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Emerging strategies of targeting lipoprotein lipase for metabolic and cardiovascular diseases

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Abstract

Although statins and other pharmacological approaches have improved the management of lipid abnormalities, there exists a need for newer treatment modalities especially for the management of hypertriglyceridemia. Lipoprotein lipase (LPL), by promoting hydrolytic cleavage of the triglyceride core of lipoproteins, is a crucial node in the management of plasma lipid levels. Although LPL expression and activity modulation is observed as a pleiotropic action of some the commonly used lipid lowering drugs, the deliberate development of drugs targeting LPL has not occurred yet. In this review, we present the biology of LPL, highlight the LPL modulation property of currently used drugs and review the novel emerging approaches to target LPL.

Introduction

Homeostatic balance of fat absorption, synthesis and breakdown is crucial to the metabolic health of humans. The dietary lipids absorbed via the small intestine and the lipids carried in the form of lipoproteins generated by the liver are the major contributors to circulatory lipid levels and comprise the exogenous and endogenous pathways of lipid transport, respectively. The circulatory lipids in exogenous and endogenous lipid transport pathways converge on a catabolic enzyme named lipoprotein lipase (LPL). The enzymatic activity of LPL is central to the maintenance of plasma lipid levels in check in the face of excessive fat intake or dysregulated lipid metabolism in the liver. As such, LPL has slowly but definitively emerged as a drug target in dyslipidemia.

Lipases are the orchestrators of fat digestion and absorption, metabolism of lipoproteins and mobilization of stored depots of fats to oxidative tissues in conditions of nutrient demand. The catalytic reaction that facilitates the role of LPL in these processes is the hydrolytic cleavage of the ester bonds of the triacylglycerols (TGs) to form glycerol and free fatty acids. This hydrolytic processing of lipids known as lipolysis is carried out in the gastrointestinal tract (pancreatic lipase, gastric lipase, among others), intracellularly [adipose triglyceride lipase (ATGL), hormone-sensitive lipase, among others], as well as in

circulatory blood vessels. In the vasculature, the key lipases are LPL, endothelial lipase (EL) and hepatic lipase (HL). These three lipases differ in the relative ratios of their triglyceride lipase to phospholipase activity [1]. Although all three enzymes are involved in triglyceride metabolism, the current review will focus on LPL as a drug target in metabolic diseases.

LPL: structure, function and regulation

LPL is primarily synthesized in the heart, skeletal muscle and adipose tissue. Other tissues with measurable LPL activity include lungs, lactating mammary glands, brain, kidney and macrophages. In all tissues, LPL is found lining the capillary endothelial lumen and its main function is to hydrolyze the core triglycerides in the triglyceride-rich lipoproteins such as the chylomicrons and the very-low-density lipoproteins (VLDLs) yielding glycerol and free fatty acids (FFAs) for uptake by tissues. Apart from TG hydrolysis, LPL in a nonenzymatic role also facilitates the uptake of lipoprotein particles into tissues by anchoring them to the vessel wall and by serving as a ligand for the lipoprotein receptors [2–7].

Human LPL, a 448 amino acid protein, is encoded by the gene found on chromosome 8p22. Although a lack of an X-ray crystal structure has hindered full exploitation of the enzyme as a drug target, the strong homology with the pancreatic lipase has enabled the development of high-fidelity molecular models of the protein structure. Characteristic features of the lipase gene family such as a heparin-binding domain and an active site α/β hydrolase fold are found in the LPL protein structure. The protein is organized into two structurally distinct domains along with a 27 amino acid signal peptide. The bigger N-terminal domain contains a binding site for heparin and the site for binding of apolipoprotein C-II (APOC2) [8–10]. Also housed in the N terminus is the catalytic site of the enzyme comprising the triad: serine 132, asparagine 156 and histidine 241 [11–13]. The smaller C-terminal domain has been shown to be important for binding lipoproteins. The active site of the LPL is covered by a 'lid'; as seen in other homologs such as HL and PL [14]. The lid is postulated to have an impact on the substrate specificity of the lipase gene family and is essential for interaction with the lipid substrates [1,15,16]. Native LPL monomers dimerize in a head-to-tail fashion to form a noncovalent active dimer [17,18]. In this orientation, the C-terminal of one monomer is in close juxtaposition to the catalytic site of the other monomer in the dimer. In the most well accepted model of LPL activity, the lipid substrates are presented to the active site via interaction with the C-terminal of the protein. This dimerization process and the head-to-tail orientation are key to the activity of the enzyme because the monomers have been shown to be inactive [19,20].

The process of LPL maturation and transport is tightly controlled. The enzyme is synthesized in the parenchymal cells of the heart, muscle and adipose tissues and transported across to the luminal surface of the vascular endothelial cells. It was believed hitherto that, at the luminal site, the protein was anchored to the cells by heparan sulfate proteoglycans (HSPGs). This ionic interaction was considered the basis of the long-established procedure of using intravenous heparin injections to release the free LPL into plasma for collection and assay of its activity. However, the role of HSPGs in anchoring LPL on the luminal surface has been called into question with the recent discovery of a novel protein, now shown to be implicated in this process (discussed below). The intricate process of expression of active

lipase is only now being elucidated. Emerging evidence points to significant post-translational processing of the inactive monomeric lipase in the endoplasmic reticulum (ER). A transmembrane lipase-specific chaperone named lipase maturation factor 1 (LMF1) was identified as having a crucial role in facilitating the assembly of inactive monomers of the enzyme into active homodimers as well as maintaining the stability of LPL dimer in the ER [21]. An intracellular loop of LMF1 is localized to the ER lumen wherein it interacts with LPL. Absence of LMF1 leads to accumulation of LPL as high molecular weight aggregates that are retained in the ER. LMF1 was also identified to be crucial for the maturation of other vascular lipases: HL and EL [22,23]. Physical interaction with each of the vascular lipases has been demonstrated. Indeed, mutation in the LMF1 gene underlies the syndrome of combined lipase deficiency (cld) and is characterized by massive hypertriglyceridemia and chylomicronemia with a 93% decrease in LPL activity [21]. Another mechanism controlling LPL activity recently emerged when an additional ER-associated factor was shown to mediate LPL secretion. This factor, suppressor of lin-12-like (Sel1L), is an ER-localized adaptor protein that forms a complex with LPL and LMF1 and is required for the release of active LPL from the ER [24]. Sel1L knockout mice displayed severe postprandial hypertriglyceridemia and retention of LPL in the ER in the form of protein aggregates.

Until recently, it was believed that HSPGs played a crucial part in LPL binding in the interstitial space, its transport across the endothelial cells and its tethering on the surface of the capillary lumen. However, this traditionally accepted model of HSPG-mediated LPL binding, transport and tethering has been challenged recently by the discovery of another key protein facilitating the transport of LPL from the subendothelial space to the luminal surface of vascular endothelial cells: glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (GPIHBP1). A small glyco-protein, GPIHBP1 has high affinity for LPL and has recently been shown to be responsible for the transendothelial transport of LPL from the parenchymal cells to the capillary lumen where it is postulated to provide a platform for lipolysis [25–27]. GPIHBP^{-/-} mice showed delayed release of LPL upon heparin injection consistent with mislocalization of LPL. Further evidence has shown that GPIHBP1 is responsible for margination of triglyceride-rich lipoproteins. By modulating LPL processing and transport, GPIHBP1 modulates triglyceride metabolism. Severe chylomicronemia has been reported in mice lacking GPIHBP1 [25]. In humans, the significance of GPIHBP1 in LPL biology has also been demonstrated with the existence of mutations in the GPIHBP1 gene that abolish its binding with LPL. In such mutants, chylomicronemia has been observed [28,29]. Additionally, LPL mutants that fail to bind GPIHBP1 show the similar phenotype of chylomicronemia [30]. Discovery and characterization of GPIHBP1 has led to a dramatic evolution of our understanding of LPL biology and will lead to further insights into the interplay between the enzyme, various accessory proteins, the apolipoproteins (APOs) and the triglyceride-rich lipoproteins.

As a crucial node in lipid metabolism and transport, LPL regulates plasma triglyceride levels. The FFAs that are the products of triglyceride hydrolysis are used by the underlying tissue in a tissue-specific manner. Whereas in heart and muscle, LPL-activity-derived FFAs are used as a source of energy, the ones in the adipose tissue are used for storage as fat depots. Adult liver can take up LPL from the circulation, wherein LPL activity can lead to increased fat deposition and compounded insulin resistance. The role of LPL in the nervous

system is slowly being unraveled. Immunostaining for LPL has been detected in the neurons, astrocytes, microglia and oligo-dendroglia throughout the central nervous system [31]. LPL has been shown to regulate energy balance and bodyweight in mice because a neuron-specific deletion of LPL exhibits obese phenotype in mice fed standard chow [32]. Further, LPL function in Alzheimer's disease pathology has been implicated in mouse studies showing cognition and memory deficits in LPL-deficient mice [33]. In addition to the adipose, muscle, heart and brain, LPL is also found in lactating mammary gland, macrophages, lung, kidney, spleen and testes [34,35]. Interestingly, macrophage-derived LPL has been ascribed a proatherogenic role with its catalytic activity and noncatalytic bridging action contributing to this effect. However, the relative contribution of macrophage LPL to overall physiological effects of LPL is unclear; a well-defined antiatherogenic activity has been noted in several mouse models of LPL overexpression probably via global lowering of plasma lipids [36–40]. Furthermore, several commercially available hypolipidemic and antidiabetic drugs (discussed below) are known to have LPL stimulatory activity that probably contributes to lowering of plasma lipids and improvement of cardiovascular outcomes [41–44]. Conversely, LPL deficiency in human studies has been shown to correlate with worsened atherosclerotic outcomes [45–47]. As such, in spite of the proatherogenic role of macrophage LPL, a large body of evidence suggests that LPL is a bona fide target in treating dyslipidemia and associated complications.

Several loss-of-function and gain-of-function studies have provided insight into the key role of LPL in metabolic homeostasis. The LPL knockout mouse models have shown a severe hypertriglyceridemic phenotype [48]. The homozygote knockouts are born with threefold higher triglyceride levels and sevenfold higher VLDL cholesterol levels. Upon suckling, the LPL knockout mice became pale, then cyanotic and finally died 18–24 hours after birth. Heterozygous LPL knockout mice survive to adulthood and have mild hypertriglyceridemia with 1.5–2-fold higher triglyceride levels than control mice [48]. Transgenic mice overexpressing LPL show markedly lower plasma TG and a resistance to diet-induced hypertriglyceridemia and hypercholesterolemia [40]. Tissue-specific LPL knockout and overexpression have facilitated a detailed understanding of LPL functions [32,49–51]. Overall, evidence points to the role of LPL in controlling partitioning of lipids toward either deposition or utilization pathways.

Regulation of LPL activity primarily occurs post-transcriptionally. A constellation of proteins plays a significant part in regulating LPL activity. These 'LPL regulatory proteins' are derived from the liver as well as other tissues (Fig. 1, Table 1). The roles of LMF1 and GPIHBP1 have already been stressed. Among other LPL regulatory proteins are the APOs and the angiopoietin-like proteins (ANGPTLs). On the basis of their function, LPL-regulatory proteins can be grouped as either LPL-stimulatory or LPL-inhibitory proteins. APOC2 and APOA5 have been described to have LPL-stimulatory properties with APOC2 recognized as a required cofactor for hydrolytic activity of LPL [52,53]. APOC2 is required for maximal rates of TG-rich lipoprotein lipolysis [52]. The C-terminal helix in APOC2 guides lipoproteins to the active site of LPL [54,55]. APOC2 deficiency is associated with marked elevation of plasma TG, VLDL and chylomicron levels and decreased LPL activity, LDL, IDL and HDL levels [56]. Interestingly, higher concentrations of APOC2 lead to hypertriglyceridemia [57]. Although mechanisms of this dose effect are not clear, it is

speculated that, at high plasma concentrations, APOC2 produces impairment of lipolysis and a defect in remnant removal.

As opposed to APOC2 and APOA5, APOC1, APOC3 and APOE inhibit LPL-dependent TG clearance [58–61]. APOC3 is especially well characterized for its LPL inhibitory activity with known human heterozygous carriers of null mutation in the APOC3 gene exhibiting lower plasma TG levels [62]. Angiopoietin-like 3, 4 and 8 are well characterized LPL activity inhibitors. Modulation of LPL-regulatory proteins is emerging as an indirect means of targeting and regulating LPL for therapeutic use (described below). Among these, APOC3 and ANGPTL proteins have received the most attention.

APOC3 is found on APOB-containing lipoproteins like VLDL and LDL but unlike APOB is much smaller (only ~9 Kda) [63]. APOC3 has multiple roles in lipoprotein metabolism. As noted earlier, APOC3 inhibits LPL activity and thus TG lipolysis. It has been proposed that APOC3 inhibits LPL by displacing the enzyme from the lipid droplets [64]. Further, APOC3 also inhibits receptor-mediated uptake of remnant lipoproteins and LDL. Large population-based genome-wide association studies (GWAS) have highlighted the linkage between APOC3 and cardiovascular outcomes [65]. These observations have stimulated great interest in targeting APOC3 for lipid lowering, as will be discussed later in this review.

Emerging evidence suggests that ANGPTLs are key regulators of triglyceride and lipoprotein levels in humans [66]. Among this subfamily, prominent roles have been ascribed to ANGPTL3, ANGPTL4 and ANGPTL8 which are secretory proteins with a distinct expression pattern [67–71]. These proteins inhibit LPL found lining capillary endothelium in many tissues. Inhibition of LPL activity by ANGPTL3 and ANGPTL4 results in elevated TGs in the bloodstream and a poor lipid profile. Injection of recombinant ANGPTL4 as well as peripheral overexpression of ANGPTL4 is associated with increased serum triglycerides, non-HDL cholesterol and nonesterified fatty acids (possibly because of stimulation of adipose tissue lipolysis) [68,72,73]. ANGPTL4 deficiency has been shown to improve total cholesterol, triglyceride and reduce foam cell formation thereby protecting against atherosclerosis [74]. However, similar to LPL, the macrophage-expressed ANGPTL4 has distinct effects on atherosclerosis progression as opposed to non-macrophage ANGPTL4. The expression of ANGPTL4 in human atherosclerotic plaques and its localization to macrophages has been demonstrated [75]. Using apolipoprotein E (ApoE)*3-Leiden (E3L) mice, authors showed that ANGPTL4 inhibits foam cell formation and promotes reduction in total lesion area. In another recent report, it was shown that ANGPTL4 expression is upregulated in foam cells [76]. Additionally, hematopoietic deficiency of ANGPTL4 was achieved by transplantation of bone marrow from *Angptl4*^{-/-} mice into the *Ldlr*^{-/-} mice. The mice with hematopoietic deficiency of ANGPTL4 showed larger atherosclerotic plaques and enhanced foam cell formation via increased CD36 expression and reduced ATP-binding cassette subfamily A member 1 (ABCA1) localization at the cell surface. In the same study, the global deficiency of ANGPTL4 was shown to be protective against atherosclerosis contrasting the effects observed with the hematopoietic deficiency of ANGPTL4. Thus, although the precise role of ANGPTL4 is still being elucidated, it is evident that ANGPTL4 is a major player in the process of circulatory lipid homeostasis.

Although the exact mechanism of ANGPTL4 inhibition of LPL remains unclear, several mechanisms have been proposed. First, it has been shown that co-incubation of ANGPTL4 with LPL increases the abundance of inactive LPL monomers [77,78]. Additional evidence shows that ANGPTL4 overexpression results in the reduction of proportion of LPL dimers. Whether ANGPTL4 drives the dimer to monomer conversion irreversibly or whether ANGPTL4 is bound to LPL monomers, thereby driving this conversion, are points that are currently debatable in this model. Alternatively, it has also been proposed that ANGPTL4 functions as a conventional noncompetitive inhibitor that binds to LPL to prevent the hydrolysis of the substrate and that a reversible complex between ANGPTL4 and LPL is formed [79]. Although three conserved polar residues within a 12 amino acid motif of ANGPTL4 are known to be required for interaction with LPL, the domains and residues of LPL involved in this interaction are unknown [77]. In yet another mechanism, evidence for ANGPTL4-mediated intracellular degradation of LPL has been proposed [80]. Co-transfection of LPL and ANGPTL4 in CHO cells resulted in reduced intracellular LPL levels and in adipocytes derived from ANGPTL4^{-/-} mice increased levels of mature LPL was found to accumulate. Moreover, it was observed that blocking ER-Golgi transport processes abolished the differences in the levels of LPL derived from wild-type and ANGPTL4 adipocytes suggesting that ANGPTL4 probably acts on LPL after its processing in the ER. Furthermore, physiological conditions of fasting and cold resulted in an inverse relationship between the ANGPTL4 and mature LPL levels in the wild-type mice but not in ANGPTL4^{-/-} mice. These findings suggest that, in addition to the intravascular inhibition of LPL, ANGPTL4 also acts intracellularly to promote LPL degradation. This novel discovery will inform the development of future small molecule and antibody approaches to targeting this protein-protein interaction.

Pharmacological targeting of LPL

The recognition of LPL as a drug target has existed for several decades. LPL modulation has been shown to be a pleiotropic effect of several clinically used drugs in metabolic disorders. However, a clinically useful drug with LPL activation as its centerpiece mechanistic effect has not yet been achieved. Only recently, the emergence of ANGPTL proteins and a renewed interest in APO proteins has reinvigorated the quest for a LPL-targeted drug. Strategies to target LPL have yielded a wide range of pharmacological tools including multiple small molecules, monoclonal antibodies, peptides, antisense oligonucleotides and even gene therapy. The remainder of this review will discuss the pharmacological manipulation of LPL achieved by currently marketed drugs or approaches as well as highly encouraging clinical and preclinical candidates and approaches in development. The drugs and strategies have been grouped as either the ones directly acting on LPL or the ones acting indirectly via LPL regulatory proteins.

Direct LPL modulation

Clinically used drugs—As mentioned above, LPL activation has been found to be a pleiotropic effect of many clinically employed drugs, some of which have been used for several decades for metabolic disorders. Fibrates, such as gemfibrozil and fenofibrate, increase LPL activity accounting for their hypotriglyceridemic property. It was discovered

that these drugs modulate the transcriptional expression of several APOs and enzymes via the peroxisome proliferator activated receptor (PPAR)- α in the liver, leading to stimulation of LPL activity [81]. Particularly, fibrates inhibit the expression of APOC-III thereby enhancing the LPL activity [82]. Other notable effects of fibrates include their property to enhance beta-oxidation of fatty acids and inhibit VLDL synthesis and release from the liver [44]. These latter effects are probably of greater significance in triglyceride-lowering effects of these drugs. Statins, a significant lipid-lowering drug class, have also been shown to have an effect on LPL physiology. Atorvastatin enhances serum LPL levels in type 2 diabetics [83]. Similar clinical observations have been made for simvastatin [84]. This increased serum LPL seen in response to atorvastatin is suggested to occur via increased LPL production in the skeletal muscle in an adenosine-monophosphate-activated protein kinase (AMPK)-dependent mechanism [85]. Interestingly, the effects of statins on LPL occur in a tissue-specific manner with a decrease in LPL mass after statin administration also reported in macrophages [86]. Statins are known to have strong cholesterol-lowering properties by virtue of inhibition of HMG CoA reductase (HMG-CoA) and downstream upregulation of LDL receptor (LDL-R) on the surface of hepato-cytes. The triglyceride-lowering property of statins is often inadequate in cases of severe hypertriglyceridemia and requires supplementation with fibrates, niacin or omega 3 fatty acids [87]. The contribution, if any, of LPL regulation in statin activity is unclear. Among the many activities ascribed to ω -3 fatty acids is their regulation of LPL. Evidence suggests that ω -3 fatty acids regulate LPL directly via increasing the production of the enzyme as well as indirectly by inhibiting the expression of APOC3 from the liver [88–91].

LPL modulation has also been observed in glucose-lowering drugs. These include thiazolidinediones (TZDs) and metformin. A PPARE identified in the promoter region of the human LPL gene has been shown to mediate the TZD-induced expression of LPL in the adipose tissue [81]. PPAR- γ is also shown to increase the expression of carbohydrate sulfotransferase 11 (CHST11/C4ST1), a protein that is known to sulfate the membrane-bound proteo-glycans that anchor the LPL protein to the cell surface [92]. Rosiglitazone was shown to induce the expression of GPIHBP1 in adipose tissue, heart and skeletal muscle. Further, knockdown of PPAR- γ was found to result in reduced GPIHBP1 expression [93]. Metformin regulation of LPL has been established in human clinical use and animal models with early evidence emerging over 30 years ago. Rats fed a fructose-rich diet and administered met-formin at 50 mg/kg/day showed higher plasma LPL activity and lower plasma TG than untreated fructose-fed rats [94]. Metformin induces the pre-heparin LPL mass in type 2 diabetic patients [95]. Cell culture studies have shown metformin activates LPL production in skeletal muscle downstream of AMPK [96]. Another indirect target of metformin in LPL physiology includes LMF1, the expression of which was increased by metformin in the heart and to a lesser extent in the muscle and adipose tissues of rats [97]. Whether LPL activation is the mechanism of lipid-lowering effects of these antidiabetic drugs has not been validated but evidence suggests that this activity probably contributes to the hypotriglyceridemic effects of the TZDs and metformin. Thus, whereas LPL modulation as a pharmacological property is seen in several pharmaceutical drugs, it is often a pleiotropic effect and not the principal mechanism of action of these drugs. Efforts to

intentionally target LPL activity have gained mainstream attention and from this point forward this review will summarize some of these promising new approaches (Table 2).

Experimental direct LPL activators—Development of direct LPL activators has been largely sporadic. An initial phase dominated by a single compound in the late 1990s was followed by prolonged inactivity in this area. Only recently has this been revived by two new molecules identified as a result of focused LPL-targeting efforts.

In 1993, researchers reported the identification of a novel compound named NO-1886 (generic name: ibrolipim) as an activator of LPL [98]. In the initial study, NO-1886 was found to be an inducer of LPL gene expression in the adipose tissue leading to increased post-heparin plasma LPL activity, lower plasma triglycerides and higher plasma HDL. Further, the compound showed the property of inhibiting atherosclerotic lesion formation in vitamin-D2-treated cholesterol-fed rats upon prolonged administration. Similar results were observed in a rabbit model of atherosclerosis [99]. Single doses of NO-1886 were found to significantly stimulate LPL activity, lower plasma triglycerides and elevate the levels of HDL-C [100]. In streptozotocin (STZ)-treated diabetic rats, NO-1886 increased LPL activity 59% over control [101]. The compound was tested in several other animal models of dyslipidemias and showed promising activity [102–107]. NO-1886 entered clinical development in Japan in the late 1990s. However, owing to unknown side effects, the clinical development of NO-1886 was halted. The compound did cause a species-specific effect on adrenal cortex steroidogenesis leading to hypertrophy of adrenal glands in rats and dogs although this effect was not observed in monkeys [108]. Nevertheless, to date, NO-1886 remains the most extensively studied direct LPL activator.

Recently, two new molecules targeting LPL directly have emerged. Both these molecules differ from NO-1886 in respect to their mechanism of LPL activation. Whereas NO-1886 primarily induces LPL mRNA resulting in an increase in LPL activity in plasma, the newer compounds primarily enhance the LPL hydrolytic activity having been identified in an *in vitro* enzyme activity screening assay. One of these newer compounds was identified in our laboratory in pilot screening of a small chemical library [109]. The initial hit compound identified in the screening assay (C10) was further optimized to yield a highly potent analog we named C10d. We compared the LPL activation property of C10d against NO-1886 and found that C10d exhibited twice the stimulation of LPL than NO-1886 did at equivalent doses. Interestingly, when we tested the efficacy of LPL activators in reversing the ANGPTL4 inhibition of LPL, C10 and C10d rescued the ANGPTL4 effects in a dose-dependent manner. NO-1886, by contrast, does not reverse the ANGPTL4 inhibition of LPL in this *in vitro* assay. However, it is probable that NO-1886 can rescue the ANGPTL4 inhibition of LPL *in vivo* owing to its stimulatory effects on LPL gene expression. C10 and C10d do not have effects on LPL gene expression and only affect the enzymatic activity of LPL. Nevertheless, C10 and C10d represent a new class of LPL activators that can be optimized and developed further. Unpublished data from our laboratory suggest robust *in vivo* stimulation of LPL activity. The precise molecular mechanisms of LPL activation by these compounds are currently being studied in our lab.

Another notable molecule that has recently been reported is an *N*-phenylthalimide derivative [110]. Identified in a small molecule screen set up to select compounds that protect LPL against inhibition by ANGPTL4, the researchers identified a molecule named 50F10 that exhibited potent activity in primary and secondary screens using different LPL substrates. Mechanistic studies showed that this molecule stabilizes the active homodimer structure of LPL and prevents its conversion to inactive monomers in the presence of ANGPTL4. *In vivo* evidence of activity in an APO A-V deficient mouse model challenged with olive oil gavage was also presented. Extensive SAR studies demonstrated that the phthalimide moiety and the lipophilic substituents as well as substitution pattern on the central phenyl ring are functionally essential to the activity of the compound [111]. SAR studies led to improvement of *in vitro* LPL activity and one such analog designated '80' was tested *in vivo* where efficient plasma TG-lowering activity was shown.

Hypolipidemic activity was also demonstrated for indole-2-carboxamide and benzofuran-2-carboxamide derivatives [112,113]. Although direct LPL activity of these compounds was not shown, their hypolipidemic activity was demonstrated in a rat model using anionic detergent Triton® WR-1339 which is known to prevent catabolism of TG-rich lipoproteins by LPL. Follow-up studies to elucidate the mechanism of these compounds were not reported.

LPL gene therapy—LPL gene deficiency (type 1 hyperlipidemia) is a rare (1–2:1,000,000) autosomal-recessive disease [114,115]. Hyper-triglyceridemia, often resulting from chylomicronemia in these patients, can lead to severe pancreatitis that is recurring and life threatening. Traditionally, the treatment has been a drastic reduction in dietary fat intake. However, such dietary compliance is difficult to sustain and often ineffective. A gene therapy designed to introduce extra copies of the functional potent enzyme in the muscles of the lower limbs of patients was introduced in Europe in 2014 [116,117]. The genetic construct used to introduce functional LPL is an adenoassociated viral vector (AAV)-LPL S447X and the therapy is named alipogene tiparvovec (Glybera®). S447X, a naturally occurring gain-of-function mutation of LPL, has been extensively studied and is associated with a lower rate of cardiovascular disease (CVD) [118]. Initial preclinical evaluation of AAV LPL^{S447X} in LPL^{-/-} mice and cats demonstrated effectiveness in reducing lipemia and plasma triglycerides [119,120]. The clinical trials of alipogene tiparvovec in 27 patients with LPL deficiency showed a transient reduction in plasma TG levels [121]. Although the plasma TG levels rebounded to pre-treatment levels in 26 weeks after alipogene administration, the therapy was found to have a long term impact on chylomicron metabolism with a reduction in chylomicron TG content [122]. Patients have also shown expression of functional copies of the LPL^{S447X} gene in long-term follow-up studies. It is postulated that chylomicrons are primarily responsible for pancreatitis. The incidence of pancreatitis declined with LPL gene therapy. Notably, the reduction in the incidence of pancreatitis was sustained up to 6 years after administration [123]. The therapy has been generally well tolerated with mild-to-moderate injection site reactions. The immune response against the AAV coat proteins has been observed which persists despite pharmacological suppression using immunosuppressants such as cyclosporine and mycophenolate mofetil. The immunosuppressants have been found not to affect the

production of the transgene in the body or impact the duration of TG lowering achieved [124]. This first of its kind gene therapy for LPL is a highly significant development for this rare genetic disease and its approval has paved the way for the development and regulatory approval of additional gene therapy products in a wide array of human diseases around the world.

Indirect LPL modulation

Apart from the excitement around novel small molecule activators of LPL and the groundbreaking gene therapy advances in LPL deficiency, there have been concerted efforts in industrial and academic laboratories to modulate LPL indirectly via regulation of expression or activity of LPL regulatory proteins. As discussed earlier and as shown in Table 1, LPL functional activity is subject to regulation by a host of LPL regulatory proteins such as APOs and ANGPTLs. The discussion below will summarize the approaches around LPL regulatory proteins that have yielded clinical candidates in pharmaceutical and biotech pipelines, as well as other promising preclinical therapies.

Inhibition of APOC3—The changing paradigm on the role of TGs in CVD, from a risk factor to a causal relationship, has triggered a quest for druggable targets involved in regulation of plasma TGs. Owing to its dual inhibitory activities – on LPL and on that of liver uptake of remnant lipoproteins – APOC3 has emerged as a bona fide target in regulating the plasma levels of TG-rich lipoproteins. In a GWAS published in 2008, Pollin *et al.* uncovered the cardioprotective effect of APOC3 in the Lancaster Amish population among carriers of the null mutation in the APOC3 gene [62]. These carriers demonstrated low fasting and post-prandial plasma TG levels as well as high HDL and low LDL cholesterol levels. Moreover, they also exhibited lower levels of subclinical atherosclerosis as measured by coronary artery calcification. Recently, a large prospective study confirmed the causal association between high plasma TGs and CVD risk [125]. Moreover, investigators found that a loss of function mutation of APOC3 which is associated with low non-fasting plasma TG levels (44% lower than non-carriers) is also correlated with a low risk of ischemic vascular disease (41% risk reduction) and ischemic heart disease (36% risk reduction). Further, in an exome sequencing study to identify genes related with plasma TG levels, aggregate gene mutations in APOC3 (four mutations) were found to correlate with lower plasma TG levels. The carriers of any of the four APOC3 mutations were found to be at 40% lower risk for coronary heart disease [126]. The findings of APOC3 loss-of-function mutation were replicated in a newer report, wherein a multiethnic US adult population was studied [127]. Although the interest and the efforts to develop therapeutics around APOC3 predate some of these large human population studies, the findings from these studies have provided additional validation of APOC3 as an attractive drug target in lipid-related risks for CVD.

Although efforts to target APOC3 using RNAi are underway, the methodology that has made the most headway is the antisense oligonucleotide platform developed by Ionis Pharmaceuticals. This antisense drug, named volanesorsen (formerly known as ISIS-APOCIII_{Rx} or ISIS 304801), is currently undergoing Phase III clinical trials in patients with hypertriglyceridemia and in patients with familial chylomicronemia syndrome (FCS). The

antisense technology used here is a chimeric oligonucleotide (a 20-mer) that binds the human APOC3 mRNA within the 3'-untranslated region and results in RNase H-mediated degradation of the APOC3 mRNA. The oligo is characterized by five 2'-O-(2-methoxyethyl) (-MOE)-modified ribonucleotides at the 5' and 3' ends that confer increased affinity for the target mRNA, increased resistance to exo- and endo-nucleases and an improved safety profile [128–130]. The first studies on volanesorsen emerged in 2013, when the identification of this antisense oligo was reported from a screen of ~350 MOE chimeric candidates [131]. Authors demonstrated robust reduction in the plasma APOC3 levels along with reductions in plasma TGs in multiple animal models treated with volanesorsen. Reduction in plasma TGs was not a result of decreased hepatic VLDL-TG secretion or intestinal TG secretion but rather an increase in plasma TG clearance. No hepatic steatosis or other toxicity was observed for inhibition of APOC3. Favorable effects on plasma lipid profile were observed in rhesus monkeys. In the same publication, a Phase I human study was reported where the animal findings of APOC3 reduction, plasma TG reduction and safety profile were found to be validated. Promising data obtained in this study laid the groundwork for further human clinical development.

Interestingly, the drug development of APOC3 antisense oligonucleotide has also greatly informed the biology related to the triglyceride-elevating properties of APOC3. Novel findings regarding the role of APOC3 in triglyceride metabolism were brought to light in a Phase II clinical trial of volanesorsen in three patients with FCS [132]. These patients have genetic loss of function of mutation of LPL or one of the proteins necessary for LPL function and, as a result, have elevated levels of chylomicrons in the circulation which predisposes them to increased risk of severe pancreatitis and life threatening lipemia. Unexpectedly, volanesorsen showed remarkable activity of lowering plasma triglycerides in these patients without any appreciable increase in the residual LPL activity. The efficacy of volanesorsen in these patients led the authors to postulate the existence of a LPL-independent pathway of triglyceride clearance and the inhibition of this pathway by APOC3. Although such APOC3 activity had earlier been proposed in a transgenic APOC3 mouse model, the findings obtained from the volanesorsen use in FCS have provided human proof-of-concept validation of this hypothesis [133].

A bigger Phase II trial on hypertriglyceridemic patients was performed in a 13-week study with either volanesorsen monotherapy (41 patients receiving the drug and 16 receiving placebo) or with volanesorsen in combination with a stable fibrate therapy (20 receiving the combination and eight receiving the placebo) [134]. A dose-dependent decrease in the plasma APOC3 levels was observed in volanesorsen-treated patients with an accompanying decrease in plasma TG levels ranging from 31.3% to 70.9% over the placebo group. Using a novel high-throughput chemiluminescent ELISA, authors reported a decrease in APOC3 associated with specific lipoproteins [135]. The therapy was generally well tolerated with no effects on renal or hepatic functions. Cutaneous erythema and pain at the injection site were the observed side effects in 13% of injections in monotherapy cohorts and 15% of injections in the combination cohort. One patient developed a serum-sickness-like reaction even though no antibodies against the antisense drug were detected. Another patient showed adverse events related to arterial graft stenosis after the final high dose (300 mg) of volanesorsen. In a recent report, the efficacy of volanesorsen in improving insulin sensitivity

was demonstrated in type 2 diabetes patients [136]. In a randomized double-blind trial, 15 patients with HbA1c >7.5% and hypertriglyceridemia (TG levels: 200–500 mg/dl) were administered subcutaneous volanesorsen weekly for 15 weeks. Significant suppression of plasma APOC3 and TG levels was seen along with a 57% improvement in whole-body insulin sensitivity as measured using a two-step hyperinsulinemic-euglycemic-clamp procedure. Sustained suppression (up to 3 months post-dosing) of HbA1c was observed. Overall, the clinical development of volanesorsen has brought attention to the roles of LPL and APOC3 in triglyceride metabolism and sparked great interest in more-rigorous studies around these two proteins.

Volanesorsen is not the only product under development to target APOC3. Examination of pharmaceutical and biotech product pipelines and reports from scientific congresses have revealed other products and strategies in development against APOC3. These include RNAi-based approaches reported by Arbutus Pharmaceuticals, using lipid nanoparticles as delivery vehicles. RNAi against APOC3 and a combination RNAi product targeting APOC3 and ANGPTL3 have been reported (Keystone Symposium 2015; <http://investor.arbutusbio.com/releasedetail.cfm?releaseid=903410>). Investigators have observed robust and durable inhibition of plasma TG and APOC3 levels *in vitro* and in an *in vivo* APOC3 transgenic mouse model treated with APOC3 RNAi. The combination RNAi (APOC3 + ANGPTL3) was observed to demonstrate super-additive effects over individual RNAi on plasma TG lowering in a high-fat-diet model of hypertriglyceridemia.

Hepatocyte-targeted delivery of APOC3 siRNA via *N*-acetylgalactosamine (GalNAc) ligand conjugation underwent preclinical development by Alnylam Pharmaceuticals [137]. GalNAc serves as the ligand for the hepatocyte-specific asialoglycoprotein receptor (ASGPR) to facilitate liver-targeted delivery of the oligonucleotide. Early studies have shown a strong persistent (up to 30 days) knockdown of APOC3 levels in the liver by single doses (3 mg/kg) of these siRNAs (ALN-APOC3) delivered by the subcutaneous route in a mouse model transduced with AAV construct expressing human APOC3 gene (<http://investors.alnylam.com/releasedetail.cfm?ReleaseID=883469>). Multi-dose studies have shown persistent knockdown of up to 35 days. In a db/db mouse model, a >90% lowering of plasma APOC3 and a >50% reduction of plasma TGs were observed. Similar efforts to inhibit ANGPTL3 via GalNAc tagging of siRNA are also being undertaken (mentioned below).

Inhibition of ANGPTL family members—The interest in developing targeted therapies against ANGPTL-mediated modulation of LPL is just as strong as for the APOC3. The strategy against ANGPTL3 that has stood out the most so far is the monoclonal antibody being developed by Regeneron Pharmaceuticals. Using their proprietary fully human antibody development platform named VelocImmune®, the investigators have developed a fully human monoclonal antibody that targets ANGPTL3 [138]. The product is named evinacumab (also called REGN1500) and is in Phase II clinical trials. Early studies have demonstrated significant activity of the antibody in extensive preclinical testing [139]. The antibody demonstrates specificity for ANGPTL3 and does not show any cross-reactivity with ANGPTL4, 5 and 8. The antibody was found to enhance post-heparin LPL activity in normolipidemic mice and lower plasma TGs by >50%. Further, chronic testing (8 weeks) in

high-fat- and high-cholesterol-fed dyslipidemic mice showed reduced plasma TG, plasma LDL-C and HDL-C levels. Administration of a single dose of the antibody in dyslipidemic cynomolgus monkeys exhibited similar activity on plasma lipids. REGN1500 has also proven to be a useful tool in the study of biological functions of ANGPTL3. Using this antibody, a study has recently demonstrated that inactivation of ANGPTL3 leads to increased clearance of APOB-containing lipoproteins and decreased production of LDL in ANGPTL3-deficient animals [140]. Indeed, emerging clinical data for REGN1500 validates these preclinical findings. Interim data from an ongoing Phase II clinical study in homozygous familial hypercholesterolemia (HoFH) patients shows that adding REGN1500 to a lipid-lowering therapy (statin + ezetimibe; one patient also received additional lomitapide) causes a mean 55% (25–90%) reduction in LDL-C at 4 weeks over baseline with no adverse effects (http://files.shareholder.com/downloads/REGN/2807302673x0x894306/C8D4BE1B-4EB3-4E8D-9DE8-8FCBACEB8975/REGN_News_2016_5_31_General_Releases.pdf).

Notably, the efforts to target a related member of the ANGPTL family, ANGPTL4, preceded the antibody efforts around ANGPTL3. However, the inhibition of ANGPTL4 produced safety concerns when genetic ablation of ANGPTL4 in mice led to intestinal abnormalities with mucosal thickening, infiltration of foamy macrophages and lipogranulomatous inflammation in the mesenteric lymph nodes [141]. Reduced viability of ANGPTL4^{-/-} mice was also observed. The surviving ANGPTL4^{-/-} mice when weaned onto a high fat diet showed decreased survival compared with wild-type mice on a high fat diet or the knockout mice on a chow diet, and developed chylous ascites. Similar pathological findings in the mesenteric lymphatics and lymph nodes were seen in high-fat-diet-fed APOE^{-/-} or LDLr^{-/-} mice treated with anti-ANGPTL4 antibody. In a follow-up study, it was indeed demonstrated that mice lacking ANGPTL4 developed fibrinopurulent peritonitis, ascites, intestinal fibrosis and cachexia upon saturated fat intake. Induction of macrophage ANGPTL4 by fatty acids was shown to inhibit the fatty acid uptake into mesenteric lymph node macrophages thereby preventing the macrophage activation and uncontrolled fat-induced inflammation [142].

Another candidate to enter clinical development against ANGPTL3 is being pursued by Ionis Pharmaceuticals, using the similar antisense approach that has been used in the development of volanesorsen. The drug is named IONIS ANGPTL3-LRx (formerly ISIS 703802 or ISIS-ANGPTL3 Rx) and is a ligand-conjugated antisense aimed to enhance the liver-specific delivery of the oligonucleotide. Studies from the unconjugated antisense product have been reported. Although modest effects of the antisense oligonucleotide were seen in normocholesterolemic mice, potent reductions in plasma TG (up to 88%) and cholesterol (up to 66%) along with reduced atherosclerosis were seen in LDLr^{-/-} mice [143]. In a Phase I study, ISIS-ANGPTL3 Rx showed an acceptable safety profile and dose-dependent reductions in plasma ANGPTL3 (82%), TG (49%) and total cholesterol (28%) [143]. Patients with higher baseline TGs showed a higher reduction in plasma TGs. The development of this drug is ongoing and is currently recruiting for a Phase II study testing safety, tolerability, pharmacokinetics and pharmacodynamics in healthy volunteers with elevated TGs and in patients with FCS (NCT02709850).

GalNAc conjugation of ANGPTL3 siRNA has also been explored in preclinical studies [137,144]. Encouraging data in female ob/ob mice have shown a dose-dependent and robust (>99%) knockdown of serum ANGPTL3. A single 3 mg/kg dose in these mice produced an 80% reduction in serum LDL-C and TGs. Another preclinical candidate reported is the combination RNAi product (Arbutus Pharmaceuticals) described earlier. Overall, we anticipate that ANGPTL3 will continue to be of pharmacological interest in dyslipidemias and hyperlipidemias for the foreseeable future.

Activation of APOC2—Several other APOs play a crucial part in post-translational modification of LPL function and activity. Among these, a stimulatory role has been ascribed to APOC2. Comprising three amphipathic helices, the C terminus of the protein is known to activate LPL. Interestingly, a marked elevation in the levels of APOC2 results in inhibition of LPL activity rather than stimulation of activity [57]. Nevertheless, APOC2 levels have been correlated with plasma LPL activity and TG levels. There is interest in developing APOC2 mimetic peptides that can enhance the activity of LPL and result in enhanced hydrolysis of lipoprotein TGs. In a 2014 publication, researchers reported the development of an APOC2 mimetic peptide named C-II-a, containing a 21 amino acid motif of the third amphipathic helix of APOC2 connected to a synthetic amphipathic peptide named 18A [145]. The resultant synthetic bi-helical peptide is bifunctional with the properties of stimulating triglyceride hydrolysis (via the third helix of APOC2) and of promotion of cholesterol efflux by the ABCA1 transporter (via the 18A peptide). The APOC2 motif in C-II-a was found to recapitulate the activity of the full length APOC2 protein in LPL activity assays. Further, *in vivo* evaluation of the peptide in the APOE^{-/-} mouse model showed significant activity of lowering plasma triglycerides and plasma cholesterol. Further evaluation of the same peptide in APOC2 mutant mice showed the ability to correct the hypertriglyceridemia in these mice [146]. Although preliminary in nature, these studies provide crucial proof-of-concept validation for APOC2 mimetic peptides as pharmacological tools.

Concluding remarks

Over the past 5–10 years, LPL has attracted significant pharmacological attention in the treatment of metabolic disease and the downstream cardiovascular sequelae. Increased efforts of understanding of LPL physiology in recent years have led to identification of ANGPTL proteins GPIHB1 and LMF1 as key players in LPL biology, revitalizing the interest in LPL as a drug target. The pursuit of the approaches outlined in this manuscript and development of other novel modalities of targeting LPL are expected to continue.

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Biography

Prabodh Sadana, PhD, is an Assistant Professor in the Department of Pharmaceutical Sciences at Northeast Ohio Medical University. Dr Sadana obtained his doctorate in pharmacology from the University of Tennessee studying transcriptional regulation of genes involved in fatty acid oxidation and glucose metabolism. Dr Sadana underwent postdoctoral training in chemical biology at the St Jude Children's Research Hospital, studying the development of novel small molecules regulating nuclear hormone receptors involved in metabolic pathways. Dr Sadana's current research focuses on the molecular pathways of regulation of lipid metabolism and the development of novel small-molecule approaches in the treatment of dyslipidemias.

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FIGURE 1.

The liver–LPL axis. Lipoprotein lipase (LPL) hydrolyzes the circulatory triacylglycerol (TG)-rich lipoproteins [very-low-density lipoproteins (VLDL) and chylomicrons derived from the liver and the intestine, respectively] with the resulting decrease in plasma TG levels. The maturation, transport and anatomical localization of LPL form a multistep process with the participation of lipase maturation factor 1 (LMF1) and glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (GPIHBP1). The liver is a primary source of several LPL regulatory proteins with either pro-LPL functions [apolipoprotein (APO)C2 and APOA5] or anti-LPL function [APOC1, APOC3, angiopoietin-like protein (ANGPTL)3, ANGPTL4 and ANGPTL8].

TABLE 1

LPL regulatory proteins			
Name	Expression	Modulation of LPL and proposed mechanisms	Refs
Pro-LPL proteins			
APOC2	Liver, colon	Stimulation of LPL activity; high concentrations reported to inhibit LPL activity	[52,57]
APOA5	Liver, intestine, placenta	Stimulation of LPL activity; multiple mechanisms have been proposed including direct interaction with LPL and heparan sulfate proteoglycans	[53]
GPIHBP1	Adipose, heart, breast, skin, endocrine glands	Transport of LPL from subendothelial space to the capillary lumen	[25–27]
LMF1	Endometrium, testis, lung, kidney, stomach, skeletal muscle, adipose, brain	Promotes maturation of LPL via assembly of inactive monomers into active dimers; transport of active LPL dimer in the secretory pathway from the cells	[21]
Sel1L	Ubiquitous, strongest in the pancreas	Forms complex with LPL and LMF1 and is required for the release of active LPL from ER	[24]
Anti-LPL proteins			
APOC1	Liver, colon	Inhibition of LPL activity; displaces LPL from the lipid droplets	[59]
APOC3	Liver, intestine	Inhibition of LPL activity; displaces LPL from the lipid droplets	[60]
APOE	Hippocampus, cerebral cortex, cerebellum, adrenal gland, liver, skin, kidney, testis	Inhibition of LPL activity	[58,61]
ANGPTL3	Liver	Inhibition of LPL activity; does not alter LPL self-inactivation rate	[66,69,71]
ANGPTL4	Adipose, skeletal muscle, lung, cerebral cortex, kidney, cerebellum, thyroid gland, adrenal gland and others	Inhibition of LPL activity; accelerates irreversible inactivation of LPL; probably mechanisms include conversion of active dimers to inactive monomers and noncompetitive reversible binding to LPL	[66,71,74,77,78]
ANGPTL8	Liver, brain, adipose tissue, lung	Inhibition of LPL activity; promotes cleavage and activation of ANGPTL3	[70]

Abbreviations: ANGPTL3, angiopoietin-like 3; ANGPTL4, angiopoietin-like 4; ANGPTL8, angiopoietin-like 8; APOA5, apolipoprotein AV; APOC1, apolipoprotein C1; APOC2, apolipoprotein C2; APOC3, apolipoprotein C3; APOE, apolipoprotein E; GPIHBP1, glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1; LMF1, lipase maturation factor 1; LPL, lipoprotein lipase; Sel1, suppressor of lin-12-like.

TABLE 2

Novel and notable LPL-targeting strategies				
Strategy	Examples	Preclinical data	Clinical data	Refs
Direct LPL activation				
Small molecule LPL activation	C10d	Activation of LPL in <i>in vitro</i> screening assay; mechanistic evidence shows stabilization of LPL dimers; <i>in vivo</i> TG lowering in APOA3 knock-out mice	None reported	[109]
	50F10 and compound 80	Activation of LPL in <i>in vitro</i> screening assay; mechanistic evidence shows stabilization of LPL dimers; <i>in vivo</i> TG lowering in APOA3 knock-out mice	None reported	[110]
	Alipogene liparivovos (Glybera®)	SAR studies performed to elucidate the key determinants of LPL activation ↑ in LPL activity in LPL ^{+/−} mice: 834 ± 133 versus 313 ± 89 mU/ml; ↑ in LPL activity in LPL ^{−/−} rats and tenfold ↓ in plasma TG	↓ in plasma TG levels for up to 26 weeks; sustained ↓ in chylomicron TG levels; immune response against viral coat proteins detected	[111] [119,120] [122,124]
Indirect LPL activation				
APOC3 inhibition	Antisense oligonucleotide: ISIS 304801 (product name: volanesorsen)	Evaluated in multiple preclinical animal models: C57BL/6 (chow and western diet), LDLR ^{−/−} (chow and western diet), Ob/Ob mice, western diet fed CETP transgenic LDLR ^{−/−} mice, fructose fed Sprague Dawley rats, ZDF rats and chow-fed cynomolgus and high-fructose supplement fed rhesus monkeys. APOC3 mRNA levels were reduced by 60–70% from 66% to 98%. TG reduction ranged from 19% to 80%. Acceptable tolerability profile reported. Favorable effects on plasma lipid profile were observed in cynomolgus and rhesus monkeys	Healthy subjects: dose dependent ↓ in plasma APOC3 and plasma TG levels with no safety concerns in a 50 day study FCS patients administered once weekly for 13 weeks and followed up for another 2 weeks: 71–90% ↓ in APOC3 mRNA and 56– 86% ↓ in TG levels	[131] [131] [132]
	siRNA: TKM-APOC3	In the human ApoC3-Tg mouse model: silencing of ApoC3 gene was accomplished, which resulted in rapid, potent and sustained ↓ in plasma TG which lasted for more than 2 weeks	Hyper TG patients: 13 week monotherapy led to 40–80% ↓ in APOC3 levels and a ↑ of 60–70% was observed when added to combination with ongoing fibrinolytic therapy Plasma TG ↓ by 31–70% Type 2 diabetes patients: 15 week volanesorsen therapy led to significant ↓ in plasma TG and APOC3; 57% improvement in insulin sensitivity after 3-month suppression of HBA1c	[134]
	siRNA: ALN-APOC3	Early studies have shown a strong persistent (up to 30 days) knockdown of APOC3 levels in the liver via single doses (3 mg/kg) of siRNA. In the ApoC3-Tg mouse model, intravenous route in mouse model transduced with AAV construct expressing human APOC3 gene. In the db/db mouse model of hyperlipidemia, plasma ApoC3 ↓ by >90% leading to a >50% ↓ of TGs	None reported	[136]
ANGPTL3 inhibition	Monoclonal antibody: REGN1500 (product name: evincumab)	↑ Post-heparin LPL activity in normolipidemic mice and lower plasma TG by >50%. Further, chronic testing (8 weeks) with high fat and high cholesterol diet in ApoE ^{−/−} mice showed that plasma TGs, plasma LDL-C, HDL-C levels, and similar activity seen in dyslipidemic cynomolgus monkeys	None reported	[137]
	Antisense oligonucleotide: IONIS ANGPTL3-LRx (ISIS 703802)	LDLR ^{−/−} mice up to 80% ↓ in plasma TG and 66% ↓ in plasma cholesterol, reduced atherosclerosis	HoFH patients: adding REGN1500 to ongoing lipid-lowering therapy ↓ LDL-C by 55% (25–90%) Phase I study: ISIS-ANGPTL3 Rx showed acceptable safety profile and dose-dependent reductions in	[139] [143] [143]

(http://files.shareholder.com/downloads/REGN/2807302/673x08894306/CSD4BE1B-4EB3-4E8D-9DE8-8FCBACEB8975/REGN_News_2016_3_1_General_Releases.pdf)

Novel and notable LPL-targeting strategies					
Strategy	Examples	Preclinical data	Clinical data	Preclinical	
				Refs	Clinical
APOC2 activation	siRNA: ALN-ANG	ob/ob mice: a dose-dependent and robust (>99%) ↓ of serum ANGPTL3. A single 3 mg/kg dose in these mice produced >80% ↓ in serum LDL-C and TGs	None reported	[137,144]	Phase 1 ANGPTL3 (82%), TG (49%) and total cholesterol (28%)
	Mimetic peptide: C-II-a	21 amino acid peptide (C-II-a) promotes TG hydrolysis, reduces TG activity to the same extent as full length APOC2. Peptide significantly ↓ plasma TG and cholesterol in APOE ^{-/-} mice. C-II-a corrects hyperTG in APOC2 mutant mice	None reported	[145] [146]	

Abbreviations: ANGPTL3, angiopoietin-like 3; ANGPTL4, angiopoietin-like 4; APOAV, apolipoprotein AV; APOC2, apolipoprotein C2; APOC3, apolipoprotein C3; APOE, apolipoprotein E; CETP, cholesteryl ester transfer protein; FCS, familial chylomicronemia syndrome; HDL-C, high-density lipoprotein cholesterol; HoFH, homozygous familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol; LDLr, low-density lipoprotein receptor; LPL, lipoprotein lipase; TG, triglycerides; ZDF, Zucker D fatty rat.