Targeting RNA With Antisense Oligonucleotides and Small Interfering RNA in Dyslipidemias: JACC State-of-the-Art Review

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Abstract

There is an unmet clinical need to reduce residual cardiovascular risk attributable to apolipoprotein B-containing lipoproteins, particularly low-density lipoprotein and remnant particles. Pharmacologic targeting of mRNA represents an emerging, innovative approach. Two major classes of agents have been developed – antisense oligonucleotides and small interfering RNA. Early problems in their use have been overcome by conjugation with *N*-acetylgalactosamine (GalNAc), an adduct that targets their delivery to the primary site of action in the liver. Using these agents to inhibit the translation of key regulatory proteins such as PCSK9, apolipoprotein CIII, apolipoprotein(a), and angiopoietin-like 3 has been shown to be effective in attenuating dyslipidemic states. Cardiovascular outcome trials with GalNAc-conjugated RNA-targeting drugs are ongoing. The advantages of these agents include long dosing intervals of up to six months and the potential to regulate the abundance of any disease-related protein. Long-term safety is yet to be demonstrated in large-scale clinical trials.

Condensed abstract

Despite contemporary treatments, residual cardiovascular risk attributable to low-density lipoprotein and remnant particles persists. Pharmacologic targeting of mRNA with antisense oligonucleotides and small interfering RNA represents an emerging, innovative approach. These drugs can be directed specifically to the liver by conjugation with *N*-acetylgalactosamine (GalNAc). Inhibiting translation of key regulatory proteins such as PCSK9, apolipoprotein CIII, apolipoprotein(a), and angiopoietin-like 3 favours normalisation of dyslipidemia. These agents have several advantages, including very long dosing intervals and the potential to regulate the abundance of any disease-related protein. Cardiovascular outcome trials are ongoing to assess their effects on clinical outcomes and long-term safety.

Key words

PCSK9 – apolipoprotein CIII – lipoprotein(a) – angiopoietin-like 3 – ASO – siRNA

Abbreviations

ANGPTL3	angiopoietin-like 3
ASCVD	atherosclerotic cardiovascular disease
ASO	antisense oligonucleotide
apoCIII	apolipoprotein CIII
ASGPR	asialoglycoprotein receptor
FCS	familial chylomicronemia syndrome
Lp(a)	lipoprotein(a)
GalNAc	*N*-acetylgalactosamine
RISC	RNA-induced silencing complex
siRNA	small interfering RNA

1 Introduction

In 2019, the *New England Journal of Medicine* published a ground-breaking report on a 6-year-old girl with a rare genetic neurodegenerative disorder due to a previously undescribed gene insertion. On the basis of the antisense oligonucleotide (ASO) drug *nusinersen*, which is approved for the treatment of spinal muscular atrophy, a splice-modulating agent specific for the patient's mutation was developed. After evaluation in cell lines from the patient and on completion of toxicology studies in rats, the modified ASO was administered for the first time within one year after first contact with the patient. Follow-up revealed clinical improvement in objective parameters such as seizure frequency and duration, with no serious adverse events (1). Although this RNA-based drug addresses a specific mutation and may be of use in a limited number of patients worldwide, its exquisite specificity for the target, rapid development, and apparent clinical efficacy illustrate the potential of a novel class of drugs targeting RNA.

It is becoming increasingly evident that RNA-targeting drugs may not only be beneficial in rare diseases caused by specific mutations, but equally in highly prevalent chronic disorders such as the atherogenic dyslipidemias, which are associated with elevated risk of atherosclerotic cardiovascular disease (ASCVD), and in hard-to-treat conditions such as severe hypertriglyceridemia which can cause pancreatitis. High plasma levels of low-density lipoprotein (LDL) – the major cholesterol transporter in the circulation (2) – are causal in the initiation and progression of ASCVD (3). Small molecule drugs such as statins are recommended as first line therapy to lower LDL cholesterol (LDL-C) in international guidelines, and abundant evidence attests to their efficacy and safety (4)–(7). However, a counter-regulatory effect limits their ability to lower LDL; statins stimulate production of proprotein convertase subtilisin/kexin type 9 (PCSK9), a hepatic protein that reduces LDL receptor activity and so impairs LDL removal from the circulation. Accordingly, a new

therapeutic approach to inhibit PCSK9 with monoclonal antibodies allows many hypercholesterolemic patients to achieve unprecedented, very low levels of LDL-C. However, antibodies against PCSK9 are costly, a factor which has limited their use to patients displaying very high LDL-C levels (such as heterozygous or homozygous familial hypercholesterolemia). Heretofore, such patients infrequently achieved guideline LDL-C goals with standard treatment. In addition, large numbers of individuals with seemingly well-treated LDL-C levels exhibit high residual risk despite optimised statin therapy and are likely to benefit from further LDL lowering (8),(9).

Recently, it has been recognised that additional lipid abnormalities can contribute to ASCVD risk, both in the general population and in those with LDL-C levels at their recommended goal. Lipoprotein(a) (Lp[a]), a lipoprotein particle similar in size and composition to LDL but possessing a second major protein component, i.e. apolipoprotein(a) (apo[a]), is now believed to be an independent causal risk factor for ASCVD (10)–(12). Furthermore, elevated levels of triglyceride-rich lipoproteins are accompanied by elevation in the circulating concentrations of cholesterol-rich remnant lipoprotein particles, themselves causal in the pathophysiology of ASCVD (13)–(15). Traditional, small-molecule agents have been of limited utility in adequately normalising the highly prevalent dyslipidemias which feature these atherogenic particles. Indeed, raised Lp(a) levels are not amenable to correction with drugs such as statins and ezetimibe, and while plasma triglycerides are reduced by statins and fibrates, their effects are moderate in most subjects. Thus, there is a clear unmet clinical need; RNA-targeted drugs offer a potency and specificity that holds promise for the treatment of these conditions.

Targeting of mRNA as a pharmacologic principle differs from traditional drug development in that it entails the deployment of highly specific agents to regulate the production of disease-causing or disease-related proteins that have previously been considered undruggable. It comes

with further advantages such us long dosing intervals of up to six months and reduced likelihood of off-target effects, but presents challenges in terms of drug delivery and topical reactions at the site of administration. The recent development of adding *N*-acetylgalactosamine (GalNAc) to the RNA-product (ASO or small interfering RNA [siRNA]) represents a significant advance. This modification facilitates the highly efficient liver-specific uptake of the drug, thereby allowing the desired pharmacodynamic effect to be achieved at markedly lower doses where the protein of interest is made principally or entirely by hepatocytes.

The genomic revolution is now delivering on its much-heralded promise to facilitate target discovery and to drive development of innovative therapeutics in the arena of cardiovascular disease. The four targets discussed here are examples of this; genetic variation in PCSK9, apolipoprotein CIII (apoCIII), apo[a] – representing the specific apolipoprotein of Lp(a) –, and angiopoietin-like 3 (ANGPTL3) are each associated with perturbation in plasma levels of atherogenic lipoproteins, and with elevated risk of ASCVD. Genetic studies of cohorts carrying loss-of-function mutations or genetically determined subnormal levels of the protein of interest have identified promising avenues for intervention (10),(11),(16)–(21). The recent report of early clinical trials involving GalNAc-conjugated drugs targeting the mRNA of these proteins has given a preliminary indication of significant therapeutic promise. However substantial further work remains to progress these agents to clinical use; most critically the demonstration of their positive effects on ASCVD outcomes.

In this review, we discuss the emerging principle of therapeutic RNA targeting, modes of delivery of RNA-based drugs, safety considerations, a comparison to antibody-based strategies, and present the current status on the use of GalNAc-conjugated RNA therapeutics in cardiovascular medicine.

2 Therapeutic targeting of RNA

2.1 General considerations

Classical small molecule drugs typically act as enzyme inhibitors (e.g. statins inhibit HMG CoA reductase), or modulate the behaviour of regulatory receptors localised either at the cell membrane or intracellularly (e.g. fibrates are agonists of the PPARα nuclear receptor). Proteins without a well-defined catalytic or regulatory domain – such as apoCIII, apo(a), or ANGPTL3 – are not amenable to this type of intervention. Their action can be modified or inhibited with monoclonal antibody-based drugs and these agents are used widely in modern medicine. However, for abundant plasma proteins, large amounts of antibodies would be needed to achieve a pharmacodynamic effect and high concentrations of immune complexes would result. These factors limit the use of monoclonal antibodies, with direct implications for their safety and cost (22).

An entirely different approach to reduction in protein abundance, and hence activity, involves blocking their production by inhibition of the translation of their mRNA (**Figure 1**). The two main classes of RNA-targeted drugs developed for this purpose are single-stranded antisense oligonucleotides (ASOs) and double-stranded small interfering RNAs (siRNAs). Differences between the two classes in terms of composition are set out below, but both share a common mechanism of action; following parenteral administration and uptake into the target tissue, release in the cytoplasm ensues, where they bind to a complementary, specific sequence within the mRNA of interest. Such interaction leads to degradation of the target mRNA and hence diminished translation of the encoded protein (23). Once the physiological functions of regulatory RNA had been discovered, this approach emerged as a conceptually elegant modality for control of the abundance of a target protein. Decades of research were however required to overcome multiple hurdles that hampered development of this concept from a theoretical possibility to clinical reality. Conceivably in the future, the range of targets may be expanded

to include non-coding RNAs which have been identified as potentially important players in ASCVD development. Modulating this class of RNA may open up the possibility of gaining leverage over atherogenic mechanisms that are fundamentally different from those mediated by classical cardiovascular risk factors (24).

2.2 Technologies for delivery of RNA-targeting therapeutics

2.2.1 Technologies for delivery in general

One major issue in therapeutic use of RNA oligonucleotide strands involves the effective delivery of the drug to the intracellular site of action. Several technologies have been developed as described in the following section (**Figure 2**). Compared to most small molecule drugs, ASOs and siRNAs are considerably larger in size; equally, siRNAs are highly charged molecules which can lead to difficulties in cellular permeability and penetration. Furthermore, naked siRNAs do not bind to proteins in plasma and are rapidly excreted. Therefore, derivatisation of the basic RNA product is needed in order to produce a compound with pharmacological potential. Phosphorothioate-substituted ASOs are amphipathic (possessing both hydrophobic and hydrophilic domains), thereby enabling them to be administered in saline solutions, and facilitating their transport in the aqueous medium of plasma in protein-bound form. While phosphorothioate-substituted ASOs have some ability to passively cross cell membranes due to their hydrophobicity, siRNAs cannot cross lipid bilayers due to their extreme hydrophilicity (resulting from the high density of negatively charged components) (25).

Thus, cellular uptake and release of the drug into the cytoplasm represented a bottleneck in the clinical application of RNA-targeting drugs for decades. Fortunately, recent developments have shown that these problems can be overcome, and present-day delivery technologies are generally based on encapsulation in nanoparticles or conjugation with ligands that facilitate specific cellular uptake.

Another approach is the use of viral vectors (26). The RNA sequence complementary to the target mRNA is integrated into e.g. adeno-associated virus and after transduction expressed by the host cell. For ASCVD and dyslipidemias, approaches using viral vectors are at very early stages of development.

2.2.2 Nanoparticles

Nanoparticles are used to encapsulate RNA-based drugs, resulting in enhanced cellular uptake and rendering them inaccessible to inactivating nucleases. The most extensively investigated example of this approach involves the use of lipid nanoparticles (LNPs). These structures are 50–100 nm in diameter and are composed of polyethylene glycol-conjugated lipids, cholesterol, and nucleic acids (27). Ionisable LNPs have been found to acquire apolipoprotein E and to be taken up primarily by members of the LDL receptor family on hepatocytes due to the binding of apolipoprotein E (28). LNPs enter cells via endocytosis; in the acidic environment of the endosome, the lipids of the nanoparticle become positively charged, enabling them to fuse with the negatively charged endosomal membrane. Subsequently, the LNP-encapsulated RNA drug is liberated into the cytosol (29). One feature of the use of nanoparticles is that they need to be administered intravenously (27). In addition, as a consequence of the pro-inflammatory effects of LNPs, the clinical use of these agents necessitates pre-treatment with high doses of corticosteroids, histamine receptor blockers, and non-steroidal anti-inflammatory agents in order to avoid infusion-related reactions (30),(31).

Another technique to enhance the delivery of siRNA which is still at the preclinical stage has been termed self-assembled micelle inhibitory RNA, abbreviated as SAMiRNA. In this approach, siRNAs are conjugated with a hydrophilic polymer, such as polyethylene glycol on the one hand, and with synthetic lipids on the other. In solution, these conjugates form self-assembled nanoparticles which do not appear to stimulate an immune response. Significantly, SAMiRNA has been successfully tested in animal models of pulmonary fibrosis (29),(32).

2.2.3 Liver-specific delivery with *N*-acetylgalactosamine (GalNAc)

Delivery can also be facilitated by conjugation of ASOs and siRNAs with ligands that improve their pharmacokinetic properties, enhance transmembrane permeation, and/or target the drug to a specific tissue. Conjugation with cholesterol for example has the aim of improving the pharmacokinetic profile of the drug and enhancing hepatic uptake, e.g. through LDL receptors (33). Of all the ligands investigated to date, *N*-acetylgalactosamine (GalNAc) has shown the most promise in the field of cardiovascular medicine. As a unique feature (compared to other approaches which lack specificity or have unfavourable pharmacokinetic properties), conjugation of siRNAs (34) and ASOs (35) with triantennary GalNAc enables their highly specific and rapid uptake into hepatocytes. The GalNAc moiety binds to the asialoglycoprotein receptor (ASGPR) which is abundantly expressed on hepatocytes with >500,000 receptors on each cell (36), but which is only minimally expressed in extrahepatic cells (37). Such binding leads to rapid endocytosis of the conjugated drug. Before the contained RNA can exert its therapeutic action however, its release from the endosome into cytoplasm is essential. For siRNAs, the mechanism is as follows: due to a drop in pH in the endosomal compartment, the GalNAc-siRNA conjugate dissociates from ASGPR and the receptor recycles back to the cell surface. Only a very small part (<1%) of the siRNA escapes the endosome through an unknown mechanism (36). Similarly, although ASOs are thought to be in part capable of passively crossing lipid bilayers (25), the exact mechanism of their release into cytoplasm is also unknown (35). This and other current areas of research surrounding use of these therapies are summarized in **Box 1**.

There are several advantages of liver-specific drug targeting. Conjugation with GalNAc increases the potency of the drug allowing the use of markedly lower doses and consequent reductions in systemic exposure, in (unwanted) uptake by non-parenchymal hepatic cells, and

in localised reactions at the injection site. The potential for systemic, liver and topical side effects is thereby reduced considerably (38). In fact, for GalNAc-conjugated ASOs, potency can be dramatically increased: for example (as discussed below) a GalNAc-conjugated ASO targeting apo(a) exhibited a 30-fold increase in potency as compared with the original preparation (39), and an ASO directed to the mRNA for apoCIII exhibited an estimated >15-fold increased potency as compared to the unconjugated agent, resulting in markedly fewer injection site reactions (38). Other benefits of the GalNAc approach as compared to that involving nanoparticles include economies in production costs and reduction in inflammatory side effects.

2.3 Classes of RNA-targeting therapeutics

2.3.1 Double-stranded small interfering RNA (siRNA)

siRNAs are double-stranded RNA molecules with 21–23 nucleotides per strand (27). To prevent naked siRNAs from degradation in the bloodstream, chemical modifications are required to stabilize the molecule and protect it from nuclease action. In designing such derivatives, activation of the immune system must be avoided, and the ability of the drug to recruit enzymes that degrade the target mRNA needs to be maintained. Selection of the optimum modification of the siRNA product is as important in maximising efficacy of siRNAs as the strategy of liver-specific targeting with GalNAc. After much trial and error, it seems that the use of 2´-*O*-methyl, 2´-fluoro derivatives and introduction of a limited number of phosphorothioate substitutions are key steps in the generation of a clinically useful agent (40). siRNAs are designed such that the antisense or guide strand is complementary to a specific sequence of the mRNA of the targeted protein, while the complementary strand, termed the passenger strand, serves as a prodrug. Following release of the siRNA into the cytoplasm, the guide strand binds to the argonaute protein Ago2 and other proteins, and in doing so initiates assembly of the RNA-induced silencing complex (RISC) (41). The passenger strand is required

as a support for the geometry of the siRNA molecule during formation of the RISC (42). After the complementary target mRNA has bound the guide strand in the RISC, the target mRNA is cleaved by Ago2. The guide strand is not cleaved, remains in the RISC, and can bind to and degrade further mRNAs for a prolonged period of time, thereby offering an explanation for the long half-life of siRNAs; indeed, while the half-life in the circulation is less than one hour, the siRNA half-life in hepatocytes is typically several weeks (30),(43) (**Figure 3**).

2.3.2 Single-stranded antisense oligonucleotides (ASOs)

ASOs are single-stranded molecules typically 15–30 nucleotides in length (23). As with siRNAs, a range of molecular modifications have been employed in attempts to improve the pharmacologic profile. The use of phosphorothioate as a substitute for phosphodiester linkages between nucleotide bases leads to resistance against nucleases and facilitates binding to plasma proteins, thereby increasing both plasma half-life and the probability of uptake in the target tissue (44). Other modifications include, but are not limited to, substitutions of the 2´-hydroxyl moiety by 2´-*O*-methyl, 2´-*O*-methoxyethyl, and 2´-fluoro groups to increase further resistance to nuclease-mediated degradation (41).

ASOs alter gene expression by two basic actions. First, ASOs can occupy the target mRNA without leading to its degradation and thereby prevent it from being translated. This is realised, among other mechanisms, through changes in RNA processing and inhibition of interaction of the target mRNA with key proteins. Second, ASOs can induce degradation of the target mRNA by several mechanisms, one of which is cleavage of the target mRNA by RNase H1. The structure best exploiting this mechanism has been identified as a DNA sequence in the middle of the ASO molecule (“gap”) flanked by 2´-*O*-methoxyethyl-modified RNA nucleotides on both sides (30),(41). Degradation of mRNA by RNase H1 is specific for RNA in an RNA-DNA duplex and takes place in both the cytoplasm and nucleus (45). In contrast to siRNAs, most

ASOs prevent translation of mRNAs on the basis of a one-to-one stoichiometry (46) (**Figure 3**).

3 Treatment targets for GalNAc-conjugated RNA-targeting therapeutics

The following section provides a synopsis of the current state of development for four treatment targets, each of which involves GalNAc-conjugated RNA-targeting therapeutics. A summary is provided in **Table 1** and the **Central Illustration**.

3.1 Proprotein convertase subtilisin/kexin type 9 (PCSK9)

3.1.1 Rationale for target identification

The earliest report of gain-of-function mutations in the *PCSK9* gene as a cause of familial hypercholesterolemia was published in 2003 (47),(48). Subsequent in-vitro studies showed that these mutations result in decreased expression of LDL receptors on the hepatocyte surface with reduction in LDL internalization (49). Contemporaneously, investigations of individuals with very low LDL-C levels led to the identification of loss-of-function mutations in *PCSK9*, which were associated with 28% lower LDL-C and 88% reduced lifetime risk of coronary heart disease (CHD) (16). Elucidation of the action of this protein followed and revealed that PCSK9 influenced LDL-C levels by regulating the abundance of the LDL receptor on cell membranes. PCSK9 binds to the LDL receptor and in doing so directs it to a degradation pathway as it cycles through the endosomal/lysosomal compartments within the cell (50).

The association of lower LDL-C concentrations with lower risk of CHD in *PCSK9* loss-of-function mutation carriers provided the rationale for the identification of PCSK9 as a target for pharmacologic inhibition. Of the different approaches to targeting of PCSK9, antibody-based strategies are the most advanced (48),(51). The fully human antibodies evolocumab and

alirocumab efficaciously lower LDL-C by ~60%; moreover, when added to background statin therapy in large-scale clinical trials in high-risk ASCVD patients, PCSK9 antibodies have been shown to provide a 15% additional relative risk reduction in ASCVD events over a period of 2.2 to 2.8 years of treatment (in the FOURIER and ODYSSEY Outcomes studies (52),(53)).

3.1.2 Pharmacology

Monoclonal antibodies targeting PCSK9 were approved for clinical use initially in 2015. There have been several attempts to develop small molecule inhibitors, as exemplified by adnectins as blocking agents; even vaccination has been considered as a therapeutic alternative (51), but the most advanced alternate strategies involve RNA-based drugs.

ALN-PCS, a PCSK9 mRNA-targeting siRNA formulated in an LNP, was shown to reduce plasma concentrations of PCSK9 by 70% and LDL-C by 40%, representing the first clinical proof of the reduction of a liver-derived protein subsequent to targeting of its mRNA in human subjects (31). However, premedication with corticosteroids, antihistamines, and paracetamol was necessary to prevent infusion-related reactions induced by the LNP formulation, which limited the utility of this product. Subsequently, a PCSK9-targeted siRNA conjugated with triantennary GalNAc was developed, called ALN-PCSsc or inclisiran. As described above, to reduce its susceptibility to degradation by endo- and exonucleases, inclisiran is modified with phosphorothioate substitutions and the inclusion of 2´-deoxy, 2´-*O*-methyl, and 2´-fluoro nucleotides. The drug in its current formulation is effective when administered subcutaneously every 3 to 6 months (42).

There is an important distinction between the mechanism of action of PCSK9 antibodies and PCSK9 siRNA; while PCSK9 antibodies bind extracellular PCSK9 to inhibit the interaction of PCSK9 and the LDL receptor, inclisiran inhibits hepatic PCSK9 production intracellularly. If, as has been reported (54), PCSK9 exerts biological functions within the cell, then these will be impacted by inclisiran, but not by monoclonal antibody-based drugs.

3.1.3 Clinical data

In a phase 1 trial involving 24 participants, doses of 300 mg inclisiran or more reduced PCSK9 levels 12 weeks after injection by up to 75%, and LDL-C levels with doses of 100 mg or more by 37–51%. PCSK9 and LDL-C levels were still reduced at day 180 after doses of 300 mg or more. No serious adverse events were reported (55).

In the phase 2 ORION-1 trial, patients (n=501) with high cardiovascular risk and elevated LDL-C were randomized to receive placebo or ascending doses of inclisiran once or twice, with the second injection 90 days after the first (56). Participants had a baseline LDL-C of >70 mg/dL (1.8 mmol/L) if they had ASCVD, or >100 mg/dL (2.6 mmol/L) if they had no history of ASVCD. Treatment with the maximally tolerated statin dose was encouraged, and patients treated with PCSK9 antibodies were excluded. Inclisiran treatment significantly reduced both circulating PCSK9 and LDL-C levels. After 6 months, LDL-C was reduced by up to 42% after a single dose, and by up to 53% when two doses had been administered. In subjects who received two doses of 300 mg inclisiran, LDL-C was reduced to <50 mg/dL (1.3 mmol/L) in half of the group. Inclisiran also reduced non-HDL cholesterol and apolipoprotein B levels (57). Furthermore, the safety profile of inclisiran was comparable to placebo (56).

Recently, three phase 3 trials of inclisiran have been reported. In all of these studies, ~90% of patients were treated with background statin. Inclisiran 300 mg or placebo were administered at baseline, after 3 months, and every 6 months thereafter. In the ORION-9 trial, 482 patients with heterozygous familial hypercholesterolemia were included (58). Baseline LDL-C was 153 mg/dL (4.0 mmol/L) on statin. At day 510, inclisiran reduced LDL-C by 47.9%, independent of the underlying genotype.

The ORION-10 and ORION-11 trials were similar in design. Patients (n=1561 and 1617 respectively) with ASCVD or at high cardiovascular risk were included (59). Inclisiran reduced

LDL-C by 52.3% and 49.9% at day 510. The occurrence of a prespecified cardiovascular safety endpoint was numerically lower in those on inclisiran.

In ORION-9, -10 and -11, total and non-HDL cholesterol, apolipoprotein B, triglycerides, and Lp(a) were reduced, and HDL cholesterol increased. In all three studies, adverse events were similar in both treatment arms while injection site reactions were more common in those receiving inclisiran, with none being severe or persistent (58),(59).

3.1.4 Ongoing studies

The ongoing cardiovascular outcome trial, ORION-4, plans to recruit 15 000 patients with ASCVD (NCT03705234)(60). Inclisiran (or placebo) will be administered at baseline, after three months, and every six months thereafter for a total of five years. The trial is expected to be completed in 2024.

3.2 Apolipoprotein CIII (apoCIII)

3.2.1 Rationale for target identification

Plasma triglyceride is a marker of the abundance of triglyceride-rich lipoproteins and their remnants. While triglyceride itself is not found in atherosclerotic lesions, elevated levels in the circulation have been causally linked to ASCVD in large part because the dysregulation that leads to accumulation of triglyceride-rich lipoproteins impacts the whole of the VLDL-LDL metabolic cascade (2),(13)–(15),(61),(62). The metabolic consequences of higher triglyceride levels are increased generation of cholesterol-enriched VLDL and chylomicron remnants, and alterations in the size and composition of LDL with the appearance of increased concentrations of small, dense LDL (2). These remnant particles and small, dense LDL have been identified as potential contributors to cholesterol deposition in growing atherosclerotic lesions.

One key regulator of triglyceride metabolism is apoCIII, which inhibits lipoprotein lipase, the main enzyme responsible for the lipolysis of core triglyceride in VLDL and chylomicron

particles (63). ApoCIII has also been implicated in retarding the clearance of VLDL and chylomicron remnants by receptor-mediated pathways (64). Plasma apoCIII levels are associated with increased risk of CHD in epidemiological studies (65)–(67). Furthermore, rare loss-of-function mutations in the *APOC3* gene are associated with 40% lower triglyceride levels and 40% lower risk of CHD (18),(19).

The cardiovascular benefits of lowering triglyceride levels are not as consistent as the benefits observed with LDL-C-lowering therapies (68),(69). However, loss-of-function mutations in the *APOC3* gene, which are strongly associated with lower risk of CHD, imply a causal relationship between apoCIII and CHD. In addition, apoCIII has been found to induce inflammation and organ damage by alternative inflammasome activation (70). Therefore, irrespective of the associated changes in triglycerides, apoCIII appears to be a promising target in prevention of ASCVD.

3.2.2 Pharmacology

The first drug specifically targeting apoCIII mRNA was volanesorsen (Waylivra; previously denoted ISIS 304801 or ISIS-APOCIIIRx). Volanesorsen is a 2´-methoxyethyl-modified ASO with phosphorothioate substitutions (71), and is given subcutaneously once per week (72),(73). Subsequently, AKCEA-APOCIII-L_{Rx} (ISIS 678354) was developed; this agent possesses the same nucleotide sequence as volanesorsen, but is conjugated with a triantennary GalNAc complex (38). It is administered subcutaneously and has been tested in dosing intervals of 1 to 4 weeks (38).

3.2.3 Clinical data

To date, volanesorsen has been tested in patients with elevated triglyceride levels and in subjects with familial chylomicronemia syndrome (FCS), a rare genetic disorder caused by deficiency of lipoprotein lipase and characterised by markedly elevated triglyceride levels (frequently >900 mg/dL [~10 mmol/L]) and recurrent pancreatitis (69).

The efficacy of this drug was established in a phase 1 trial in healthy individuals (71). Subsequently, it was tested in patients with elevated triglyceride levels (n=85) for 13 weeks with or without background fibrate therapy (72). Patients were required to have triglyceride levels in the untreated state of 350–2000 mg/dL (4.0–22.6 mmol/L) or if treated of 225–2000 mg/dL (2.5–22.6 mmol/L). At the highest dose of 300 mg weekly, apoCIII was reduced by 80% when volanesorsen was given as monotherapy and by 71% when the agent was added to fibrate therapy. Triglyceride levels were reduced by up to 71%, and HDL cholesterol levels, which were subnormal at baseline, increased. Elevation of LDL-C occurred in the monotherapy group in a dose-dependent manner (from an overall mean level of 80 mg/dL [2.1 mmol/L] to a mean of 128 mg/dL [3.3 mmol/L] after treatment). No safety issues were reported.

In the randomized COMPASS trial, participants (n=75) with triglyceride levels of at least 500 mg/dL (5.6 mmol/L) were treated with 300 mg volanesorsen weekly, resulting in a mean reduction in triglyceride level of 73% from baseline, and an absolute reduction of 869 mg/dL (9.8 mmol/L). Reactions at the injection site were common (24%). No definitive serious adverse events occurred however, including no cases of thrombocytopenia (74).

In FCS patients, volanesorsen was initially investigated in a pilot study of three subjects (75), and subsequently tested in the randomized APPROACH trial of 66 participants treated for 52 weeks (73). Patients were required to have genetically proven FCS and triglyceride levels of at least 750 mg/dL (8.5 mmol/L). Following treatment with 300 mg volanesorsen weekly, apoCIII was reduced by 84% at 3 months and triglycerides were reduced by 77%, corresponding to mean absolute decreases of 1712 mg/dL (19.3 mmol/L). The majority (77%) of the patients in the volanesorsen group achieved triglyceride levels <750 mg/dL (8.5 mmol/L). LDL-C increased by 136% (note that in FCS the baseline LDL-C is typically low, and in this instance, the mean value was 28 mg/dL [0.7 mmol/L] in the trial subjects); in addition, apolipoprotein B increased by 20%, and HDL cholesterol by 46%; non-HDL cholesterol was reduced by 46%. There were adverse reactions to the drug; 61% of the volanesorsen-treated patients exhibited

side effects at the injection site, and 45% developed thrombocytopenia with platelet levels of <100 000/µL. Platelet levels in subjects with thrombocytopenia returned to normal after interruption of volanesorsen and the side effect appeared to be dose-dependent. It appears that platelet counts in patients with FCS are naturally highly variable (even when not treated with volanesorsen) and there have been reports of both thrombocytopenia and thrombocytosis (76). Furthermore, a genetic analysis suggested that thrombocytopenia is not a direct consequence of decreased apoCIII function (77). A tentative conclusion concerning this important side effect is that there may be an interaction between volanesorsen and the disease. Volanesorsen has been approved in the European Union for the treatment of FCS in patients with high risk of pancreatitis if dietary measures and other triglyceride-lowering therapies do not lower triglyceride levels sufficiently (78).

The use of much lower doses of the drug when the conjugated form – volanesorsen with GalNAc – is used may potentially overcome the problems of thrombocytopenia and injection site reactions. In a phase 1/2a trial in 67 healthy volunteers with mildly elevated triglyceride levels, AKCEA-APOCIII-L_{Rx} reduced apoCIII by up to 92%, and triglyceride levels by up to 77% (38). Modification of the lipid profile was more favourable as compared to the APPROACH trial with the original form of volanesorsen (73): apolipoprotein B was significantly reduced by AKCEA-APOCIII-L_{Rx}. However, this comparison is confounded by the distinct study populations involved in the two studies. AKCEA-APOCIII-L_{Rx} was tested at intervals of 1 to 4 weeks at doses of up to 120 mg in a single-dose arm, and up to 30 mg weekly or 60 mg every 4 weeks for 3 months – compared to the 300 mg weekly dose of volanesorsen. No relevant safety signals occurred; there was only one injection site-related adverse event which did not lead to discontinuation of the therapy, and there were no cases of thrombocytopenia. Importantly, however, the participants in this study did not have FCS.

3.2.4 Ongoing studies

Volanesorsen is currently being tested in patients with familial partial lipodystrophy (NCT02527343)(79).

AKCEA-APOCIII-L_{Rx} will be further assessed in a phase 2 dose-ranging trial in 115 participants with established ASCVD or high cardiovascular risk and triglyceride levels greater than 200 mg/dL (2.3 mmol/L) to pave the way for cardiovascular outcome trials (NCT03385239)(80).

3.3 Apolipoprotein(a) (apo[a])

3.3.1 Rationale for target identification

Apolipoprotein(a) (apo[a]) is covalently bound to the apolipoprotein B moiety of an LDL-like particle, forming Lp(a) (81). In prospective studies, Lp(a) concentration was associated with myocardial infarction, stroke, and aortic valve stenosis (12),(82). Two genetic variants in the *LPA* gene explain 40% of variation in the plasma concentration of this lipoprotein, and both are strongly associated the risk of CHD (10). Similarly, the number of kringle IV type 2 repeats in apo(a), which is inversely related to the Lp(a) concentration, exhibits a negative association with the risk of myocardial infarction (11). Genetic variants in *LPA* are equally associated with aortic stenosis (83), reinforcing the epidemiological findings, and elevated Lp(a) has been associated with higher all-cause mortality (84).

There are no therapeutic options approved for specific lowering of Lp(a) to date. In studies of approved drugs that lower Lp(a) non-specifically, including niacin, mipomersen, PCSK9 antibodies, and estrogen, no cardiovascular benefits attributable to lowering of the lipoprotein could be demonstrated. However, patients in these studies were not selected on the basis of elevated Lp(a). Further, due to the skewed distribution of Lp(a), the mean Lp(a) concentration and the absolute reduction in the lipoprotein were predictably too low to result in a reduction in cardiovascular events (85).

To summarize, there is robust observational and genetic evidence supporting a causal role of Lp(a) in the development of ASCVD.

3.3.2 Pharmacology

The first drug specifically targeting apo(a) mRNA was IONIS-APO(a)$_{Rx}$ (previously denoted ISIS-APO(a)$_{Rx}$). IONIS-APO(a)$_{Rx}$ is an ASO modified by substitution of all phosphodiester linkages with phosphorothioate, the inclusion of five 2´-*O*-methoxyethyl RNA nucleotides at both ends, and ten 2´-*O* DNA nucleotides at its centre (86); it is administered subcutaneously. AKCEA-APO(a)L$_{Rx}$ (previously denoted IONIS-APO(a)L$_{Rx}$, and also known as TQJ230) is a modified version of IONIS-APO(a)$_{Rx}$, and in addition, contains a covalently-bound triantennary GalNAc complex and substitution of 6 of the 19 phosphorothioate linkages with phosphodiester linkages (39).

A GalNAc-conjugated siRNA targeting apo(a) mRNA, AMG 890 (formerly ARO-LPA) is also in clinical development (87),(88).

3.3.3 Clinical data

In a phase 1 trial of IONIS-APO(a)$_{Rx}$ in participants (n=47) with mildly elevated Lp(a), Lp(a) was reduced by up to 78% (86). In a phase 2 trial of this agent, in a cohort with baseline Lp(a) levels of 50–175 mg/dL (125–437 nmol/L), Lp(a) levels were reduced by 67%, and by 72% in a cohort with baseline levels of ≥175 mg/dL (438 nmol/L) (39). Doses of up to 300 mg weekly were used.

The GalNAc-conjugated and modified compound, AKCEA-APO(a)L$_{Rx}$, was investigated in a phase 1 trial in 58 volunteers. This agent reduced Lp(a) by up to 92% (39), and its potency was estimated to be about 30-fold higher as compared to the original compound. In a placebo-controlled phase 2 trial of AKCEA-APO(a)L$_{Rx}$ in patients with established ASCVD and Lp(a) levels of >60 mg/dL (150 nmol/L) (n=286), the drug was administered in ascending doses at intervals of 1 to 4 weeks. After six months, AKCEA-APO(a)L$_{Rx}$ reduced Lp(a) by 72% as

compared to placebo using a dose of 60 mg every 4 weeks, and by 80% with 20 mg weekly doses (89). Apolipoprotein B and LDL-C were reduced in all dose regimens. With AKCEA-APO(a)L_{Rx} doses of 20 mg given weekly, 98% of trial participants achieved Lp(a) levels below 50 mg/dL (125 nmol/L). Apart from reactions at the injection site in 27% of the participants, there were no relevant safety signals.

3.3.4 Ongoing studies

A cardiovascular outcome trial, "Lp(a) Horizon", was initiated at the end of 2019 (NCT04023552)(90). It is planned to include 7680 patients with established ASCVD and Lp(a) levels of at least 70 mg/dL. Study participants will be randomized to placebo or AKCEA-APO(a)L_{Rx} 80 mg monthly; this specific dose was not tested in the phase 2 trial of this compound (89), but represents the cumulative dose of the 20 mg/week regimen. The study is estimated to be completed in 2024.

The GalNAc-conjugated siRNA targeting apo(a) mRNA, AMG 890, is currently under evaluation in a double-blind, randomized, phase 2 study to assess efficacy, safety, and tolerability in subjects with elevated Lp(a) (NCT04270760/NCT03626662)(87),(88).

3.4 Angiopoietin-like 3 (ANGPTL3)

3.4.1 Rationale for target identification

The ANGPTL3 protein was first linked to lipid metabolism in a mouse model in 2002 (91). Subsequently, animal and human studies showed that loss-of-function mutations in the *ANGPTL3* gene were associated with lower triglycerides levels (92), lower LDL and HDL cholesterol (93). Carriers of heterozygous loss-of-function mutations in *ANGPTL3* were found to have 34–41% lower risk of CHD (20),(21). Therapeutic inhibition of Angptl3 in mice with the antibody evinacumab resulted in a reduction of atherosclerotic lesion surface area, while inhibition of ANGPTL3 in humans led to decrease in triglyceride concentrations (76%) and

LDL-C (up to 23%) (20). In a mouse model, targeting *Angptl3* with an ASO resulted in reductions of both hepatic triglyceride content and atherosclerosis, together with an increase in insulin sensitivity (94).

ANGPTL3, produced in the liver and secreted into the circulation, inhibits lipoprotein lipase and endothelial lipase and thereby influences both triglyceride and HDL cholesterol level (21). LDL-C is also perturbed but the mechanism by which this occurs is unknown. Importantly, however, it appears to be distinct from, and possibly synergistic with, mechanisms implicated in LDL-C reduction by statins, ezetimibe, and PCSK9 inhibitors (95). This notion is underlined by the fact that in a small series of patients with homozygous familial hypercholesterolemia (due to homozygous or compound heterozygous LDL receptor deficiency), treatment with the anti-ANGPTL3 antibody evinacumab reduced LDL-C by a mean of 49% despite stable and aggressive background lipid-lowering therapy (96). These findings were recently corroborated by a study involving 65 patients with homozygous familial hypercholesterolemia. Evinacumab reduced LDL-C by 49% compared to placebo including patients without residual LDL receptor activity (97).

3.4.2 Pharmacology

The GalNAc-conjugated ASO, AKCEA-ANGPTL3-L_{Rx} (previously denoted IONIS-ANGPTL3-L_{Rx}, or ISIS 703802), specifically targets ANGPTL3 mRNA. AKCEA-ANGPTL3-L_{Rx} contains 20 nucleotides that are linked by 13 phosphorothioate and 6 phosphodiester bonds; it includes five 2´-*O*-methoxyethyl-modified RNA nucleotides at each end, and ten DNA nucleotides in the centre of the nucleotide sequence (94). AKCEA-ANGPTL3-L_{Rx} is administered subcutaneously, and was dosed once weekly in a phase 1 trial (94).

3.4.3 Clinical data

In a phase 1 trial of AKCEA-ANGPTL3-L_{Rx}, study participants (n=44) with triglyceride concentrations >90 mg/dL (1.0 mmol/L) were treated with single doses of AKCEA-ANGPTL3-

L_{Rx} of up to 80 mg, or weekly doses of up to 60 mg for 6 weeks (94). Treatment reduced circulating ANGPTL3 protein levels by up to 85%, triglycerides by up to 63%, and LDL-C by up to 33%. Reductions were also seen in VLDL cholesterol (up to 60%), non-HDL cholesterol (up to 37%), apolipoprotein B (up to 26%), and apoCIII (up to 59%). No serious adverse events occurred.

3.4.4 Ongoing studies

A phase 2 study with AKCEA-ANGPTL3-L_{Rx} has been completed, but has not been reported to date (NCT03371355)(98). According to information from the manufacturer, the study included participants with hypertriglyceridemia, type 2 diabetes, and non-alcoholic fatty liver disease (n=105) and met the primary endpoint of significant triglyceride reduction with a favourable safety profile (99). Furthermore, the siRNA targeting ANGPTL3 mRNA ARO-ANG3 is currently being tested in a phase 1 trial in healthy volunteers and dyslipidemic patients (NCT03747224)(100).

4 Overall safety considerations

Typical side effects of RNA-based therapeutics are reactions at the injection site, which in most cases are mild and do not lead to discontinuation of therapy. A side effect specific for volanesorsen in patients with FCS is thrombocytopenia, possibly resulting from a drug-disease interaction. Apart from this, to date, clinical trials involving ASOs and siRNA demonstrate a favourable safety profile. However, length of follow-up and the number of trial participants is as yet limited. Indeed, in the case of long-acting drugs such as inclisiran, it will be of crucial importance to demonstrate long-term safety, as after one single injection, drug activity cannot be abolished for a number of months.

In principle, potential side effects of RNA-based drugs can be expected to occur as a result of an interaction of the drug with the target, or as off-target effects.

For the treatment targets described here, on-target side effects appear to be unlikely: loss-of-function mutations in PCSK9, apoCIII, and ANGPTL3 have been associated with lower risk of CHD with otherwise no apparent impact on health; and genetic variants in *LPA* are limited to association with ASCVD outcomes (101). Thus, to date, there do not appear to be negative consequences which result from the lowering of the circulating concentrations of PCSK9, or apoCIII, or ANGPTL3, or lipoprotein(a).

A potential off-target effect shared by all RNA-targeting therapeutics may involve the interaction of the antisense strand with a partially complementary mRNA other than the target mRNA. In preclinical studies, hepatotoxicity at supratherapeutic doses of siRNAs could be attributed to these interactions; the compounds taken forward into clinical use have been successfully modified in order to reduce hepatotoxicity (102). Other postulated off-target effects are attributed to chemical modifications such as phosphorothioate substitutions, which might lead to hepatotoxicity, nephrotoxicity, or immune-stimulatory effects (103). The latter are attributed to the interaction of specific sequence motifs with toll-like receptors; to minimize immune responses through these pathways, the respective sequence motifs are avoided and alternative chemical modifications introduced (23). To date, no signs of genotoxicity have been observed for siRNAs (104) and ASOs (105).

5 Comparison with antibody-based strategies

Circulating PCSK9 can be targeted directly with the monoclonal antibodies evolocumab and alirocumab, or indirectly with the siRNA inclisiran; similarly, ANGPTL3 can be targeted by the monoclonal antibody evinacumab or the ASO AKCEA-ANGPTL3-L_{Rx}. It is likely that as yet unidentified, future therapeutic targets can also be addressed by either approach. As compared to monoclonal antibodies, the use of RNA-targeting therapeutics may have potential advantages and disadvantages (**Table 2**).

One major advantage of RNA-based drugs is the possibility of targeting proteins with high plasma concentrations such as apo(a) as a component of the Lp(a) particle. As discussed above, large amounts of antibodies would be needed to neutralize a pharmacologically relevant proportion of the protein, resulting in large amounts of immune complexes, which may lead to organ toxicity (22). Furthermore, the development and production of RNA-based drugs is relatively straight forward and less onerous than the production of antibodies. Moreover, oligonucleotides, in contrast to antibodies, are stable at room temperature, a feature which facilitates their storage and distribution (42). Another advantage of RNA-based drugs is that potentially every protein and even non-coding regions of the genome can be suppressed; in contrast, antibodies can only target proteins that are secreted or are located extracellularly. The longer dosing interval of RNA-based drugs, such as monthly or 6-monthly administration, is a further beneficial feature compared to antibodies which need to be administered every few weeks. Moreover, antibody use is sometimes limited by the development of auto-antibodies, while adaptive immune responses have not as yet been demonstrated against RNA-based drugs (46).

However, one important drawback of RNA-based drugs is our limited clinical experience in long-term treatment of large populations. Furthermore, for drugs with a prolonged half-life such as inclisiran, potential drug-related side effects may be sustained as long as the drug is active; importantly however, modalities for the effective neutralisation of such agents are not currently available. However, the design of antidotes with the complementary sequence of the drug is considered feasible (46).

6 Conclusions

Targeting specific RNA species in hepatocytes is emerging as a new and efficacious pharmacologic strategy for patients at high risk for cardiovascular events. Assuming positive

findings in the ongoing cardiovascular outcome trials involving these novel strategies, and equally assuming critical confirmation of their safety, this innovative nanotechnology has the potential to fundamentally change our traditional concepts which are centred on daily administration of medication to prevent and treat ASCVD. In principle, the GalNAc-siRNA/ASO technology can be applied to specifically inhibit any one of multiple proteins of hepatic origin. Such targeted inhibition is potentially scalable, may be personalised, and has enhanced potency. In theory, targeting of mRNAs should be mutually exclusive, and specific agents should not interact with each other; indeed, they may, in all likelihood, be independent of metabolic, renal and other factors which have been found to influence the pharmacology of traditional oral therapies. The temporal distance of the subcutaneous application, e.g. every 6 months, could permit implementation of new concepts of pharmacotherapy. For example, a visit to the doctor's office (or pharmacy, or an automatic remote reminder) twice per year could replace daily blister packs of pills. The cost of production is likely to be moderate as compared to antibody treatments. The first representatives of this class, as exemplified by the PCSK9 siRNA inclisiran, are likely to become available within the next few months and could be beneficial in very large numbers of individuals with hypercholesterolemia. Clearly, efficacy and safety data derived from ongoing trials will need to be carefully assessed before treatment recommendations can be made. Without doubt, and as a result of the highly specific targeting of these therapies, the findings in these trials will greatly advance our understanding of the pathophysiological role of defined lipoprotein particles in ASCVD, an excellent example of which is Lp(a).

Bullet points

- RNA targeting as a novel therapeutic approach in cardiovascular prevention.
- Liver-specific drug delivery enhanced by *N*-acetylgalactosamine conjugation.
- Specific targeting of PCSK9, apoCIII, apo(a), and ANGPTL3 mRNA.

- Effects on clinical outcomes and safety are under assessment in ongoing trials.

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Figure legends

Figure 1: Conceptual comparison of different pharmacologic strategies

Traditional small molecule drugs target enzymes or receptors in the extra- and/or intracellular space by affecting enzyme function or signalling, respectively. Biologics such as monoclonal antibodies bind specific sites of receptors or proteins extracellularly and by this, affect their function or neutralize them. mRNA-targeting drugs cross the cell membrane and are released into cytoplasm (see **Figure 2**). After binding complementary target mRNA, degradation of the mRNA is initiated (see **Figure 3**). In consequence, the target protein is not translated.

Abbreviation: ASGPR: asialoglycoprotein receptor

Figure 2: Modes of delivery of RNA-targeting drugs

Different approaches have been developed to enable RNA-targeting drugs to cross the cell membrane. These include, but are not limited to, lipid nanoparticles, self-assembled micelle inhibitory RNA (SAMiRNA), and conjugation with *N*-acetylgalactosamine (GalNAc) for liver-specific targeting. GalNAc-conjugated siRNAs and ASOs are specifically taken up by hepatocytes via the asialoglycoprotein receptor (ASGPR). After endocytic uptake, pH drops in the endosome, and a small amount of the RNA-targeting drug is released into cytoplasm via unknown mechanisms. Upon entering the cytoplasm, the drug interacts with the complementary mRNA (see **Figure 3**).

Abbreviations: ASGPR: asialoglycoprotein receptor, ASO: antisense oligonucleotide, GalNAc: *N*-acetylgalactosamine, SAMiRNA: self-assembled micelle inhibitory RNA, siRNA: small interfering RNA

Figure 3: Mechanisms of mRNA degradation with ASOs and siRNAs

After entering cytoplasm, the siRNA is loaded into the RNA-induced silencing complex (RISC). The guide strand (antisense strand) dissociates from the passenger strand and binds to complementary target mRNA, which leads to enzymatic cleavage of the target mRNA. After cleavage, the RISC can degrade further target mRNAs, recognized by the same guide strand, for a prolonged period of time. The elimination half-life of siRNAs and 2´-*O*-methoxyethyl phosphorothioate-substituted ASOs in hepatocytes is several weeks (30). In contrast to siRNAs, ASOs usually degrade mRNA in a 1:1 fashion by a variety of mechanisms, one of which is degradation of the complementary mRNA by RNase H1. The end result of both pathways of drug action is to prevent the mRNA from being translated.

Abbreviations: ASO: antisense oligonucleotide, RISC: RNA-induced silencing complex, siRNA: small interfering RNA

Central Illustration: Targets of RNA-based drugs for atherosclerotic disease prevention

Reducing the abundance of any of the four shown proteins through degradation of their mRNA leads to changes in plasma lipids as indicated.

Abbreviations: ANGPTL3: angiopoietin-like 3, ApoCIII: apolipoprotein CIII, ASO: antisense oligonucleotide, Lp(a): lipoprotein(a), PCSK9: proprotein convertase subtilisin/kexin type 9, siRNA: small interfering RNA

Box and tables

Box 1: RNA-targeting therapeutics: current areas of research (23),(30),(36)

- Oral availability of RNA-targeting drugs
- Specific targeting to tissues other than liver
- Cellular uptake by not yet utilized endocytotic and non-endocytotic mechanisms
- Introduction of further chemical modifications to improve pharmacokinetics and pharmacodynamics
- Elucidation of the endosomal escape mechanism of ASOs and siRNAs (only <1% of siRNA escape the endosome)
- Approaches to address RNA species other than mRNA (e. g., microRNA)
- Novel approaches to address RNA (e. g., RNA editing)

Abbreviations: ASO: antisense oligonucleotide, siRNA: small interfering RNA.

Table 1: Targeting of PCSK9, apolipoprotein CIII, apolipoprotein(a), and angiopoietin-like 3 mRNA with GalNAc-conjugated therapeutics

Target	PCSK9	Apolipoprotein CIII	Apolipoprotein(a)	Angiopoietin-like 3
Rationale for target identification	• gain- and loss-of-function mutations in *PCSK9* gene • Mendelian randomization studies • positive outcome trials with PCSK9 antibodies	• observational evidence • loss-of-function mutations in *APOC3* gene	• observational and genetic evidence • Mendelian randomization studies	• loss-of-function mutations in *ANGPTL3* gene • animal studies, beneficial changes in lipid profile after pharmacologic inhibition of ANGPTL3
Pharmacology	• inclisiran: GalNAc-conjugated siRNA • subcutaneous injection • administration every 3–6 months	• volanesorsen (ISIS 304801, ISIS-APOCIIIRx): ASO, weekly subcutaneous injection • AKCEA-APOCIII-L_{Rx} (ISIS 678354): GalNAc-conjugated volanesorsen • subcutaneous injection • administration every 1–4 weeks	• AKCEA-APO(a)L_{Rx} (IONIS-APO[a]L_{Rx}, TQJ230): GalNAc-conjugated ASO • subcutaneous injection • administration every 1–4 weeks (ongoing outcome trial: monthly dosing)	• AKCEA-ANGPTL3-L_{Rx} (IONIS-ANGPTL3-L_{Rx}, ISIS 703802): GalNAc-conjugated ASO • subcutaneous injection • administration every 1–4 weeks • ARO-ANG3: siRNA, subcutaneous injection
Clinical data	• phase 1 and 2 trials: reduction in LDL-C of up to 53% • phase 3 trials ORION-9/-10/-11: LDL-C reduction of 50% on top of statin therapy in patients with HeFH,	• volanesorsen: significant reductions in apoCIII and triglycerides (>70%) in hypertriglyceridemia and FCS • volanesorsen: injection site reactions and thrombozytopenia in FCS	• phase 1 and 2 trials: reduction of lipoprotein(a) by up to 80%	• phase 1 study of AKCEA-ANGPTL3-L_{Rx} with reductions in triglycerides (63%) and LDL-C (33%)

	ASCVD, or at high cardiovascular risk • injection site reactions more common with inclisiran, but generally mild	• AKCEA-APOCIII-L_{Rx}: significant reductions in apoCIII, triglycerides, and apoB in mild hypertriglyceridemia		
Ongoing studies	• cardiovascular outcome trial ORION-4, expected to be completed in 2024 (NCT03705234)	• volanesorsen in hypertriglyceridemia and familial partial lipodystrophy (NCT02527343) • AKCEA-APOCIII-L_{Rx} in patients with ASCVD and elevated triglycerides (NCT03385239)	• cardiovascular outcome trial "Lp(a) Horizon", expected to be completed in 2024 (NCT04023552)	• phase 2 study of AKCEA-ANGPTL3-L_{Rx} in patients with hypertriglyceridemia, type 2 diabetes, and non-alcoholic fatty liver disease (NCT03371355) • phase 1 study of ARO-ANG3 in healthy volunteers and dyslipidemic patients (NCT03747224)

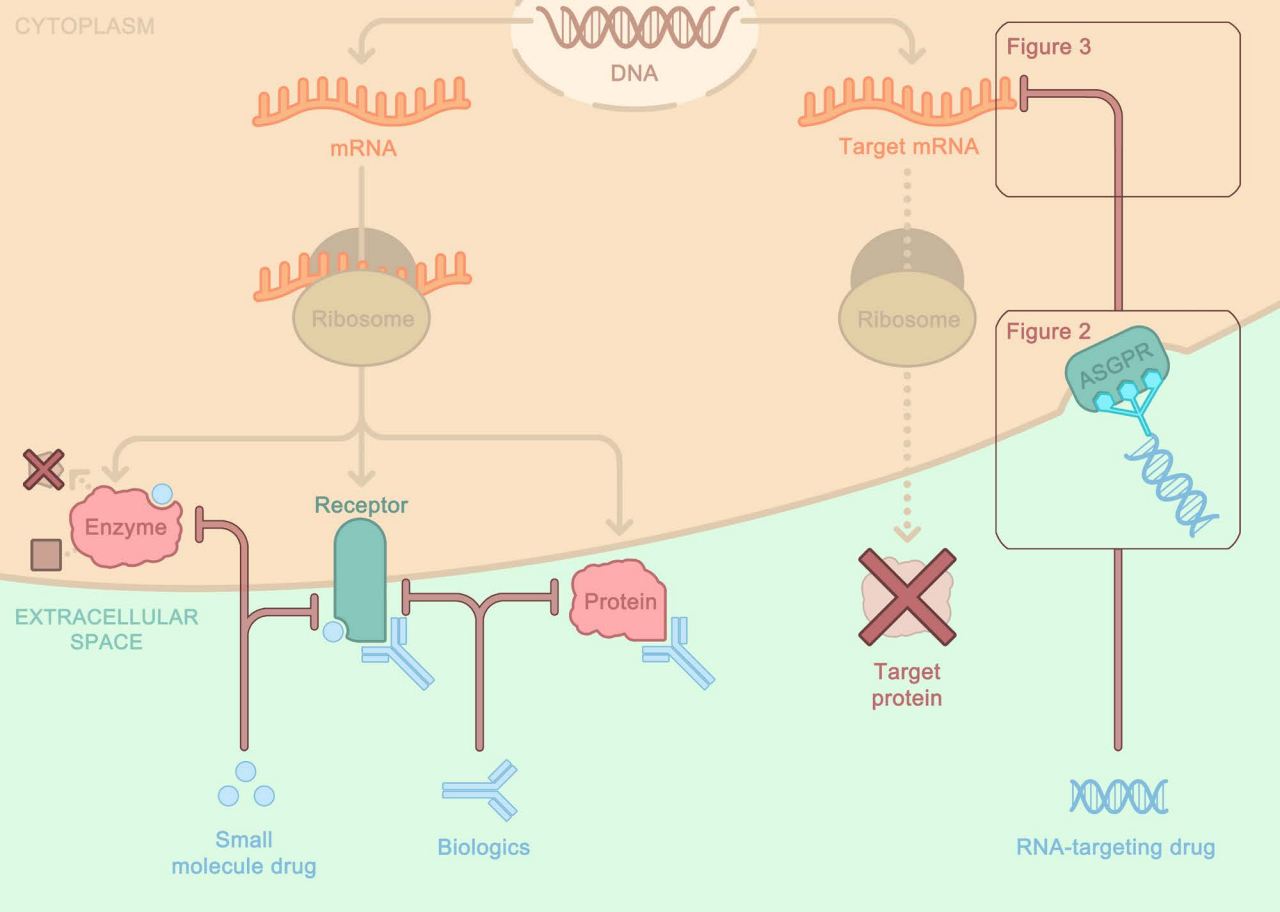
Abbreviations: apoB: apolipoprotein B, apoCIII: apolipoprotein CIII, ASCVD: atherosclerotic cardiovascular disease, ASO: antisense oligonucleotide, FCS: familial chylomicronemia syndrome, GalNAc: *N*-acetylgalactosamine, HeFH: heterozygous familial hypercholesterolemia, LDL-C: low-density lipoprotein cholesterol, PCSK9: proprotein convertase subtilisin/kexin type 9, siRNA: small interfering RNA

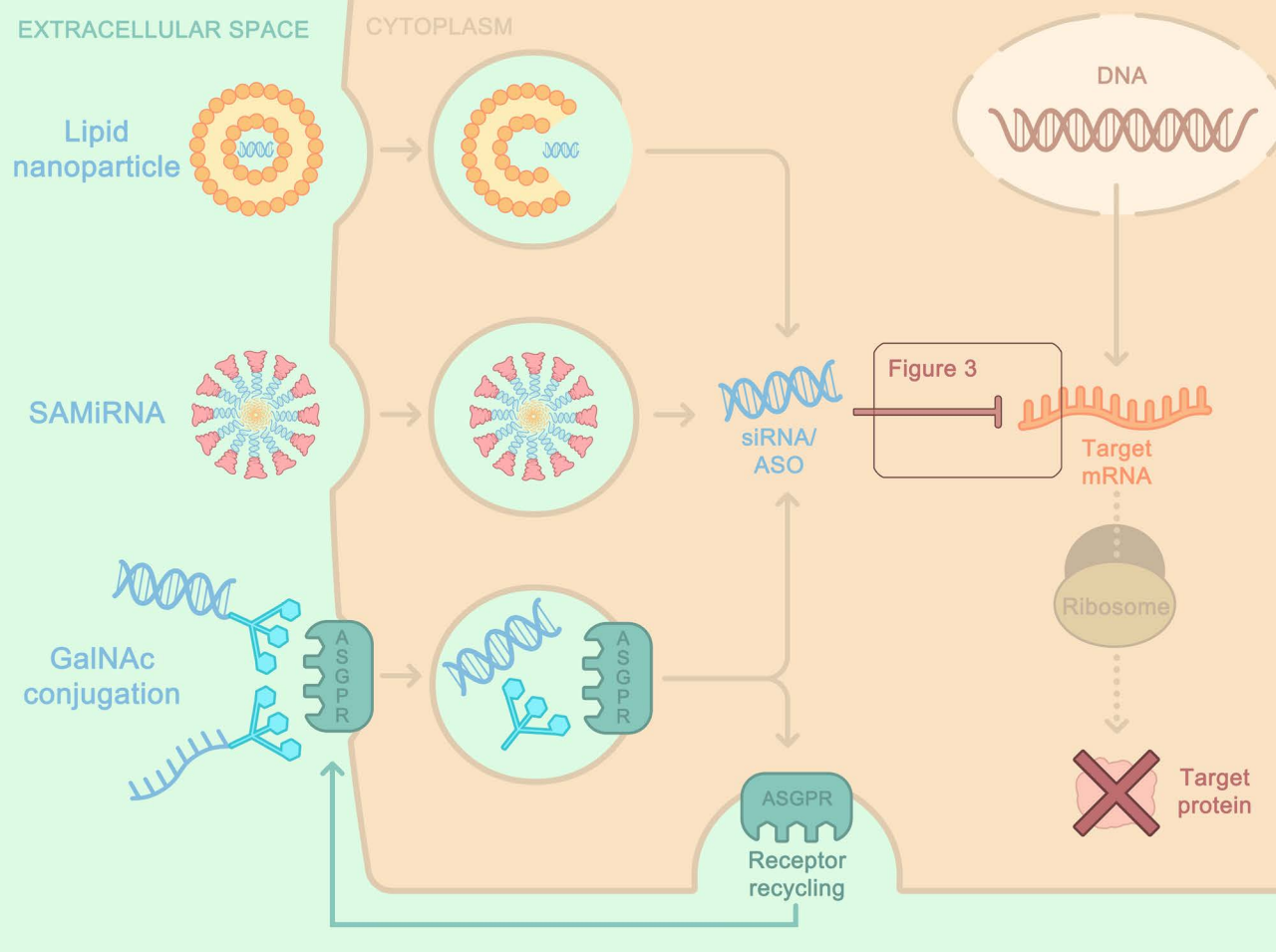
Table 2: Comparison of monoclonal antibodies and RNA-based drugs

	Monoclonal antibodies	RNA-based drugs
Targeting strategy	• specific binding of extracellular proteins (enzymes, receptors, signalling proteins) which modifies their function or neutralizes them	• specific binding of intracellular target mRNA to prevent translation of the encoded protein • targeting potentially more versatile
Site of action	• extracellular	• intracellular
Targeting of proteins with high plasma concentration	• limited – risk of generating large amounts of immune complexes with potential organ toxicity	• possible
Mode of delivery	• subcutaneous (PCSK9 MAB)	• subcutaneous
Dosing and dosing intervals*	• PCSK9 antibodies evolocumab/alirocumab: 140 mg/75–150 mg biweekly, 420/300 mg monthly • ANGPTL3 antibody evinacumab in a phase-1 trial: 20 mg/kg intravenously	• PCSK9 siRNA inclisiran: 300 mg every 6 months • ApoCIII ASO AKCEA-APOCIII-L_{Rx}: 30–60 mg every 1–4 weeks • ANGPTL3 ASO AKCEA-ANGPTL3-L_{Rx}: up to 60 mg weekly • Lp(a) ASO AKCEA-APO(a)L_{Rx}: 80 mg monthly
Metabolism	• PCSK9 MAB: elimination predominantly through binding of PCSK9 and additionally through proteolytic pathways in higher concentrations • half-life 11–17 days (106)	• short half-life (<1 h) in circulation • elimination half-life from hepatocytes: several weeks • degradation by endo- and exonucleases • renal excretion (43)
Efficacy	• for PCSK9 inhibition: comparable	
Potential for side effects	• generally low • injection site reactions	• injection site reactions • on-target side effects

	• on-target side effects • off-target side effects unlikely • organ toxicity with high amounts of immune complexes	• potential off-target side effects due to chemical modifications of the drug, effects of the passenger strand (siRNA), non-specific RNA interactions, alteration in balance of other RNA species
Adaptive immune responses	• development of auto-antibodies may attenuate therapeutic efficacy during course of therapy	• no adaptive immune responses shown yet
Production costs	• comparably high	• comparably low
Development and production process	• comparably complex	• comparably simple

Notes: * Examples of typical doses, restricted to GalNAc-conjugated RNA-based drugs and monoclonal antibodies discussed in this review.
Abbreviations: ANGPTL3: angiopoietin-like 3, apoCIII: apolipoprotein CIII, ASO: antisense oligonucleotide, Lp(a): Lipoprotein(a), GalNAc: *N*-acetylgalactosamine, MAB: monoclonal antibodies, PCSK9: proprotein convertase subtilisin/kexin type 9, siRNA: small interfering RNA


CYTOPLASM
DNA
mRNA
Target mRNA
Figure 3
Ribosome
Ribosome
Figure 2
ASGPR
Enzyme
Receptor
Protein
EXTRACELLULAR SPACE
Target protein
Small molecule drug
Biologics
RNA-targeting drug


EXTRACELLULAR SPACE
CYTOPLASM
Lipid nanoparticle
SAMiRNA
GalNAc conjugation
ASGPR
ASGPR
DNA
siRNA/ ASO
Figure 3
Target mRNA
Ribosome
Target protein
ASGPR
Receptor recycling