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A NEW DAWN FOR MANAGING DYSLIPIDEMIAS: THE ERA OF RNA-BASED THERAPIES

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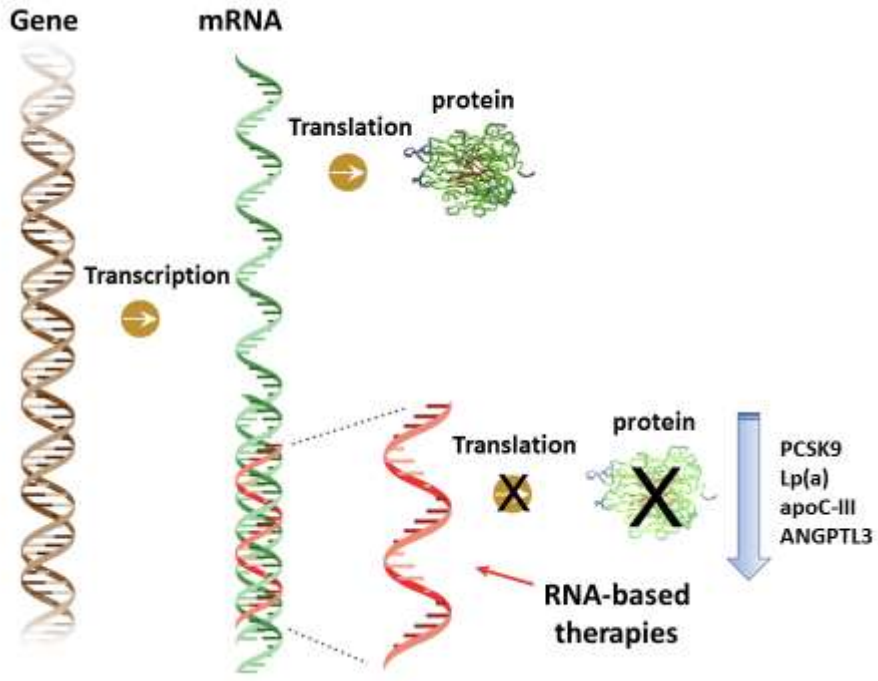
Graphical abstract



ABSTRACT

The high occurrence of atherosclerotic cardiovascular disease (ASCVD) events is still a major public health issue. Although a major determinant of ASCVD event reduction is the absolute change of low-density lipoprotein-cholesterol (LDL-C), considerable residual risk remains and new therapeutic options are required, in particular, to address triglyceride-rich lipoproteins and lipoprotein(a) [Lp(a)]. In the era of Genome Wide Association Studies and Mendelian Randomization analyses aimed at increasing the understanding of the pathophysiology of ASCVD, RNA-based therapies may offer more effective treatment options. The advantage of oligonucleotide-based treatments is that drug candidates are targeted at highly specific regions of RNA that code for proteins that in turn regulate lipid and lipoprotein metabolism. For LDL-C lowering, the use of inclisiran - a silencing RNA that inhibits proprotein convertase subtilisin/kexin type 9 (PCSK9) synthesis - has the advantage that a single s.c. injection lowers LDL-C for up to 6 months. In familial hypercholesterolemia, the use of the antisense oligonucleotide (ASO) mipomersen, targeting apolipoprotein (apoB) to reduce LDL-C, has been a valuable therapeutic approach, despite unquestionable safety concerns. The availability of specific ASOs lowering lipoprotein (a) [Lp(a)] levels will allow rigorous testing of the Lp(a) hypothesis; by dramatically reducing plasma triglyceride levels, volanesorsen (apoC-III) and angiopoietin-like 3 (ANGPTL3)-LRx will further clarify the causality of triglyceride-rich lipoproteins in ASCVD. The rapid progress to date heralds a new dawn in therapeutic lipidology, but outcome, safety and cost-effectiveness studies are required to establish the role of these new agents in clinical practice.

Keywords: angiopoietin-like 3, antisense oligonucleotide, apoC-III, dyslipidemias, inclisiran, mipomersen, LDL-C, lipoprotein (a), PCSK9, short small interfering RNA, volanesorsen

1. INTRODUCTION

Atherosclerotic cardiovascular disease (ASCVD) is still the leading cause of death worldwide despite excellent pharmacological approaches and revascularizations (1, 2). Elevated low-density lipoproteins (LDL) represent the most significant causal risk factor for the development of atherosclerosis (3), statins being the most successful and widely used therapy. However, despite their widespread use and undeniable cost-effectiveness, a considerable residual risk remains (4, 5), much of which is still attributable to lipids and lipoproteins (6).

The extent of LDL-cholesterol (LDL-C) lowering and ensuing cardiovascular (CV) risk reduction has been proven across different statin and non-statin therapies. The relative risk reduction of major vascular events was similar for all drug classes (statins, bile acid sequestrants, ezetimibe, proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors and fibrates), and the achieved lowering of LDL-C was directly associated with a reduced incidence of major ASCVD events (7). Besides raised LDL-C (8), dysregulated lipoprotein metabolism and elevated lipoprotein(a) [Lp(a)] levels remain important risk factors that contribute to the development of atherosclerosis (9, 10). Other important contributors to the development of ASCVD are the cholesterol content of triglyceride (TG)-rich lipoprotein (TGRLs) remnants (11). Observational and genetic studies clearly indicate that remnant cholesterol is a causal risk factor for CV disease (12).

Rare loss-of-function mutations in the apolipoprotein C3 *(APOC3)*, Angiopoietin-like protein 3 *(ANGPTL3)*, and *ANGPTL4* genes, that encode for natural inhibitors of lipoprotein lipase (LPL), have been observed to associate with lower TG levels and a corresponding lower risk of ASCVD (13-15). Residual risk is also being linked to inflammation, as proven by the proof-of-concept CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcomes Study) trial demonstrating that reduction of interleukin-1 β in subjects with established ASCVD leads to a 15% reduction in the risk of recurrent events (16). Insulin resistance, dysglycemia, hypertension, platelet aggregation and thrombosis also collectively contribute to residual risk of a recurrent ASCVD (17).

The present review will investigate novel therapeutic approaches targeting ribonucleic acids, *i.e.* single stranded antisense oligonucleotides (ASO) or double-stranded small inhibiting RNA (siRNA) that lower circulating levels of lipoproteins, *e.g.* LDL-C, Lp(a) and TG. Indeed, although the use of monoclonal antibodies is highly effective, as in the case of PCSK9 antagonism (18, 19), for proteins without enzyme activity, *e.g.* Lp(a), apoC-III or ANGPTL3, this approach would require the use of a large mass of antibodies that may generate large amounts of immune complexes, thus raising safety concerns (20). For this purpose, by using Pubmed.gov, we revised available English-language studies published from January 2005 – when the first siRNA clinical trial started (21) - to June 2019 and relevant to the key clinical questions discussed in this review. Search terms included, inclisiran, mipomersen, APO-(a)Rx, APO-(a)L_{RX}, APOCIII-Rx, APOCIII-$L_{RX,}$ volanesorsen, and ANGPTL3-$L_{RX.}$

2. RNA-BASED THERAPEUTIC APPROACHES: ASO AND siRNA

Antisense oligonucleotides (ASOs) are synthetic single-stranded strings of nucleic acids (typically 5-25 nucleotide-long) designed to form hybrids with target transcripts that have complementary sequences; the accuracy of the binding relies on a Watson-Crick-base-pairing interaction (22, 23). ASOs are used to control gene expression by a degrading mechanism which involves the RNase H1, an endoribonuclease that preferentially binds to the DNA-RNA hetero-duplex over RNA-RNA and DNA-DNA homo-duplexes (Figure 1). Hybridization of an ASO with a central "gap" of DNA bases (gapmer ASO) to the targeted mRNA mimics the DNA–RNA pairing, leading to the activation of RNase H1 that selectively hydrolyzes the RNA strand of the ASO-RNA duplex (24). The end result is the selective cleavage of the RNA strand while the synthetic DNA strand remains intact and free to bind additional target mRNAs (21). ASOs can also act through non-degrading mechanisms by pairing with the target mRNA but, given the design, they do not initiate the direct degradation of mRNA (24).

The therapeutic use of ASOs, however, has required a number of chemical modifications in order to improve stability, resistance to nucleases, bioavailability and ability to reach a precise cellular compartment (21). In the "first-generation" ASOs, the phosphorothioate backbone was the most widely used modification; the replacement of a non-bridging phosphodiester oxygen by sulfur reduced ASO hydrophilicity, enhanced their resistance to nuclease activity and conferred pharmacokinetic benefits, *e.g.* a raised binding to plasma proteins, a feature that leads to a delayed renal clearance and an increased half-life (25). However, the ability to interact with proteins has led to several side effects, such as complement activation, inhibition of the intrinsic coagulation pathway and immune stimulation. In this respect, first-generation ASOs were typically associated with platelet activation, pro-inflammatory changes and a raised incidence of glomerulonephritis and vasculitis (26). In an attempt to overcome these side effects, in the "second generation ASOs" the 2'-hydroxyl position of the sugar backbone moieties was substituted by 2'-O-methyl (2'-OMe) and 2'-O-methoxyethyl (2'-MOE) and 2'-fluoro (2'-F) residues. These modifications conferred ASOs a high nuclease resistance, increased binding affinity to target mRNAs, lower toxicity, and an improved lipophilicity which foster the lipid bilayer diffusion and increase plasma half-life. On the other hand, the introduction of chemical modifications into ASOs often interferes with their RNase H1-inducing capacity, a limitation exceeded by the introduction of a phosphorothioate backbone core, flanked by nuclease-resistant arms consisting of 2'-O-Me or 2'-MOE oligonucleotides (27). These chemical modifications also impact on the pharmacokinetic properties of ASOs. The dose frequency administration ranges between once a week to once every 4 months with a bioavailability

of 50-100%. After the s.c. injection, peak plasma concentrations will be achieved within 3-4 hours with a rapid plasma clearance due to the fast tissue distribution and slow clearance from the tissues. Terminal elimination half-life is long, *i.e.* from 2 weeks to 6 months (21). Moreover, as ASOs are metabolized by cellular nucleases and not by the cytochrome P450 system, they can be co-administered with traditional therapeutic agents with different modes of action (28)

Since ASOs typically show broad distribution and the organs with the highest distribution are the kidney and the liver, researchers were prone to developing ASOs primarily for the treatment of liver-related disorders. Among these strategies, the conjugation with triantennary *N*-acetyl galactosamine (GalNAc) has been the most widely used in the treatment of hyperlipoproteinemias (29). GalNAc binds to the hepatocyte-specific asialoglycoprotein receptor (ASGPR) with high affinity. ASGPR recognizes a wide variety of ligands containing terminal galactose or GalNAc residues (30). This binding occurs at the sinusoidal surface of the hepatocytes containing about 500,000 receptors per cell. Once internalized, the complex is dissociated, allowing ASGPR to return to the membrane, and ASO to be released in the intracellular compartment (29).

RNA interference (RNAi), also known as post-transcriptional gene silencing, is a conserved biological process allowing a mRNA to be destroyed in response to double-stranded RNA (dsRNA) (31). The starting event for the RNAi pathway is the cleavage of long dsRNA molecules into short small interfering RNA (siRNA) fragments, 21–23 bp in length, by a member of the ribonuclease (RNase) III family called DICER. Thus, synthetic siRNAs, aimed at silencing specific target genes, mimic the structure of DICER products. siRNAs are composed of two strands: (i) the guide containing the information for target-gene recognition and (ii) the passenger supporting the geometry required to be loaded into the RISC (RNA-induced silencing complex) (32). Once in the cytoplasm the two strands are separated with the guide loaded into the RISC and the passenger removed and degraded. RISC uses the guide RNA to find complementary mRNA sequences via Watson-Crick base pairing (33). When the complementary target-mRNA has hybridized with part of the guide strand, an endonucleolytic cleavage of the mRNA is driven by a component of RISC, the Argonaute 2 (Ago2) protein, that belongs to a protein superfamily consisting of an endonucleolytic-capable protein (Ago2) and non-catalytic proteins (Ago1, Ago3 and Ago4) (34) (Figure 1). The mRNA cleavage occurs between nucleotides 10 and 11 on the complementary antisense strand, relative to the 5'-end (35).

In spite of the promising efficacy of siRNAs, the pharmacological application has required chemical modifications or formulations to increase stability, reduce innate immunity and foster delivery to target tissues (36). Naked siRNAs undergo degradation by plasma nucleases or are

filtered by the glomerulus with a rapid renal clearance leading to a short half-life, *i.e.* from 15 min to 1 hour. To improve stability, the most widely used modifications are (i) the 2' position of the sugar ring which includes 2'-OMe, 2'-F, 2'-MOE, and (ii) the introduction of phosphorothioate modifications in place of the phosphodiester linkages of siRNA (37).

The optimization of siRNA delivery consists in improving tissue targeting and cellular uptake, *e.g.* by binding siRNA to ligands that promote uptake by target cells. A breakthrough in conjugate-mediated delivery was the development of the GalNAc conjugated siRNA targeting ASGPR in hepatocytes. After administration, the absorption is rapid with an elimination half-life from hepatocytes of several weeks (21).

Another strategy fostering the liver uptake of siRNA was the conjugation to lipophilic molecules which allows siRNA to be taken up by hepatocytes (31). The bioconjugation with cholesterol and fatty acids improves tissue specificity uptake via LDL receptor (LDLR) and protects siRNAs against nuclease activity. Other important issues rely on (i) prevention of renal clearance, achieved by the use of nanoparticles with a diameter above 20 nm which prevents renal filtration, (ii) the negative charge of siRNA, a feature not allowing these hydrophilic molecules to diffuse across the negatively charged cell surface, (iii) the avoidance of non-specific interactions with serum proteins and non-target cells (by coating siRNA with the hydrophilic polymer polyethylene glycol, it is possible to minimize macrophage uptake, non-specific interactions and immune recognition), (iv) the escape from lysosomal degradation by the fusion with cationic lipids (38). With regards to safety profile, siRNAs can cause off-target effects by silencing unintended genes which have a partial homology with the target one (39) or can induce a nonspecific activation of the innate immune system mediated or not by Toll-like receptor 3, 7 and 8. The use of nanoparticle carriers can protect siRNA from interaction with Toll-like receptors, preventing possible immune-mediated toxicities (40).

3. Therapeutic approaches to reduce LDL-C

3.1 Inclisiran (in later clinical trials). In the era of monoclonal antibodies against PCSK9, that dramatically reduce LDL-C by 45–60% when used alone or in combination with a statin (41), a different therapeutic approach inhibiting PCSK9 synthesis has been also developed (42). The ALN-PCS was an N-galactosamine conjugated siRNA formulated in lipid nanoparticles, a biodegradable coating for siRNA whose hepatic specificity was increased by conjugation with an oligosaccharide moiety (43). The safety and efficacy of ALN-PCS was tested in a phase 1 dose-escalation study

enrolling 32 healthy adult volunteers with LDL-C > 116 mg/dL. Subjects were given either one i.v. dose of ALN-PCS (ranging from 0.015 to 0.400 mg/kg) or placebo. A single-administration of this siRNA reduced in a dose-dependent manner plasma PCSK9 protein, higher doses resulting in a more prolonged reduction. The highest dose (0.400 mg/kg) on day 3 gave the maximal reduction of PCSK9, namely -70% from baseline relative to placebo. At the same dose and compared to placebo, LDL-C was reduced by 40%. Concerning safety issues, no clinically significant changes in liver function tests, troponin, or C-reactive protein (CRP) were found (44).

A major setback was the formulation of ALN-PCS, flawed for clinical use because of inadequate duration of effects; this led to the development of inclisiran, whose safety and efficacy were tested in a phase 1 trial (45). Inclisiran, designed to target the 3' UTR of the PCSK9 mRNA, is a long-acting siRNA whose 3' end of the passenger strand is functionalized with triantennary GalNAc. The chemical configuration foresees also a phosphorothioate in the backbone and one 2'-deoxy, eleven 2'-fluoro, and thirty-two 2'-O-methyl modified nucleotides (46).

Safety of inclisiran was evaluated in healthy volunteers with LDL-C> 100 mg/dL, randomized to receive either a single ascending dose (25, 100, 300, 500, or 800 mg) or multiple doses (125 mg weekly for four doses, 250 mg every two weeks for two doses, or 300 or 500 mg monthly for two doses). PCSK9 levels were maximally reduced by 74.5% either at the dose of 300 mg (in the single dose regimen) or at the dose of 500 mg monthly for two doses (in the multiple-dose regimen). Concerning LDL-C lowering, the largest magnitudes of reduction were 50.6% in volunteers receiving the 500 mg single dose and 59.7% in those receiving the 300 mg monthly injection for two months. These effects were maintained for at least six months with no serious adverse events (47). Inclisiran is rapidly distributed in plasma with peak concentrations occurring at the end of the infusion, with roughly dose-proportional increments (48). Single doses of inclisiran have pharmacologic activity for more than 3 months allowing dosing as infrequently as quarterly or even twice a year (49).

A further phase 1 trial (NCT03159416), the ORION-7 (A Study of Inclisiran in Participants With Renal Impairment Compared to Participants With Normal Renal Function) was completed in November 2018 and results have been presented in a poster form. This trial was aimed to test safety, tolerability, pharmacokinetics and pharmacodynamics of a s.c. injection of a single dose of inclisiran in subjects with mild, moderate and severe renal impairment compared to those with normal renal function. A combined analysis from the ORION-1 [Trial to Evaluate the Effect of ALN-PCSSC Treatment on Low Density Lipoprotein Cholesterol (LDL-C)] and ORION-7 trials demonstrated

that this agent achieved consistent reductions in LDL-C in patients with a wide range of renal function, with no dose adjustment necessary for those with renal impairment (50).

The ORION-1 study was a phase 2 dose-ranging trial lasting 6 months. Six different doses were tested, *i.e.* 3 single-dose (200, 300 and 500 mg) and 3 two-dose (100, 200 and 300 mg) starting regimens administered 90 days apart. In high risk ASCVD patients or ASCVD-risk equivalent, already at maximally tolerated statin therapy, siRNA was overall superior to placebo in improving dyslipidemia (Table 1). The largest LDL-C reduction (-52.6%) was achieved by the two-dose 300-mg regimen, a percentage similar to the one achieved with mAbs. Soon after the inclisiran injection (14 days), PCSK9 levels were reduced by a mean between 59.6% to 68.7%, changes that remained stable up to 6 months (range: 47.9-59.3%). The advantage of the two-dose regimen was clear at day 180 when PCSK9 levels dropped from baseline between 53.2% and 69.1%. Adverse events occurred in 11% of the patients who received inclisiran and in 8% of those at placebo (51). The presence of diabetes at baseline did not change the effectiveness of inclisiran in the management of dyslipidemias; LDL-C dropped between 28% and 52 % in patients without diabetes, and between 28% and 55% in diabetics (52). Concerning the effect of a single dose inclisiran (300 mg) on other lipoproteins, non- high-density lipoprotein (HDL-C) decreased by 35% and apolipoprotein (apo)B by 31%; a second dose at day 90 allowed non-HDL-C and apoB to be reduced by 46% and 41%, respectively, at day 180 (53). Based on these findings, the developers have chosen a dose regimen for all future studies of 300-mg on day 1 and day 90 and then every 180 days (52).

The efficacy, safety and tolerability of long-term dosing of inclisiran has been assessed in the ORION-3 trial, an open-label extension (OLE) study of the ORION-1. The interim analysis showed that inclisiran reduced LDL-C by 64 mg/dL from baseline (51%) and by 59.4 mg/dL vs placebo, the effect being independent of the doses of inclisiran previously given in the ORION-1 study. Over approximately a period of 3 years from the first dose of inclisiran, no safety issues were described, including rises in liver enzyme or changes in renal function (54).

Very recently, data on ORION-2, a pilot study that tested inclisiran in four Homozygous Familial Hypercholesterolemia (HoFH) patients, showed that inclisiran (300 mg/two regimen-dose) lowered LDL-C up to 30%. The efficacy lasted for up to 6 months in three out of four subjects in the study. Two HoFH subjects with identical LDLR mutations had variable responses highlighting that, despite comparable effects on PCSK9 reduction (about -80% in all), different degrees of LDL-C reduction may be observed in HoFH patients with the same causal mutations. Although promising, these results need to be confirmed in the larger ORION-5 study involving 45 HoFH patients (55).

The clinical development of the ORION program currently includes phase 3 trials that will evaluate the efficacy of 300-mg inclisiran in Heterozygous Familial Hypercholesterolemia (HeFH) (ORION-9) or established ASCVD (ORION-10; conducted in US) or ASCVD and ASCVD risk equivalent (ORION-11; mainly conducted in Europe) patients; a long-term cardiovascular outcomes trial involving approximately 15,000 ASCVD risk patients has been also planned (ORION-4).

Besides inclisiran, SPC5001 is an oligonucleotide acting as an antisense PCSK9 inhibitor. This 14-mer oligonucleotide with locked nucleic acid modifications allows an increase of binding affinity to the target sequence and a rise of nuclease resistance (56). Although pharmacodynamic results in 24 volunteers with LDL > 100 mg/dL were encouraging, *i.e.* -49% in PCSK9 levels over a period of 49 days and maximal LDL-C lowering at day 28, severe safety issues may limit future development. SPC5001 dose-dependently increased serum creatinine with the appearance of urinary granular casts. One case of acute tubular necrosis 5 days after the last SPC5001 administration was also reported (57).

3.2. Mipomersen – Kynamro. The manufacturing of mipomersen has been discontinued (2018) and the product is not clinically available. From the Federal Register notice (FDA-2019-N-2040-0001), the Kastle Therapeutics notified the FDA that the drug product is no longer marketed and requested the approval of the drug to be withdrawn. Approval has been withdrawn since August 2, 2019. Mipomersen is a second generation phosphorothioate oligonucleotide, 20 nucleotides in length (5'-GCCUC AGTCTGCTTC GCACC-3'), that contains MOE groups allowing a high resistance to exonucleases (58). Generally given as weekly s.c. doses (200 mg), in HoFH, severe hypercholesterolemia, HeFH with established coronary artery disease, or hypercholesterolemia with high risk for coronary heart disease (CHD), mipomersen consistently reduced all LDL particle numbers and preferentially small LDL (59). Besides LDL-C, mipomersen improved the overall lipid profile with no changes in high-sensitivity (hs)CRP levels (60, 61): apoB (-26.8%), total cholesterol (TC; -21.2%), non-HDL-C (-24.5%), Lp(a) (-31.1%), and HDL (+15.1%) (60, 62). Mixed results were reported when the reduction of TG levels was considered (63, 64), with a lowering effect on apoC-III and apoC-III-containing lipoproteins (9). Of note, the lowering effect on Lp(a) (65) is apparently secondary to an increased Lp(a) fractional catabolic rate (FCR) (66).

Mipomersen has been also tested in pediatric patients, enrolled in the OLE study (52 or 104 weeks). At the semiannual intervals over the course of OLE, the overall decrement of LDL was between -27% and -28% and that of apoB between -28% and -31%. However, after 40 weeks, levels

of apoB-containing lipoproteins tended to fluctuate, thus leaving the possibility of poor adherence as also reflected by plasma drug concentrations (67).

A retrospective analysis evaluated the potential of mipomersen to lower major adverse cardiovascular events (MACEs): in high-risk FH patients, a significant 85% reduction in the incidence of new MACEs was noted during a follow-up of 4.5 years, compared to the 2 years preceding therapy (68).

Concerning safety, major adverse effects have been injection-site reactions, flu-like symptoms, nausea, headache and elevations in serum transaminases, specifically alanine aminotransferase (ALT) (69). Hepatic enzyme elevations and liver fat content increased initially, with a trend towards baseline in many cases with extended dosing and in follow-up, suggestive of adaptation (62). Liver biopsies showed hepatic steatosis without inflammation (70).

4. Therapeutic approaches to lower Lp(a)

4.1 APO-(a)Rx and APO(a)-L$_{Rx}$ (in early clinical trials). Lp(a) consists of a cholesterol-rich lipid particle, analogous to LDL, with apoB-100 linked by a disulphide bond to a highly glycosylated apoprotein, called apo(a) (71). While the apo(a) component is synthesized almost exclusively in the liver, the site of Lp(a) assembly has not been confirmed yet (72). Lp(a) clearance from plasma has an undefined mechanism, with the LDLR playing only a modest role. In addition, five key classes of receptors have been described to play a role in the uptake of Lp(a), *i.e.* 'classical' lipoprotein receptors, scavenger receptors, toll-like receptors, carbohydrate receptors or lectins and plasminogen receptors (73); other possibilities include the proteolytic cleavage of apo(a) (72).

Based on the knowledge that up to 90% of the variability in plasma Lp(a) concentrations is genetically determined (74), the use of an ASO that reduces liver apo(a) synthesis and consequently reduces hepatic synthesis and secretion of Lp(a) particles into the circulation seems a feasible approach. The first developed ASO was the APO-(a)Rx with the following characteristics: five 2'-MOE modified ribonucleosides at the 5' and 3' ends and ten 2-O deoxyribonucleosides within the central portion. APO-(a)Rx was designed to perfectly match only the exon 24-25 splice sites, *i.e.* first exon CTTGTTC and second exon TGCTCCGTTGGTG, of the mature human apo(a) transcript at position 3901-3920 bp (75).

In this ASO, internucleotide phosphates were chemically modified with a phosphorothioate substitution, in which one of non-bridging oxygen atoms is replaced with sulphur (76). In a double-

blind phase 1 study, APO(a)$_{Rx}$ was tested at doses between 50 and 400 mg/day in patients with Lp(a) ≥ 100 mg/dL. Patients were assigned to receive a single-dose s.c. injection of the drug (50 mg, 100 mg, 200 mg or 300 mg) compared to placebo. Among these patients, 31 were assigned to six s.c. injections in order to reach a final dosage of 600 mg, 1200 mg or 1800 mg. The primary goal of the study was to test percentage changes in fasting Lp(a) levels at day 30 in the single-dose cohort and at day 36 in the multi-dose cohort. Whereas single doses did not lead to any reduction of Lp(a) at day 30, six doses resulted in a dose-dependent percentage reductions of Lp(a) concentrations: -39.6% (600 mg group), -59% (1200 mg group) and -77.8% (1800 mg group). Similar changes were found in the amount of oxidized phospholipids associated with apoB-100 and apo(a), known to mediate the pro-inflammatory potential of Lp(a): -26.1%, -55.1% and -61.3%, respectively (76) (Table 2). In a phase 2 study, 64 participants with elevated Lp(a) were given APO-(a)$_{Rx}$ at escalating-doses of 100mg, 200 mg and 300 mg once a week (s.c.) for 4 weeks each with a sequentially up-titration until week 12. Absolute percentage changes in fasting Lp(a) ranged between 66.8 and 71.7% (77).

The APO(a)-L$_{RX}$ was later produced in order to increase the efficacy of this ASO. It is a ligand-conjugated antisense nucleotide variant with a GalNAc covalently attached as well as with the replacement of six of the 19 phosphorothioate linkages with phosphodiester linkages at positions 2, 3, 5, 16 and 17. APO(a)-L$_{Rx}$ was tested in a proof-of-concept phase1/2a trial in healthy volunteers with Lp(a) levels ≥ 30 mg/dL. Injections of ascending doses of 10, 20, 40, 80 or 120 mg led to an absolute stepwise reduction in fasting Lp(a) levels with the maximal benefit occurring at day 30: -24.8% (10 mg), -35.1% (20 mg), -48.2% (40 mg), -82.5% (80 mg) and -84.5% (120 mg). The same dose-dependent trend was found for OxPL-apo(a) and OxPL-apoB. In the follow-up six dose-ascending study, at day 36, APO(a) L$_{Rx}$ resulted in mean reductions from baseline of 66% in the 10 mg group, 80% in the 20 mg group and 92% in 40 mg group, all highly significant. The oligonucleotide proved to be safe. Overall, these findings show that APO(a)-L$_{Rx}$ is 30 times more effective than APO(a)$_{Rx}$ allowing for very small injection doses (77) (Table 2).

Most recently, Tsimikas has provided data from a multicenter international dose-ranging phase 2b trial on 286 patients with pre-existing CVD, *i.e.* coronary artery disease, stroke/TIA, or peripheral arterial disease including carotid artery disease, and with baseline Lp(a) > 60 mg/dL. Patients were randomized to APO(a)L$_{Rx}$ 20, 40, 60 mg or placebo every 4 weeks (Q4W), or 20 mg every 2 weeks (Q2W) or 20 mg once weekly (QW). Duration was at least six months. The largest Lp(a) reduction was with 20 mg QW, *i.e.* -80% followed by 60 mg Q4W (-72%), 20 mg Q2W (-58%)

and the Q4W doses (-35% with 20 mg and -56% with 40 mg). These changes were associated to larger or lower achievements of Lp(a) ≤ 50 mg/dL, *i.e.* from 97.7% to 62% (Figure 2). Reductions of a similar extent were reported in oxidized phospholipid–apoB; the most effective dose gave LDL-C and apoB reductions of 20.5% and 14.5%, respectively. With respect to safety, compared to placebo, administration of APO(a)-L_{rx} resulted in more treatment emergent adverse events (TEAE), injection site erythema being most common (25%). Pooled APO(a)-L_{rx} dose regimen analyses showed more often ≥ 1 TEAE (89.1% vs 83%), ≥ 1 serious TEAE (10.5% vs 2.1%), ≥ one related serious TEAE (0.8% vs 0%) and ≥ 1 TEAE leading to discontinuation (4.6% *vs* 4.3%). There were no safety concerns related to platelet count, liver tests or renal function (78). The possible impact of Lp(a) lowering with APO(a)L_{Rx} further on denominated TQJ230 80mg once a month *vs* placebo injections in patients with previous ASCVD and Lp(a) ≥ 70 mg/dL will be tested in the (Lp(a)Horizon) trial (NCT04023552).

5. Therapeutic approaches to lower TG

5.1 Volanesorsen (recently approved in Europe). ApoC-III, mainly secreted by the liver and to a lesser extent by the intestine (79), is a 79 amino acid glycoprotein encoded by the *APOC3* gene whose expression is regulated by many metabolic stimuli, *e.g.* glucose, insulin and fatty acids (80). ApoC-III is a surface protein associated with TG-rich lipoproteins (chylomicrons and VLDL) and, to a lesser extent, with LDL and HDL particles. On the basis of its inhibitory effects on apoB lipoprotein catabolism, apoC-III has been proposed as a prominent negative regulator of TG catabolism (81-84).

Concerning the clinical association between apoC-III and ASCVD risk, carriers of loss-of-function mutations of *APOC3*, characterized by low levels of serum TG, display a reduced risk of myocardial infarction (13, 85) and a low risk of ischemic vascular disease (86). Consistent with this evidence, carriers of *APOC3* R19X null mutation with a 50% reduction in plasma apoC-III have a higher rate of both lipolysis of VLDL-TG and conversion of VLDL to LDL with little effect on direct liver uptake of VLDL (87). Therefore, specific therapy aimed at reducing apoC-III levels may be of interest in order to reduce ASCVD risk. Conversely, the *APOC3* gain-of-function mutation Gln38Lys is associated with a 32% elevation of TG (88), and, when expressed in mice, stimulates production of the $VLDL_1$ via the process of *de novo* lipogenesis (89).

Thus, considering that no synthetic drugs can specifically target apoC-III (90-92), the inhibition of apoC-III by a specific ASO has been the therapeutic approach proposed for patients with familial chylomicronemia syndrome (FCS), a rare inherited disorder, characterized by impaired

clearance of TG-rich lipoproteins from plasma, leading to severe hypertriglyceridemia and to a markedly increased risk of acute pancreatitis (93). In FCS patients there is either a lack of LPL function, as the result of recessive loss-of-function mutations in the genes coding *LPL*, or an impairment in LPL modulators (94).

Volanesorsen (formerly IONIS-APOCIIIRx) is a second generation ASO (5'-AGCTT CTTGTCCAGC TTTAT-3') which is administered s.c. and that specifically binds the *APOC3* mRNA, thereby promoting its degradation. In patients with fasting TG between 350 mg/dL and 2000 mg/dL, 13 weeks of treatment with volanersorsen, as a monotherapy or in combination with fibrates, led to dose-dependent and prolonged decreases in plasma apoC-III (up to 79.6%) and TG (-71%) levels as a monotherapy, with a concomitant increase of HDL-C of about 46%. When administered on top of fibrates, the changes in apoC-III and TG were up to -70.9% and - 64%, respectively (95).

The APPROACH Study (A Study of Volanesorsen in Patients With Familial Chylomicronemia Syndrome) evaluated the efficacy and safety of volanesorsen compared with placebo in 66 patients with FCS (confirmed either genetically or by LPL activity < 20% of normal and fasting TG $\geq$ 750 mg/dL) on a restricted low-fat diet (< 20 g fat per day). Consistent with the diagnosis of FCS, median fasting TG was 1985 mg/dL and median chylomicron-TG 1849 mg/dL. In these patients, the bulk of cholesterol seemed to be carried by chylomicrons since VLDL-C was relatively low, 41 mg/dL. ApoC-III levels were markedly raised at 30.18 mg/dL (96). After 3 months of therapy (300 mg/weekly), mean plasma apoC-III levels were reduced by 84% from baseline (25.7 mg/dL) with TG below 750 mg/dL in 77% of volanosersen-treated patients. In this arm, 3-month of therapy led to a 76.5% reduction from baseline in TG levels compared with a 17.6% rise in the placebo group. This effect remained significant after 6 and 12 months, with between-group relative differences of -77.8% and -49.1%, respectively. Concerning other variables, volanesorsen was superior to placebo in reducing chylomicron TG (-82.7%), apoB-48 (-75.9%), non-HDL-C (-45.9%), and VLDL-C (-58.3%). Conversely, a rise was found in HDL-C (+46.1%), apoA1 (+14.2%), LDL-C (+ 135.6%), and apoB (+19.5%). The most common side-effect was injection-site reactions, most of which were mild-to-moderate. Twenty-five patient who received volanesorsen developed platelet counts <140,000/μL and 16 patients had platelet count < 100,000/μL. Owing to a case of severe thrombocytopenia, *i.e.* platelet count < 25,000/μL, 2 patients were withdrawn; neither of these experienced major bleeding events and the platelet counts returned to normal values after discontinuation of the drug. Overall, among patients given volanesorsen, 14 did not terminate the 52-week trial for the following reasons: nine due to adverse events [platelet counts decrease (n=5) and volanesorsen-related effects, *i.e.* site-reaction

injections or fatigue (n=4)], four voluntarily left the trial, and one was withdrawn for nonadherence (97). Four episodes of acute pancreatitis were documented in three patients assigned to placebo compared with one episode in one patient receiving volanesorsen (Table 3).

At the end of the trial, eligible patients were asked to receive volanesorsen in an OLE study. This trial [Approach Open Label Study: A Study of Volanesorsen (Formerly IONIS-APOCIIIRx) in Patients With Familial Chylomicronemia Syndrome; NCT02658175] is aimed at evaluating the efficacy of extended dosing of volanesorsen as measured by the percent change in fasting TG from baseline. Notably, a survey including patients from this OLE study, who received volanesorsen for at least 3 months, reported an improvement of several components of health-related quality of life, *e.g.* physical, emotional and cognitive symptoms (98).

The COMPASS (The COMPASS Study: A Study of ISIS APOCIIIRx in Patients With Hypertriglyceridemia) trial was designed to test the percent changes from baseline in fasting TG, in 114 patients with hypertriglyceridemia, as assessed by fasting TG at screening ≥500 mg/dL. Three months of volanesorsen (300 mg/once weekly) decreased TG by 73% from baseline, a stable effect across 26 weeks. The most common side effect was injection site reactions and no serious platelet event was reported (99). Combining data from the APPROACH and the COMPASS studies show that 9 pancreatitis events occurred in 6 patients on placebo compared to the only event in 1 patient on volanosersen. At the end of the study, *i.e.* after 26 weeks, patients have been planned to enter a 13-week post-treatment evaluation period (100). Concerning safety issues, injection-site reactions were the most common side-effects, with most patients exhibiting a gradual and mild decline in platelet count (-30% within 6 months). Some patients experienced a rapid and unpredictable reduction in platelets to extremely low levels ≤15,000/uL (Table 3). The switch to a biweekly dose or interruption of drug administration did not always lead to a sufficient timely recovery of platelet count and some patients required hospitalization and/or treatment with prednisone and/or intravenous immunoglobulins. Finally, in patients with type 2 diabetes (HbA_{1c} > 7.5%) and hypertriglyceridemia (TG > 200 and < 500 mg/dL), volanesorsen reduced apoC-III and TG by 88% and 69%, respectively, along with an improvement in HbA_{1c} (-0.44%). Conversely, HDL-C was raised by 42% (101).

Future studies, *i.e.* the BROADEN trial, will evaluate the efficacy of volanesorsen in patients with familial partial lipodystrophy (A Study of volanesorsen, formerly ISIS APOCIIIRx, in Patients With Partial Lipodystrophy, NCT02527343). These patients frequently display moderate to severe hypertriglyceridemia and elevated apoC-III levels. Primary aim will be to determine whether

volanesorsen reduces TG; secondary and tertiary endpoints will be the improvement of insulin resistance, diabetes and liver steatosis.

On May 2019, the EMA has authorized the marketing of the ASO anti-APOC3 volanesorsen as an adjunct to diet in adult patients with genetically confirmed FCS and at high risk for pancreatitis, in whom response to diet and TG lowering therapy was inadequate (102).

To improve the efficiency of APOCIII-Rx, this was conjugated with a GalNAc moiety leading to the design of APOCIII-L_{RX}. In healthy volunteers, with TG ≥200 mg/dL, 6-week-administration of APOCIII-L_{RX} (15 and 30 mg) reduced in a dose-dependent fashion apoC-III up to 84% and TG up to 71%, with a good safety and tolerability profile. ApoC-III protein levels stayed reduced by 50% for 90 days after the last dose; significant changes were also found for apoB (up to -30%) and HDL-C (up to +100%) (103) (Table 3). An on-going phase 2 study is enrolling patients with hypertriglyceridemia and established ASCVD (104).

5.2. ANGPTL3-L_{RX} (in the pipeline). ANGPTL3 is a secreted protein primarily expressed in the liver. It contains two domains, the N-terminal coiled-coil region and a C-terminal fibrinogen domain. ANGPTL3 inhibits LPL activity by inducing a conformational change in LPL which results in (i) increased susceptibility to cleavage by proprotein convertases, (ii) dissociation of LPL from the cell surface, and (iii) inhibition of its catalytic activity (105). Besides LPL, ANGPTL3 inhibits the activity of endothelial lipase (EL), an enzyme responsible for the hydrolysis of HDL phospholipids (106); consistent with this observation, carriers of *ANGPTL3* loss-of-function mutation have low HDL-C levels (107). Interestingly, these patients are diagnosed as carriers of familial hypobetalipoproteinemia, a metabolic disorder characterized by low plasma LDL-C, low plasma HDL-C and low plasma TG (107).

From a genetic perspective, in carriers of two ANGPTL3 loss-of-function alleles the VLDL apoB production rate is significantly lower and LDL apoB FCR higher (107). Another possible hypothesis of an increased LDL clearance could be a raise in the inactive form of PCSK9, *i.e.* the furin-cleaved one, found in carriers of *ANGPTL3* mutation p.S17* (108). Other genetic evidence, from the DiscovEHR study, reported that people heterozygous for *ANGPTL3* had approximatively 50% lower ANGPTL3 circulating levels which translate into lower TG (-27%), LDL-C (-9%), HDL-C (-4%), and a 41% lower odds of CVD; no statistical benefit was found for myocardial infarction (109). Conversely, individuals with complete *ANGPTL3* deficiency had reduced odds of myocardial infarction with no evidence of coronary atherosclerotic plaques (14). Interestingly, besides the overall TG and LDL-C

lowering effects, in both homozygous and heterozygous carriers of the S17X loss-of-function mutation, ANGPTL3 deficiency resulted in reduced cholesterol content in TG-rich lipoproteins and their remnants (110). Based on these findings, ANGPTL3 inactivation appears to be a promising target for decreasing CV risk.

A specific ASO targeting hepatic ANGPTL3 mRNA has been developed and safety and efficacy tested. ANGPTL3-L_{Rx} is composed of 20 nucleotides with 2'-O-MOE groups at 3' and 5' ends. These modifications confer increased affinity to the target mRNA, increased stability in tissues and appeared to ameliorate some of the high dose toxicity related to first generation ASO. The nucleotide sequence is 5'-GGACA TTGCCAGTAA TCGCA-3' and contains three GalNAc moieties attached to the 5' end (111). In a phase 1 trial, ANGPTL3-L_{RX} administered in a multiple-dose design (weekly for 6 weeks) was effective at day 43 to lower TG (range: 33.2%-63.1%), LDL-C (range: 1.3%-32.9%), VLDL-C (range: 27.9%-60%), non-HDL-C (range: 10%-36.6%), apoB (range: 3.4%-25.7%) and apoC-III (range: 18.9%-58.8%) than in the placebo group. The mean percentage reductions in ANGPTL3 levels from baseline at day 43 were: -46.6% (10 mg), -72.5% (20 mg), -81.3% (40 mg) and -84.5% (60 mg) (Table 3). There were no clinical signs of prothrombotic effects, bleeding episodes, significant decreases in platelet counts and on liver or renal function (111).

Finally, although not strictly linked to the topic of this review article, another byosinthetic approach targeting ANGPTL3 has been tested. Evinacumab - a fully human monoclonal antibody against ANGPTL3 - when administered to homozygous FH for the LDLR already on an aggressive lipid-lowering regimen, reduced LDL-C levels by -49%, apoB by -46%, non-HDL-C by -49%, TG by -47% and HDL-C by -36% (112). When given to subjects with TG between 150 and 450 mg/dL and LDL-C > 100 mg/dL, it led to lipid changes similar to those observed in *ANGPTL3* loss-of-function mutations, *i.e.* about - 88% and -20% for TG and LDL-C, respectively (both *vs* placebo) (113).

6. Limitations. With the exception of mipomersen and volanesorsen that underwent a fully clinical development, safety and proof-of-concept studies involving a larger range of dislypidemic patients are needed for the other drugs reviewed above. In the case of mipomersen, never approved in Europe (114), Kastle Therapeutics notified the FDA that the drug product is no longer marketed and requested the approval of the application to be withdrawn. Approval has been withdrawn since August 2, 2019 (115). As far as the use of inclisiran is concerned**,** since the effect of intracellular inhibition of PCSK9 remains unclear, it will be crucial to understand the safety profile, as previously assessed for PCSK9 monoclonal antibodies, *e.g.* leading or not to an increased risk of diabetes (116,

117), leading or not to cognitive function impairment (118), leading or not to myalgia (119). Moreover, since PCSK9 seems to be required for the hepatic integrity (120), it becomes necessary to understand if the PCSK9 inhibitory strategy in subject with a genetic architecture susceptible to liver disease may lead to detrimental consequences, *i.e.* in the case of carriers of polymorphism in microsomal triglyceride transfer protein, superoxide dismutase or tumor necrosis factor-α (121). Concerning ASOs against ANGPTL3, the precise mechanisms of TG lowering, *e.g.* TG-rich lipoproteins clearance or changes in LPL activity, need to be exactly determined (122).

7. Conclusions

In the context of current and future approaches to handle dyslipoproteinemias, the reviewed biosynthetic drugs hold promise for further improvements in the foreseeable future. Indeed, significant progress has been made in drug development using RNA-based therapies aimed at treating difficult to target lipid disorders. In the field of hypercholesterolemias, compared to other biosynthetic LDL-C lowering agents, *i.e.* PCSK9 monoclonal antibodies which require once- or twice-monthly s.c. injections (123), inclisiran has the advantage that a single injection dose leads to a long-lasting and durable LDL-C reduction. Moreover, looking at waterfall plots of atherogenic lipoproteins of patients enrolled in the ORION-1 trial, it is clear that inclisiran guarantees, at least for LDL-C (52), non-HDL-C and apoB (53) levels equal or below baseline, an effect not to be found in statin trials (124). Finally, relative to the possible cost of inclisiran over monoclonal antibodies, it has been calculated that the manufacturing cost for oligonucleotides is on par with that of small molecule drugs and probably much lower than that of monoclonal antibodies (46).

Although the marketing of mipomersen is being discontinued in US, targeting the translation of apoB-100 mRNA as a tool to lower apoB-100 atherogenic lipoproteins, still remains a potentially viable approach. This emerged from the observation that loss-of-function *APOB* mutations were causally linked to familial hypobetalipoproteinemia, an autosomal dominant condition characterized by very low LDL-C and apoB levels (125). Since the GalNac modification allows a rapid liver absorption with an elimination half-life of several weeks (21), the design of anti apoB-100 ASO carrying this modification may be a future strategy to be pursued.

Relative to the risk factors contributing to the development of ASCVD, epidemiological studies (126) and large clinical trials reported that elevated Lp(a) remains a strong risk factor regardless of the reduction of LDL-C achieved by statins (127). Testing for elevated Lp(a) during cascade screening for FH identified relatives with high Lp(a) and raised risk of ASCVD, particularly in

the case of probands having both FH and elevated Lp(a) (128). No available agent (statins, nicotinic acid or PCSK9 inhibitors) (129-131) can lower Lp(a) to the extent required to achieve a CV benefit (132), *i.e.* approximately 100 mg/dL (133), corresponding to the 38.7 mg/dL LDL-reduction obtained with statins (134). Thus, the selected strategy to treat patients with markedly elevated Lp(a) with a specific ASO against Lp(a) may start a new era in this field, so far paved with limited therapeutic options. Indeed, the (Lp(a)Horizon) trial (clinicaltrials.gov indentifier NCT04023552) will test the effect of a monthly injection of 80 mg of the ASO TQJ230 against apo(a) in patients with previous myocardial infarction, stroke or symptomatic peripheral artery disease and Lp(a) ≥ 70 mg/dL where LDL-C lowering therapy is optimized. As of now, for patients with progressive ASCVD and high plasma Lp(a), a potentially effective therapeutic option is the apheretic procedure, that can effectively and safely remove Lp(a) by 60%- 80% (135).

In the case of atherogenic dyslipidemia, the causal role for TG-rich lipoproteins in CHD development has been supported by the definition of rare variants, *e.g.* those of *APOC3* or *ANGPTL3* associated with a reduced ASCVD risk (13, 14). Thus, ASOs against APOC3 or ANGPTL3 appear to offer promise to treat a substantial number of patients with hypertriglyceridemia with or without statin therapy and with or without the metabolic syndrome. In this condition, in fact, LDL-C does not account for all of the risk conferred by atherogenic plasma lipids; among individuals with the lowest LDL-C levels on statin, the subclass of the smallest VLDL has been most strongly associated with residual CV risk (136). This evidence has been further supported by results of a *post hoc* analysis of the Treating to New Targets (TNT) study showing that TG-rich lipoprotein cholesterol concentration was an independent marker of residual ASCVD risk (137).

In conclusion, since lipoprotein metabolism is complex and plasma concentrations are the result of a balance between rates of production and/or catabolism of the various lipoprotein particles, kinetic studies could provide mechanistic insight into the mode of action of these drug classes, *e.g.* anticipating an increased risk of hepatic steatosis (10). Overall, despite considerable progresses, the major hurdles for these drugs are safety and delivery and these should not be underestimated.

Conflicts of interest declaration

RDS has received honoraria for consulting, speaker activities, or research from Amgen, Akcea, AstraZeneca, Biolab, Esperion, Kowa, Merck, MSD, Novo Nordisk, and Sanofi/Regeneron.

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Figure 1. *RNA-based therapeutic approaches: ASO and siRNA*. *Panel a)* antisense oligonucleotides (ASOs) control gene expression by the involvement of the RNase H1, an endoribonuclease that preferentially binds to the DNA-RNA hetero-duplex over RNA-RNA and DNA-DNA homo-duplexes. The final result is the selective cleavage of the RNA strand while the synthetic DNA strand remains intact and free to bind additional target mRNAs; *Panel b)* siRNAs are composed of two strands, the guide and the passenger. Once in the cytoplasm the two strands are separated with the guide loaded into the RISC and the passenger removed and degraded. When the complementary target mRNA has hybridized with part of the guide strand, an endonucleolytic cleavage of the mRNA is driven by a component of RISC, the Argonaute 2 (ago 2) protein.

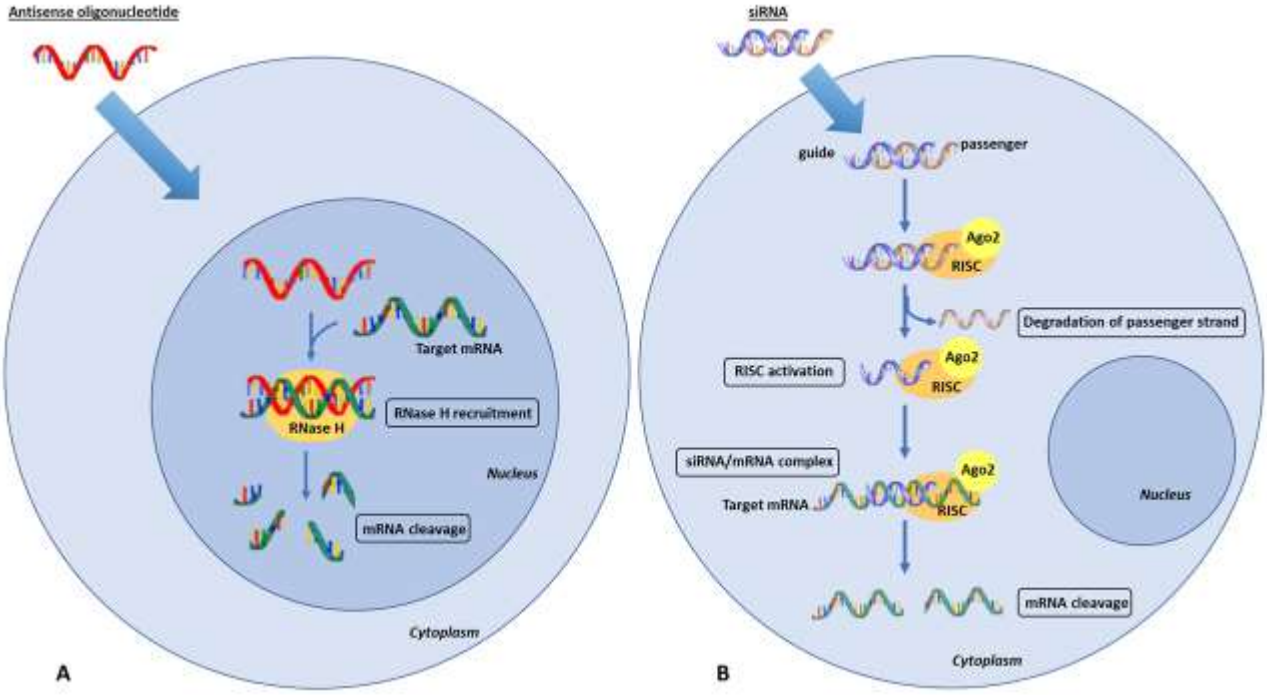
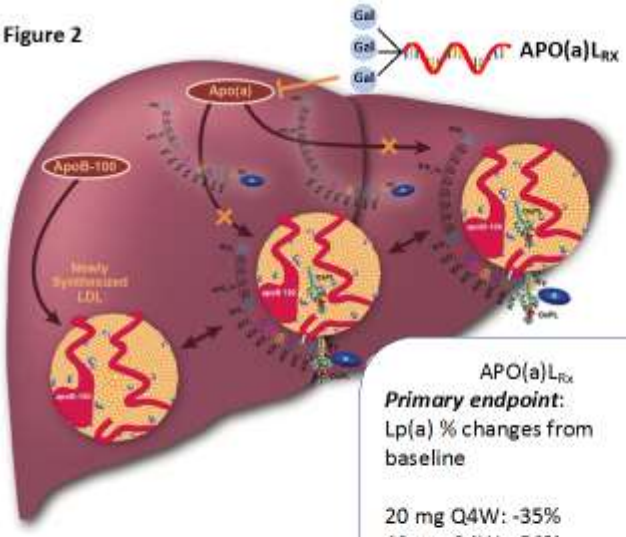


Figure 2. *Dose-ranging phase 2b trial on patients with pre-existing cardiovascular disease***.** APO(a)L_{Rx} is an antisense oligonucleotide (ASO) conjugated with the triantennary *N*-acetyl galactosamine (GalNAc). GalNAc binds to the hepatocyte-specific asialoglycoprotein receptor (ASGPR) with high affinity. ASGPR, a transmembrane C-type lectin, recognizes a wide variety of ligands that contain either terminal galactose (Gal) or GalNAc residues. APO(a)L_{Rx} reduces liver apo (a) synthesis and consequently reduces hepatic synthesis and secretion of Lp(a) particles into the circulation. Partially modified with permission of Elsevier (138).

Figure 2



APO(a)L$_{Rx}$
Primary endpoint:
Lp(a) % changes from baseline

20 mg Q4W: -35%
40 mg Q4W: -56%
20 mg Q2W: -58%
60 mg Q4W: -72%
20 mg QW: **-80%**

APO(a)L$_{Rx}$
Secondary endpoint:
% patients achieving Lp(a) ≤50 mg/dL

20 mg Q4W: -25%
40 mg Q4W: -62.5%
20 mg Q2W: -64.6%
60 mg Q4W: -80.9%
20 mg QW: **-97.7%**

Table 1. Silencing RNA approach to lower LDL-C

Target	*Drug*	*study*	*Efficacy*
PCSK9	Inclisiran (siRNA with GalNac modification)	*ORION-1 (Phase 2):* ASCVD or ASCVD-risk equivalent already at maximally tolerated dose [51, 52]	**PCSK9** single dose regimen (200, 300 and 500 mg): -47.9% to 59.3% vs placebo (at day 180) two-dose regimen (100, 200 and 300 mg): -53.2% to -69.1% vs placebo (at day 180) **LDL-C** single dose 300-mg regimen: -38.4% (whole cohort) -36.9% (without diabetes) -52.0% (with diabetes) two-dose 300-mg regimen: -52.6% (whole cohort) -52.3% (without diabetes) -55% (with diabetes)

ASCVD, atherosclerotic cardiovascular disease; GalNac, triantennary *N*-acetyl galactosamine; LDL-C, low-density lipoprotein cholesterol; siRNA, silencing RNA; PCSK9, proprotein convertase subtilisin/kexin type 9

Table 2. Antisense oligonucleotide approaches to lower Lp(a)

Target	*Drug*	*study*	*Efficacy*
Lipoprotein (a)			
Apo(a)	APO(a)$_{Rx}$ (ASO)	*Phase 1 (European Clinical Trials Database: 2012-004909-27):* Lp(a) ≥ 100 mg/dL (31 patients with). Six s.c. injections in order to reach a final dosage of 600 mg, 1200 mg or 1800 mg [77]	**Lp(a) and Lp(a) oxidized phospholipids-apoB** -39.6% and -26.1% vs placebo (600 mg group) -59% and -55.1% vs placebo (1200 mg group) -77.8% and -61.3% vs placebo (1800 mg group)
		Phase 2 (NCT-02160899)[77]: Cohort A (51 patients): Lp(a) ≥ 50 and < 175 mg/dL Cohort B (13 patients): Lp(a) ≥ 175 mg/dL	**Lp(a)** -66.8 to -71.7% *vs* placebo
	APO(a)-L$_{Rx}$ (ASO with GalNac modification)	*Phase1/2a (NCT-02414594)[77]:* Lp(a) levels ≥ 30 mg/dL (58 healthy volunteers)	**Lp(a)** -24.8% vs placebo (10 mg; single-dose) -35.1% vs placebo (20 mg; single-dose) -48.2% vs placebo (40 mg; single-dose) -82.5% vs placebo (80 mg; single-dose) -84.5% vs placebo (120 mg; single-dose) **Lp(a)** -59.4 vs placebo (10 mg; multiple-ascending-dose) -72.3 vs placebo % (20 mg; multiple-ascending-dose) -82.4 vs placebo % (40 mg; multiple-ascending-dose)

ASO, antisense oligonucleotide; GalNac, triantennary *N*-acetyl galactosamine; Lp(a), lipoprotein (a); s.c., subcutaneous

Table 3. Antisense oligonucleotide approaches to lower triglycerides

Target	*Drug*	*study*	*Efficacy*
APOC3	Volanesorsen	NCT01529424 (Phase 2) [95]; 100, 200 and 300 mg/once weekly (as a monotherapy); 200 and 300 mg in combination with fibrates Patients with fasting TG 350 - 2000 (mg/dL)	*As a monotherapy (*)* **apoC-III**: -40% (100 mg), -63.8% (200 mg) and -79.6% (300 mg) **TG**: -31.3% (100 mg), -57.7% (200 mg) and -70.9% (300 mg) **HDL-C:** 26.6% (100 mg), +36.2% (200 mg) and +45.7% (300 mg) *In combination with fibrates (*)* **apoC-III**: -60.2% (200 mg) and -70.9% (300 mg) **TG**: -51% (200 mg) and -64.0% (300 mg) **HDL-C:** 50.6% (200 mg) and +51.8% (300 mg)
		APPROACH (Phase 3); 300 mg/once weekly [97] Patients With FCS (n= 66) on restricted low-fat diet: mean fasting TG= 2209 mg/dL; median chylomicron-TGs= 1621 mg/dL; median apoC-III= 30.18 mg/dL [96]	**apoC-III (*)** -84% after 3 months -83% after 6 months **TG (*)** -76.5% after 3 months - 53% after 6 months -40% after 12 months
		COMPASS (Phase 3); 300 mg/once weekly for 26 weeks patients (n= 114) with fasting TG ≥500 mg/dL	**TG:** -73%

		BROADEN (Phase 3) Patients With Partial Lipodystrophy who display moderate to severe hypertriglyceridemia and elevated apoC-III levels	On going
	APOCIII-L_{RX} (ASO GalNac conjugated)	NCT03385239 (phase1/2a) [103]: single ascending dose 10, 30, 60, 90, and 120 mg multidose design 15 and 30 mg (weekly) for six weeks; 60 mg every 4-week for 3 months Healthy volunteers with TG ≥200 mg/dL	**apoC-III** (*) -4% (10 mg, single dose), -32% (30 mg, single dose), -65% (60 mg, single dose), -78% (90 mg, single dose), -91% (120 mg, single dose) -65% (15 mg/weekly for six weeks) and -84% (30 mg/weekly for six weeks) -83% (60 mg, every 4-week for 3 months) **TG** -12% (10 mg, single dose), -11% (30 mg, single dose), -43% (60 mg, single dose), -68% (90 mg, single dose), and -77% (120 mg, single dose) -61% (15 mg/weekly for six weeks) and -71% (30 mg/weekly for six weeks) -65% (60 mg, every 4-week for 3 months) **apoB:** up to -30% **HDL-C:** up to +100%
ANGPTL3	ANGPTL3-L_{RX} (ASO GalNac conjugated)	Phase 1 [111]; multiple dose design: 10, 20, 40, or 60 mg/weekly (for 6 weeks) Healthy volunteers (n=32)	**ANGPTL3** (*) -46.6 (10 mg), -72.5 (20 mg), -81.3 (40 mg), -84.5 (60 mg) **TG** (*) -33.2 (10 mg), -63.1 (20 mg), -53.8 (40 mg), -50.4 (60 mg) **LDL-C** (*) -1.3 (10 mg), -4.3 (20 mg), -25.4 (40 mg), -32.9 (60 mg) **apo C-III** (*) 18.9 (10 mg), -57.8 (20 mg), -50.7 (40 mg), -58.8 (60 mg)

**vs baseline*; APOC3, apolipoprotein C3; ANGPTL3, angiopoietin-like 3; ASO, antisense oligonucleotide; GalNac, triantennary *N*-acetyl galactosamine; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; VLDL-C, very-low density lipoprotein cholesterol.