reductions in triglycerides of 36%, 53% and 47% at 6 months were observed with 40 mg every 4 weeks (Q4W), 80 mg Q4W and 20 mg weekly, respectively, of SC vupanorsen. However, there was no improvement in indices of insulin resistance, glycaemia and hepatic steatosis. Despite these findings, in a press release on 31 January 2022, Pfizer announced the discontinuation of the clinical development programme for vupanorsen. This was based on two findings from the TRANSLATE-TIMI 70 study: first, the magnitude of lowering triglycerides and non-HDL cholesterol was considered sub-optimal; second, there was an increase in quantity of liver fat with higher dose of the investigational product [87]. In a phase I study of healthy individuals with fasting triglycerides > 100 mg/dL, repeated doses of ARO-ANG3 given SC at day 1 and day 29 achieved a mean decrease of 66% in serum triglycerides with the 300-mg SC dose after 16 weeks [83, 84]. There were no serious adverse events and no clinically significant adverse changes in platelet count and liver function tests.

### 9.3 ApoC-III Nucleic Acid Therapies

The APPROACH study was a phase III, double-blind randomized trial to evaluate the safety and efficacy of volanesorsen in patients with FCS and plasma triglycerides >500 mg/dL [79]. At 3 months there was an 84% decrease in plasma apoC-III levels in patients receiving the ASO compared with placebo, which was accompanied by a 77% decrease in triglycerides and a 136% increase in LDL-cholesterol concentrations. A significant portion of the volanesorsen group experienced injection-site reactions, which were not observed in the placebo group, and 45% of the volanesorsen group also experienced severe thrombocytopenia [79].

The COMPASS study was a multi-centre, placebo-controlled phase III trial of the efficacy and safety of subcutaneously administered volanesorsen in patients with predominantly MCS and plasma triglycerides > 500 mg/ dL [80]. Given the findings from APPROACH, a protocol change was made in COMPASS after 13 weeks of treatment that required the dose of volanesorsen to be decreased, which helped offset thrombocytopenia. After 3 months, volanesorsen markedly lowered plasma apoC-III ($-76\%$) and triglyceride ($-71\%$) concentrations, but increased plasma LDL cholesterol ($+96\%$), which were sustained over 6 months of treatment. This was associated with a reduction in acute pancreatitis. Mild injection-site reactions and mild thrombocytopenia were again more frequently seen in patients receiving volanesorsen [80].

Table 4 also summarizes other key studies utilizing apoC-III inhibitors in subjects with HTG. The findings from the COMPASS trial augment the findings of these trials with volanesorsen [81, 88–90] and demonstrate that this intervention is efficacious across a wide range of plasma triglyceride concentrations and particularly in patients with MCS. Furthermore, the efficacy of treatment appears to be independent of the type of genetic variants impairing the activity of LPL, supporting the notion that volanesorsen can operate via LPL-independent mechanisms [90], including by inhibition of TRL production. Another important finding was that volanesorsen was equally efficacious in lowering triglycerides in patients both taking and not taking lipid-lowering treatments, including fibrates, statins and fish oils [88]. A potential limitation of these studies, however, is the increase in LDL-cholesterol concentration with volanesorsen treatment (see Table 3), although this can be offset with use of statin therapy. Whether such increase in plasma LDL cholesterol is clinically relevant remains to be determined. In contrast to findings in the APPROACH and COMPASS trials, volanesorsen or olezarsen ASO treatment did not significantly alter plasma LDL-cholesterol

**Table 3** Summary of clinical trials targeting ANGPTL3 in various patient populations

| Study | Intervention | Design | Population | Dose | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | TG | LDL-C | HDL-C | ApoB | VLDL-C | Non-HDL-C |
| Dewey et al. [50] | Evinacumab (mAb) | Phase I Double-blind, placebo-controlled, single ascending dose | 83 healthy participants TG 150–450 mg/dL LDL-C ≥100 mg/dL | Placebo | −4 | +3 | +3 | | | |
| | | | | 75 mg SC | −11 | −3 | +3 | | | |
| | | | | 150 mg SC | −11 | −5 | −7 | | | |
| | | | | 250 mg SC | −32 | −14 | −12 | | | |
| | | | | Placebo | −18 | −5 | −1.9 | | | |
| | | | | 5 mg/kg IV | −49 | −17 | −18 | | | |
| | | | | 10 mg/kg IV | −60 | −19 | −27 | | | |
| | | | | 20 mg/kg IV | −63 | −28 | −20 | | | |
| Ahmad et al. [69] | Evinacumab (mAb) | Phase I Double-blind, placebo-controlled, multiple ascending dose | 139 participants TG 150–450 mg/dL LDL-C ≥100 mg/dL | Placebo | −6 | +5 | +4 | +1 | −10 | −5 |
| | | | | 75 mg SC | −22 | −1 | −8 | +1 | −15 | −4 |
| | | | | 150 mg SC | −23 | −12 | −8 | −6 | −16 | −7 |
| | | | | 250 mg SC | −45 | −17 | −10 | −9 | −32 | −18 |
| | | | | 5 mg/kg IV | −58 | −14 | −16 | +1 | −52 | −24 |
| | | | | 10 mg/kg IV | −70 | −19 | −24 | −20 | −58 | −32 |
| | | | | 20 mg/kg IV | −62 | −25 | −17 | −27 | −72 | −37 |
| | | | | Placebo | −5 | 0 | +5 | 0 | −7 | −2 |
| | | | | 150 mg QW SC | −10 | −6 | −9 | −5 | −10 | −28 |
| | | | | 300 mg Q2W SC | −28 | −18 | −20 | −10 | −13 | −18 |
| | | | | 300 mg QW SC | −42 | −11 | −12 | −7 | −42 | −42 |
| | | | | 450 mg Q2W SC | −47 | −18 | −9 | −18 | −40 | −24 |
| | | | | 450 mg QW SC | −45 | −6 | −20 | −10 | −41 | −32 |
| | | | | 20 mg/kg Q4W | −78 | −35 | −8 | −35 | −73 | −44 |
| Graham et al. [76] | Vupanorsen (ASO) | Phase I Double-blind, placebo-controlled, single & multiple ascending | 44 healthy participants TG >150 mg/dL LDL-C >100 mg/dL | Placebo | +179 | 0 | −19 | | +182 | +37 |
| | | | | 20 mg SC SD | −17 | +10 | +13 | | −25 | +4 |
| | | | | 40 mg SC SD | −17 | −10 | −1 | | +12 | −10 |
| | | | | 80 mg SC SD | −56 | −24 | −7 | | −58 | −31 |
| | | | | Placebo | −11 | +14 | −6 | +11 | −4 | +9 |
| | | | | 10 mg SC QW | −33 | −1 | −4 | −3 | −28 | −10 |
| | | | | 20 mg SC QW | −63 | −4 | −6 | −13 | −60 | −18 |
| | | | | 40 mg SC QW | −54 | −25 | −7 | −26 | −49 | −31 |
| | | | | 60 mg SC QW | −50 | −33 | −27 | −22 | −49 | −37 |
| Gaudet et al. [86] | Vupanorsen (ASO) | Phase II Double-blind, placebo-controlled, multiple ascending dose | 105 patients with T2DM, hepatic steatosis TG >150 mg/dL | Placebo | −16 | 0 | +7 | −3 | −14 | −4 |
| | | | | 40 mg SC Q4W | −36 | +6 | −2 | −7 | −35 | −13 |
| | | | | 80 mg SC Q4W | −53 | −7 | −18 | −12 | −47 | −21 |
| | | | | 20 mg SC QW | −47 | −12 | −4 | −10 | −40 | −22 |

**Table 3** (continued)

| Study | Intervention | Design | Population | Dose | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | TG | LDL-C | HDL-C | ApoB | VLDL-C | Non-HDL-C |
| Watts et al. [83, 84] | ARO-ANG3 (siRNA) | Phase I Double-blind, placebo-controlled, single ascending dose | 40 healthy participants TG >100 mg/dL LDL-C >70 mg/dL | Placebo | +31 | +8 | +6 | +9 | +30 | +9 |
| | | | | 35 mg SC | −12 | −23 | −9 | −21 | −12 | −20 |
| | | | | 100 mg SC | −29 | −24 | +8 | −21 | −29 | −25 |
| | | | | 200 mg SC | −47 | 0 | −14 | −2 | −46 | −13 |
| | | | | 300 mg SC | −52 | −8 | −16 | −11 | −52 | −17 |
| | | Open label, multiple-ascending dose | 12 healthy participants TG >100 mg/dL LDL-C >70 mg/dL | 100 mg SC (day 1 & 29) | −61 | −37 | −14 | −31 | −62 | −41 |
| | | | | 200 mg SC (day 1 & 29) | −66 | −46 | −21 | −39 | −66 | −49 |
| | | | | 300 mg SC (day 1 & 29) | −67 | −361 | −37 | −28 | −65 | −49 |

*ApoB* apolipoprotein B, *apoC-III* apolipoprotein C-III, *ASO* antisense oligonucleotide, *HDL-C* high-density lipoprotein cholesterol, *HTG* hypertriglyceridaemia, *IV* intravenous, *LDL-C* low-density lipoprotein cholesterol, *mAb* monoclonal antibody, *QW* every week, *Q2W* every 2 weeks, *Q4W* every 4 weeks, *SC* subcutaneous, *SD* single dose, *siRNA* small interfering RNA, *TG* triglycerides, *T2DM* type 2 diabetes mellitus, *VLDL-C* very-low-density lipoprotein cholesterol

**Table 4** Summary of clinical trials targeting APOC-III in various patient populations.

| Study | Intervention | Design | Population | Dose | Results | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | TG | LDL-C | HDL-C | ApoB | VLDL-C | Non-HDL-C |
| Witztum et al. [79] | Volanesorsen (ASO) | Phase III Double-blind, placebo-controlled | 66 patients with FCS TG >750 mg/dL | Placebo | +18 | +6 | +7 | +4 | +15 | +7 |
| | | | | 300 mg SC | −77 | +136 | +46 | +20 | −58 | +46 |
| Gouni-Berthold et al. [80] | Volanesorsen (ASO) | Phase III Double-blind, placebo-controlled | 114 patients with severe HTG or FCS, TG >500 mg/dL | Placebo | +1 | +5 | +4 | −2 | −12 | +1 |
| | | | | 300 mg SC | −71 | +96 | +61 | +6 | −72 | −27 |
| Alexander et al. [81] | Olezarsen (ASO) | Phase I/IIa Double-blind, placebo-controlled, single and multiple ascending dose | 67 healthy participants with TG ≥90 or ≥200 mg/dL | Placebo | +22 | −8 | +4 | −14 | +5 | −6 |
| | | | | 10 mg SC | −12 | +3 | +7 | | −23 | −5 |
| | | | | 30 mg SC | −11 | −7 | +19 | | −2 | −6 |
| | | | | 60 mg SC | −43 | +2 | +34 | | −65 | −12 |
| | | | | 90 mg SC | −68 | +8 | +63 | −16 | −81 | −24 |
| | | | | 120 mg SC | −77 | −7 | +62 | −26 | −68 | −26 |
| | | | | Placebo | +18 | −1 | +3 | +3 | +6 | −1 |
| | | | | 15 mg SC QW | −71 | −3 | +50 | −15 | −71 | −22 |
| | | | | 30 mg SC QW | −73 | −17 | +56 | −26 | −73 | −30 |
| | | | | 60 mg SC Q4W | −65 | −22 | +76 | −30 | −40 | −31 |
| Digenio et al. [88] | Volanesorsen (ASO) | Phase II Double-blind, placebo-controlled | 15 patients with T2DM TG 200–500 mg/dL | Placebo | −10 | −6 | −7 | −10 | −14 | −8 |
| | | | | 300 mg SC | −69 | 0 | +42 | −21 | −73 | −22 |
| Clifton et al. [85] | ARO-APOC3 (siRNA) | Phase I Double-blind, placebo-controlled, multiple ascending dose | 32 patients with severe HTG (TG ≥300 mg/dL) | Placebo | −19 | | +9 | | | 0 |
| | | | | 10 mg SC | −74 | | +95 | | | −41 |
| | | | | 25 mg SC | −92 | | +116 | | | −60 |
| | | | | 50 mg SC | −85 | | +96 | | | −45 |
| | | | | 100 mg SC | −87 | | +110 | | | −39 |
| | | | 16 patients with chylomicronaemia (TG >880 mg/dL) | 50 mg SC | −88 | | +120 | | | −59 |
| Tardif et al. [91] | Olezarsen (ASO) | Phase II Double-blind, placebo-controlled, dose-ranging | 114 patients with moderate HTG and established ASCVD or at high risk of ASCVD (TG 200–500 mg/dL) | 10 mg Q4W | −23 | +8 | +11 | 0 | −27 | −6 |
| | | | | 15 mg Q2W | −56 | −6 | +33 | −17 | −48 | −24 |
| | | | | 10 mg QW | −60 | +16 | +40 | −7 | −56 | −15 |
| | | | | 50 mg Q4W | −60 | +3 | +29 | −12 | −58 | −19 |

*ApoB* apolipoprotein B, *apoC-III* apolipoprotein C-III, *ASCVD* atherosclerotic cardiovascular disease, *ASO* antisense oligonucleotide, *FCS* familial chylomicronaemia syndrome, *HDL-C* high-density lipoprotein cholesterol, *HTG* hypertriglyceridaemia, *LDL-C* low-density lipoprotein cholesterol, *mAb* monoclonal antibody, *QW* every week, *Q2W* every 2 weeks, *Q4W* every 4 weeks, *SC* subcutaneous, *siRNA* small interfering RNA, *TG* triglycerides, *T2DM* type 2 diabetes mellitus, *VLDL-C* very-low-density lipoprotein cholesterol

concentration in patients with mild-to-moderate HTG [81, 88]. This suggests that the effect of apoC-III inhibition on LDL cholesterol is dependent on fasting triglyceride or TRL concentrations. There is currently an ongoing clinical trial (BALANCE study) which investigates the effect of olezarsen in patients with FCS (ClinicalTrials.gov identifier: NCT04568434) and is due for completion in mid-2023.

In a phase I study to evaluate the effect of ARO-APOC3 (50 mg SC Q4W), significant reductions at 16 weeks were observed in apoC-III (98% and 96%), triglycerides (85% and 88%) and non-HDL cholesterol (45% and 59%) in patients with HTG (≥300 mg/dL) or chylomicronaemia (>800 mg/dL), respectively [85]. There were no serious adverse events reported. More recently, a phase II, dose-ranging study investigating the effect of olezarsen in patients with fasting triglycerides 200–500 mg/dL and were at high risk for or had established ASCVD was conducted. Treatment with olezarsen resulted in reductions in triglycerides of 23% for 10 mg Q4W, 56% for 15 mg Q2W and 60% for 10 mg weekly and 50 mg Q4W. Significant reductions were also seen for apoC-III, VLDL, non-HDL cholesterol and apoB [91].

## 10 Implications and Conclusions

Significant evidence supports the causal role of elevated TRLs, specifically remnant particles, in the development of ASCVD in both primary and secondary prevention

[1–4, 8]. International guidelines have highlighted increased plasma TRL and apoB concentrations as risk-enhancing factors for the prevention of ASCVD [8, 35, 36]. Elevated TRLs are also related to the increased risk of metabolic-associated fatty liver disease, aortic stenosis and acute pancreatitis [3, 22, 33, 34]. Despite best standard of care, including optimal LDL cholesterol concentrations, a significant proportion of patients with ASCVD remain at increased residual risk due to increased plasma concentrations of TRLs [25, 26]. The therapeutic use of high-dose pure EPA is supported by clinical trial evidence for benefit in secondary prevention of ASCVD [37]. However, the value of this trial has been challenged, with evidence suggesting that benefits are not mediated by reduction in TRLs and that the positive findings may relate to acceleration in risk of ASCVD in the group treated with 'placebo' oil [38]. New therapeutic targets, such as ANGPTL3 and apoC-III [5, 6], are therefore welcomed for addressing gaps in the management of HTG in secondary prevention, as well as for addressing the management of severe HTG as a risk factor for acute pancreatitis [8]; a particular advantage of these agents is the durability of effects that may address poor adherence to orally administered therapies.

The recent clinical trial evidence that we have reviewed suggest that ANGPTL3 and apoC-III inhibitors may be equally effective in lowering markedly elevated plasma triglyceride concentrations (see Tables 3 and 4), with possibly greater efficacy with the latter agent. However, ANGPTL3 inhibitors may have the advantage of greater
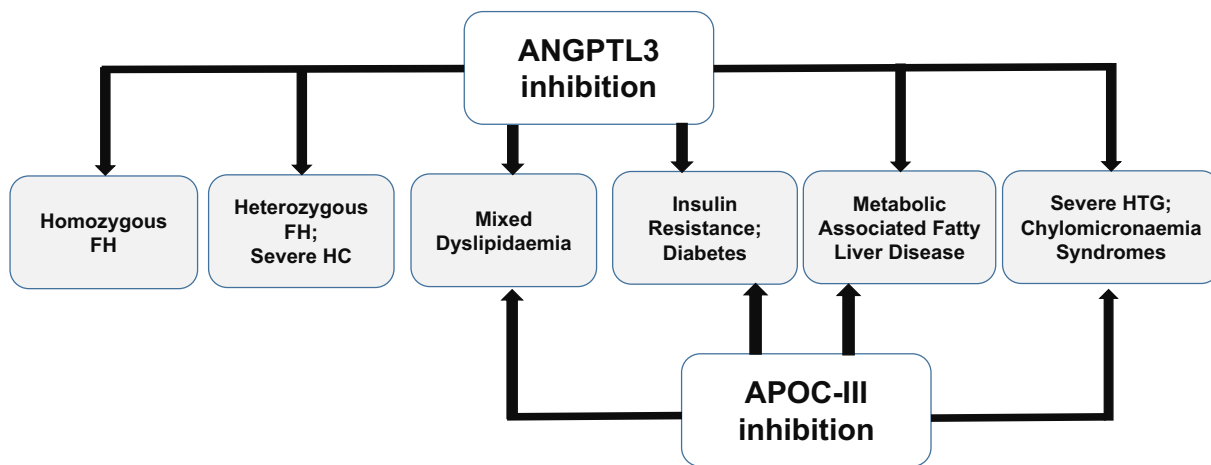


ANGPTL3: angiopoietin-like 3; APOC-III: apolipoprotein C-III; FH: familial hypercholesterolaemia; HC: hypercholesterolaemia; HTG: hypertriglyceridaemia; LDL: low-density lipoprotein

**Fig. 2** Angiopoietin-like protein 3 (ANGPTL3) and apolipoprotein C-III (apoC-III) inhibition may be equally effective in correcting hypertriglyceridaemia in chylomicronaemia syndromes, metabolic-associated fatty liver disease, insulin resistance/diabetes and mixed hyperlipidaemia, but ANGPTL3 inhibition may more effectively lower low-density lipoprotein (LDL) cholesterol and have extended applications in mixed atherogenic dyslipidaemia and more severe forms of familial hypercholesterolaemia

lowering of plasma LDL concentrations and this may be particularly relevant to patients with mixed atherogenic dyslipidaemia. Both agents need to be tested in a wide range of lipid disorders characterized by impairment in the metabolism of TRLs. Relevant trials are currently underway. Figure 2 compares the putative application for ANGPTL3 and apoC-III inhibitors for treating lipid disorders ranging from severe HTG through mixed atherogenic dyslipidaemia to severe FH [92]. Such applications are contingent on use of best background standard of care, especially lifestyle measures and proven pharmacotherapies including statins, ezetimibe, fibrates and PCSK9 inhibitors [8].

Confirmation of the safety of long-term inhibition of ANGPTL3 and apoC-III with RNA-based therapies is evidently essential. Notwithstanding the small numbers of studies, current evidence confirms safety and good tolerability following the utilization of GalNAc$_3$ conjugated nucleic acids [81, 83–85]. The costs of these potent agents and their effectiveness needs to be clearly demonstrated in different clinical contexts. Nucleic acid is likely to be more effective than mAb-based therapies for inhibiting both ANGPTL3 and apoC-III. Whether there are synergistic effects of ANGPTL3 and apoC-III inhibition in correcting severe HTG remains to be demonstrated. siRNA may in turn also be less expensive than ASO, owing to the former intrinsic mode of action via RISC and hence the lower frequency of subcutaneous drug administration. In aggregate, whilst it may be premature to speculate, given safety, tolerability and cost effectiveness remain to be confirmed, siRNA inhibition of ANGPTL3 may offer a futuristic panacea for targeting HTG in a wide range of dyslipidaemic settings, including residual elevation in LDL cholesterol in FH [92].

## Declarations

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