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Recent advances in novel therapies for lipid disorders

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Abstract

The prevalence of lipid disorders is alarmingly increasing in the Western world. They are the result of either primary causes, such as unhealthy lifestyle choices or inherited risk factors, or secondary causes like other diseases or medication. Atypical changes in the synthesis, processing and catabolism of lipoprotein particles may lead to severe hypercholesterolemia, hypertriglyceridemia or elevated Lp(a). Although cholesterol-lowering drugs are the most prescribed medications, not all patients achieve guideline recommended cholesterol levels with the current treatment options, emphasising the need for new therapies. Also, some lipid disorders do not have any treatment options but rely only on stringent dietary restriction. Patients with untreated lipid disorders carry a severe risk of cardiovascular disease, diabetes, non-alcoholic fatty liver disease and pancreatitis among others. To achieve better treatment outcome, novel selective gene expression and epigenetic targeting therapies are constantly being developed. Therapeutic innovations employing targeted RNA technology utilise small interfering RNAs, antisense oligonucleotides, long non-coding RNAs and microRNAs to regulate target protein production whereas viral gene therapy provides functional therapeutic genes and CRISPR/Cas technology relies on gene editing and transcriptional regulation. In this brief review, we will discuss the latest advances in clinical trials for novel lipid-lowering therapies and potential new targets in pre-clinical phase.

Introduction

Lipids are packaged into lipoproteins for transportation in blood and due to the intricate nature of lipoprotein metabolism, lipid disorders are a heterogeneous group of diseases that are characterised by abnormal levels of cholesterol and/or triglycerides in blood. They can originate from aberrant synthesis, processing, clearance or characteristics of lipoprotein particles, which in turn increases the risk of cardiovascular disease (CVD) and other metabolic syndromes such as diabetes and non-alcoholic fatty liver disease (NAFLD) (1). Severe lipid disorders are hypercholesterolemia, hypertriglyceridemia, mixed hyperlipidemia, elevated lipoprotein(a) (Lp(a)) and low high-density lipoprotein (HDL) cholesterol. Familial hypercholesterolemia (FH) is a hereditary lipid disorder that highly elevates low-density lipoprotein cholesterol (LDL-C) levels and predisposes patients to premature CVD (2). The prevalence of heterozygous FH (HeFH) worldwide is between 1:200 and 1:250 making it a major public health concern. Homozygous FH (HoFH) is a rarer and
more severe form of the disease with total cholesterol levels >13 mmol/l and estimated frequency of 1:160,000–1:300,000.

The first successful reports on lowering LDL-C levels are from the 1980s and since that, there have been multiple new lipid-lowering therapies (3). To date, the most widely used drugs are statins, small molecules that act by inhibiting cholesterol synthesis and by increasing LDL uptake. Other existing treatments aim to increase the availability of LDL receptors, such as antibodies targeting proprotein convertase subtilisin kexin type 9 (PCSK9), or to inhibit the absorption of cholesterol from the small intestine, such as small molecule drug ezetimibe. In addition, niacin and bile acid resin are used as a treatment for patients at high risk of CVD. While efficient, these traditional therapies have also some significant limitations when treating lipid disorders since they do not allow selective gene expression or epigenetic regulation but rely on inhibiting enzyme function or receptor activity. However, many of the severe lipid disorders are characterised by high circulating protein concentrations such as Lp(a) and apolipoprotein C3 (ApoC3), making the traditional treatment options unfeasible concerning both safety and administration frequency. In addition, substantial residual cholesterol risk has still been present in many studies with high dose statins or combined statins and ezetimibe, suggesting the traditional treatment options are not always satisfactory (4). In summary, there is a high need for alternative treatments and here we will discuss the status of novel therapies for the management of severe lipid disorders.

**Current novel lipid-lowering therapies in clinical trials**

**1) Small interfering RNAs**
Small interfering RNAs (siRNA) are double stranded RNAs that induce cleavage of the target mRNA and subsequently inhibit the target protein production (Figure 1.) (5). siRNAs function by incorporating into a cytoplasmic RNA-induced silencing complex (RISC) to complementary bind target messenger RNA (mRNA) and activate its cleavage. The cleaved mRNA is degraded and thus unavailable for protein translation, which results in the decreased levels of the target protein synthesis. siRNAs can be modified by conjugation to a targeting ligand such as N-acetylgalactosamine (GalNac) resulting in more efficient hepatic cellular uptake and distribution by the asialoglycoprotein receptors (5). One of the GalNac modified siRNA drugs is Inclisiran, which targets PCSK9, an enzyme that negatively regulates levels of the LDL receptor. Recent encouraging phase 2 trial showed that in the two-dose 300-mg Inclisiran group, a mean 52.6% LDL-C reduction was achieved at day 180 (6). Inclisiran was administered subcutaneously at day 1 and day 90, highlighting the benefit of chemical modification of the drug and therefore permitting infrequent dosage (Table 1.). Currently, Inclisiran is in phase 3 of development (NCT03705234).

**2) Antisense oligonucleotides**
Another powerful RNA targeting method utilises antisense oligonucleotides (ASO), which are short single stranded nucleic acid molecules that bind to their target mRNA through Watson-Crick interactions and lead to changes in translation (Figure 1.) (7). ASOs can be utilised to enzymatically cleave the target mRNA, change mRNA splicing pattern or alter the function of a regulatory RNA. ASOs are chemically modified to facilitate effective cell delivery and distribution (GalNac) as well as to enhance molecule stability. ASO technology has evolved rapidly and it has led to several commercially available ASO drugs and clinical trials (Table 1).
Mipomersen (KYNAMRO®, Kastle Therapeutics), a FDA approved ApoB targeting ASO drug for the treatment of HoFH, binds to the mRNA encoding ApoB and prevents ApoB production, and thus reduces hepatic VLDL, LDL and Lp(a) production decreasing LDL-C in a dose-dependent manner (8). Mipomersen is administered subcutaneously at 200 mg weekly and in a randomised, double-blind, placebo-controlled, phase 3 study it reduced LDL-C levels by 24.7% after 26-week treatment period. However, Mipomersen is prescribed only to a small group of patients and due to some side effects such as injection site-reactions, hepatic steatosis and flu-like symptoms, there is an increased risk of discontinuation of the treatment and low clinical use (5). Another lipoprotein possessing major CVD risk is Lp(a), which consists of an LDL like particle with ApoB and Apo(a). Oligonucleotide drug IONIS-APO(a)-LRx targets Apo(a) mRNA sequence and induces target cleavage and prevents the generation of Lp(a) (9). There is no specific approved therapy to lower Lp(a) and therefore results from a phase 2 trial showing potent, dose-dependent Lp(a) reduction were encouraging. Subjects with elevated Lp(a) levels receiving 100-300 mg subcutaneous injections weekly showed a mean Lp(a) reduction of 71.6%. At present, IONIS-APO(a)-LRX is in a randomised, double-blind, placebo-controlled, dose-ranging phase 2 study for subjects with elevated Lp(a) and established CVD (NCT03070782).

Volanesorsen, targeting ApoC3 mRNA, is an effective oligonucleotide drug reducing triglyceride levels in patients with hypertriglyceridemia or familial chyomicronemia syndrome (FCS) (10). FCS is characterised by severe hypertriglyceridemia due to a deficiency in lipoprotein lipase (LPL) or abnormalities in proteins promoting LPL activity and it is mainly treated with stringent dietary fat restriction (11). ApoC3 has been shown to act as an inhibitor of lipoprotein lipase and hepatic triglyceride-rich lipoprotein (chylomicrons, VLDL and their remnants) clearance inducing hypertriglyceridemia. In a randomised, double-blind, placebo-controlled, phase 3 study, weekly 300 mg subcutaneous Volanesorsen injections were given to FCS patients (12). By week 13, triglycerides decreased by a mean of 77% without any significant side effects. Currently, Volanesorsen is in a phase 3 open-label trial (NCT02658175).

Another target for triglyceride metabolism is ANGPTL3, a secretary protein that regulates plasma lipid levels by inhibiting LPL and endothelial lipase (4). ANGPTL3 loss-of-function mutations have been associated with low levels of plasma LDL-C, HDL cholesterol and triglycerides, suggesting ANGPTL3 could be a potential target to treat combined hyperlipidemia. In a phase 1 study, multiple doses of oligonucleotide drug IONIS-ANGPTL3-LRx reduced triglycerides by 33.2-63.1%, LDL-C by 1.3-32.9% and VLDL-C by 27.9-60% in patients with elevated triglyceride levels (13). A phase 2 trial of IONIS-ANGPTL3-LRx in subjects with hypertriglyceridemia, type 2 diabetes and NAFLD is ongoing (NCT03371355).

(3) Viral vector mediated gene therapy
Viral-based gene therapy involves the delivery of DNA encoding a therapeutic gene to treat underlying disease (Figure 1.). Adeno-associated viruses (AAVs) are some of the most widely used viral vectors due to their high transfection efficiency and their ability to transduce both dividing and non-dividing somatic cells (14). AAV gene therapy vectors are non-enveloped recombinant AAV particles that lack the ability to reproduce and can hold up to 5 kb of genetic material. Because of their biology, simple structure, and the fact that AAVs are not associated to any known disease, AAVs are considered one of the most alluring and safest gene therapy approaches (Table 1.).
A majority of FCS patients suffer from LPL deficiency (LPLD), a very rare but devastating autosomal recessive disorder that predisposes the patients to hypertriglyceridemia, xanthomas and life-threatening complications such as pancreatitis (15). Alipogene tiparvovec (Glybera®, uniQure) is an AAV serotype 1 vector/therapeutic designed effectively reduce circulating plasma triglyceride levels by introducing to the body a functional LPL gene. Before the commercialisation of alipogene tiparvovec as the first European Medicines Agency -accepted gene therapy, no treatment for LPLD was available (16). However, 5 years after its release to the market in 2012, the manufacturer of alipogene tiparvovec decided not to pursue the renewal of the marketing authorisation in Europe because of the treatment’s record-breaking price.

A vast majority (>90%) of HoFHs are caused by defects in the LDL receptor (LDLR) gene, making LDLR an optimal target for gene therapy of HoFH (2). AAV8.TBG.hLDLR (RGX-501, RegenXBio), a recombinant AAV8 vector containing the human LDLR gene, has been extensively studied in mice and non-human primates (17, 18). AAV8.TBG.hLDLR delivers a functional LDLR gene to patients suffering from mutations in both LDLR alleles. The results of the preclinical studies are very promising: in a pharmacology/toxicology study, LDLR deficient mice were administered with a single intravenous injection to the tail vein of either the mouse LDLR gene (at low, medium or high dose) or the human LDLR gene (high dose) (18). All AAV-treated mice demonstrated a marked reduction in plasma cholesterol levels and no dose-limiting toxicities were reported. Serum cholesterol levels reduced over 80% in the medium dose (7,5x10^{12} GC/kg of mouse LDLR) group, which is the highest proposed dose for the clinical trial. AAV8.TBG.hLDLR is currently in phase 1/2 open label clinical trial (NCT02651675) and results of this trial are expected later in 2019.

Novel gene therapeutics in pre-clinical studies

(1) CRISPR/Cas9 based therapeutics
CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 is a novel gene editing system, which allows direct modification and repair of the target sequences and knocking out genes. As naturally occurring inactivating mutations in PCSK9 (19), ApoC3 (20) and ANGPTL3 (21) have shown to be athero-protective, mimicking these phenotypes with the aid of CRISPR/Cas9 technology is a promising novel treatment option for hyperlipidemia and atherosclerosis (Figure 1.). First encouraging results were obtained from mice studies showing that the inhibition of PCSK9 with S. pyogenes Cas9 and guide RNA packaged in adeno-viral vector (22) as well as smaller S. aureus Cas9 in adeno-associated virus (AAV) vector resulted in reduction of PCSK9 protein concentrations by 90 % and significantly reduced plasma cholesterol levels without considerable off-target effects (23, 24). Importantly, PCSK9 inhibition was also achieved in mice transplanted with human hepatocytes suggesting that CRISPR/Cas9 technology could be utilised in human cells and tissues (25). Furthermore, the base editing variation of CRISPR/Cas9 in adenovirus vector demonstrated efficient ANGPTL3 downregulation and triglyceride and cholesterol lowering suggesting another method to treat combined hyperlipidemia (26).

(2) Other viral vectors for gene therapy
Lentiviruses (LV) are capable of integrating into the genome of non-dividing cells providing stable and long-term transgene expression (27). LV gene therapy vectors are an attractive option since they have a packaging capacity up to 8 kb and they induce only a moderate
immune response. In a recent study, LV carrying LDLR as a therapeutic gene for hyperlipidemia were injected in Watanabe heritable hyperlipidemic (WHH) rabbits, which are naturally lacking functional LDLR (28). Encouraging results show that LV LDLR gene transfer to WHH rabbit liver reduced total cholesterol levels by 18 % four weeks after the gene transfer and by 47 % at one-year time point. FDA approved the first LV gene therapy tisagenlecleucel in 2017 for treating acute lymphoblastic leukemia, suggesting that LV could provide an efficient tool also for the treatment of lipid disorders.

(3) Non-coding RNA therapy
Since the discovery of non-coding RNAs and increased understanding of their regulatory function, the use of several different non-coding RNAs as a therapeutic tool has widely expanded. MicroRNAs (miRNAs) are endogenous, single-stranded RNAs of around 22 nucleotides long and they function in post-transcriptional regulation of gene expression. miRNAs have been reported to regulate several key genes related to lipid metabolism and a recent publication demonstrated their therapeutic potential by lentivirus injection of miR-98, a SREBP-2 regulator, via tail vein, which significantly reduced serum and liver cholesterol levels in mice without inducing liver toxicity (29, 30). Other therapeutically suitable transcripts for treating lipid disorders are long non-coding RNAs, which consist of 200 nucleotides or more and regulate gene expression in multiple ways. Tail vein injection of long non-coding RNA LeXis in AAV8 expression vector (AAV8.hTBG.LeXis) led to significant decrease in total cholesterol and triglyceride levels combined with lowered atherosclerotic burden in mice, highlighting the therapeutic potential of long non-coding RNAs for treating lipid disorders (31).

Alternative targets
In addition to well-established treatment targets, such as LDLR, PCSK9 and ApoB, novel mediators of lipid metabolism are continuously explored to establish more specific ways to diagnose and prevent the development of CVD. Genome- and exome-wide association studies provide an insight to potential new targets by identifying genetic variants. Novel loci associated with dyslipidemias are found frequently, both within protein coding genes and non-coding RNAs (32, 33). However, genome wide association studies do not provide functional characterisation of the target loci and therefore, traditional in vivo studies are performed to find functional associations between genes and lipid disorders. For example, vascular endothelial growth factor receptor 3 (VEGFR3) and its ligands VEGF-C and VEGF-D are a well-known angiogenic and lymphangiogenic factors but have recently also shown to regulate lipoprotein metabolism by modifying lipid absorption and the expression of hepatic receptors (34–36). Interestingly, abnormal levels of VEGF-C and VEGF-D in the plasma of patients with CVD have been shown to predict higher mortality (37) as well as heart failure (38), atrial fibrillation and stroke (39), suggesting the importance of these growth factors in different stages of CVD.

Conclusions
CVD remains the leading cause of death in the Western world and dyslipidemia is the number one risk factor. The development of novel RNA based therapeutics and viral gene therapy is providing more specific and potent therapies for tackling complex and devastating lipid disorders. These advances might provide powerful treatment for patients that have not benefitted from the traditional therapies, consequently easing the burden of
CVD. Many drugs are in phase 1-3 of development and several novel therapies are explored in vivo, of which some are expected to be approved for clinical use. Lipid metabolism is a complex and multifactorial system, and hence the evaluation of the side effects of different gene targets as well as long-term safety of the therapy must be carefully evaluated in every novel treatment strategy before widespread implementation.

Acknowledgements

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References


**Figure 1.** Schematic overview of putative viral vector and RNA targeted therapeutics for lipid disorders. Viral like particles can be used to deliver vectors encoding therapeutic and
functional proteins or to introduce CRISPR/Cas9 to repress or activate genes that are associated with lipid disorders. RNA therapeutics relies on targeting mRNA with siRNAs or ASOs for degradation hence reducing the targeted protein and subsequently affecting circulating lipoprotein levels. AAV indicates adeno-associated virus; ASO, antisense oligonucleotide; gRNA, guide RNA; siRNA, small interfering RNA; RISC, RNA inducible silencing complex.
<table>
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<td>AAV8.TBG.hLDLR</td>
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ASCVD, atherosclerotic cardiovascular disease; CVD, cardiovascular disease; FCS, familial chylomicronemia syndrome; HC, hypercholesterolemia; HeFH, heterozygous familial hypercholesterolemia; HoFH, homozygous familial hypercholesterolemia; LPLD, lipoprotein lipase deficiency.