Research Review™
EDUCATIONAL SERIES

RNA therapies in cardiovascular medicine

Making Education Easy 2021

Independent expert commentary provided by

Associate Professor John Amerena

Associate Professor Amerena FRACP, FACC, FCSANZ, trained in Melbourne before spending four years in the United States at the University of Michigan. Over that period of time, he worked in the fields of hypertension and hyperlipidaemia, before returning to Australia where he is now a Cardiologist at Barwon Health. He currently has a joint appointment in the Department of Clinical and Biomedical Sciences at the University of Melbourne and the Department of Epidemiology and Preventive Medicine at Monash University. He is the director of the Geelong Cardiology Research Unit, which is currently involved in many phase II-III clinical trials. While still actively researching in hypertension, his focus has changed to research in antithrombotic/antiplatelet therapies, particularly in the context of acute coronary syndromes and atrial fibrillation. Heart failure is also a major interest, and he is also the Director of the Heart Failure Programme at Barwon Health. He is well published in these areas, as well as in many other areas of cardiovascular medicine.

Clinical Associate Professor David Sullivan

Clinical Associate Professor Sullivan, MBBS, FRACP, FRCPA, is a physician / chemical pathologist in the Department of Chemical Pathology at Royal Prince Alfred Hospital and an Associate Professor, Faculty of Medicine at the University of Sydney Central Clinical School. David has a long-term interest in lipid metabolism with particular emphasis on the environmental component (especially dietary) of gene – environment interactions contributing to cardiovascular disease. He has been involved in the early use of many forms of lipid-lowering intervention. He supports the improvement of detection and management of severe inherited dyslipidaemia, such as that seen in familial hypercholesterolaemia. David has experience in several international clinical posts, including World Health Organization (WHO) Fellowship at the MRC Lipoprotein Unit, Royal Postgraduate School of Medicine, Hammersmith Hospital, London. He co-ordinated international clinical studies from the WHO reference lipid laboratory in Wageningen, Netherlands where he worked with the originator of the technique known as Mendelian Randomisation. In addition to his clinical and teaching activities, he has served on numerous clinical committees including the management committees of the LIPID and FIELD trials. Current research interests include biomarkers and post-prandial metabolism.

This education review will outline the various forms of RNA therapies. In particular, this review will focus on RNA therapies that work to silence genes in cardiovascular medicine through the use of single-stranded antisense oligonucleotides (ASOs) and double-stranded small interfering molecules that operate through RNA interference (siRNA therapies). Other types of RNA therapies will also be briefly discussed, although to date these alternative forms of RNA therapy have largely been investigated in other therapeutic indications.

Cardiovascular disease

Cardiovascular disease (CVD), of which atherosclerotic cardiovascular disease (ASCVD) is the leading component, is a major cause of death worldwide.[1-3] In Australia, an estimated 1.2 million adults aged ≥18 years had one or more conditions related to heart or vascular disease in 2017-2018, with more than one in four deaths considered to be related to CVD in 2018.[2]

A person's CVD risk is frequently the result of multiple, interacting risk factors including age, gender, smoking behaviour, systolic blood pressure, total cholesterol, documented ASCVD, type 1 or 2 diabetes mellitus, chronic kidney disease, and familial hypercholesterolaemia (FH)/family history of CVD.[3] Various risk assessment systems are available to determine a person's CVD risk,[4] including those of the European Society of Cardiology and the European Atherosclerosis Society, and the Australian CVD Risk Calculator.[5]

Dyslipidaemia is widely recognised as one of the most important risk factors in ASCVD. Numerous genetic studies, prospective epidemiologic cohort studies, and randomised clinical trials have demonstrated a positive dose-dependent relationship between the exposure of the vasculature to low-density lipoprotein cholesterol (LDL-C) and the risk of ASCVD, with increasing duration of exposure to LDL-C amplifying this effect.[6] Other apolipoprotein-B (ApoB)-containing lipoproteins, including very low-density lipoproteins (VLDL) and their remnants, and lipoprotein(a) [Lp(a)], are also implicated in the development of ASCVD.[3, 7-9]

Lipid-lowering therapy

LDL-C lowering has become the mainstay of drug treatment for patients at high risk of ASCVD.[10-12] Given their ability to reduce CV events,[11] statins have become the pharmacological intervention of choice for the prevention of ASCVD, according to various international guidelines.[3, 14, 15] However, despite the proven efficacy and safety profile of statins, there remains a need for additional or alternative lipid-lowering therapies. Many patients fail to reach their recommended target LDL-C level while on statin therapy,[16] due to a variety of reasons including poor adherence,[17] drug discontinuation related to side effects,[17, 18] and the high variability in individual responses to statin therapy.[19] As a consequence various other lipid-lowering therapies have been developed (**Table 1**, page 2). An increased understanding of the genes involved in regulating atherogenic lipoproteins has led to the development of molecular therapies (including RNA therapeutics) targeting these pathways.[10, 20-22]

Overview of RNA-targeting strategies

Until recently, treatment strategies have largely relied on the ability of small molecule drugs to target active sites of proteins by inhibiting or altering their function. However, only a proportion of proteins have active binding sites that are "druggable" targets for small molecules.[29] As a consequence, RNA has increasingly been investigated as a potential mechanism for modifying the level of target proteins.[29, 30]

Not only does RNA serve as a direct link between DNA and proteins, but RNA molecules also play direct effector roles by binding to various ligands, including proteins, DNA, other RNAs, and metabolites.[30-32] Consequently, RNA can mediate cellular processes such as the regulation of gene transcription and the enhancement or inhibition of protein activity.[31, 32]

RNA species include:[32]

- coding RNA i.e. messenger RNA (mRNA) that is translated into proteins, and
- non-coding RNA which includes long non-coding RNAs such as transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), circular RNA, and small non-coding RNAs such as micro RNAs (miRNAs) and small interfering RNAs (siRNAs).

Armed with a greater knowledge of the RNA molecule, advances in the generation, purification, and cellular delivery of RNA have been made and have enabled the development of RNA-based therapeutics for use in a broad range of indications.[22, 29-33]

Different types of RNA therapeutics have reached clinical trials across a wide variety of indications including CV. These various therapies include (**Figure 1**): [21, 29-31]

- Aptamers, short single stranded nucleotides, which bind to a receptor or inhibit signal transduction.[29-31]
- Antisense oligonucleotides (ASOs), siRNA, or miRNA that result in the "silencing" of endogenous mRNA.[21, 29-31] Each of these RNAs, bind to their complementary sequences on target mRNA and prevent its translation, but the mechanism involved is different for each.[21, 29-31] This group of therapies have been investigated for use in CV disease and will be the focus of discussion in this article.
- mRNA therapeutics, in which exogenous mRNA is introduced into a cell in order to generate a therapeutic protein/peptide or to introduce transcripts not naturally present in the cell (e.g. in vaccines).[21, 29-31, 34-36]

Antisense oligonucleotides (ASOs)

ASOs are short, single-stranded oligonucleotides that are complementary to a target mRNA to which they hybridize and thereby modulate protein expression.[29, 30] ASO can serve as substrates for RNase H, or sterically block mRNA processing and translation.[29, 30] An example of an ASO that has reached clinical trials is volanesorsen.[37] Volanesorsen can be injected subcutaneously, and is an inhibitor of apolipoprotein CIII (Apo-CIII) mRNA.[37-39] Rare loss-of-function mutations in the *ApoCIII* gene are associated with 40% lower triglyceride levels and 40% lower risk of CHD.[40, 41] Volanesorsen is being investigated as a lipid-lowering agent in patients with hypertriglyceridaemia and familial partial lipodystrophy.[37, 38, 42] Another ASO in clinical trials is pelacarsen developed to decrease Lp(a) by targeting the *LPA* gene mRNA.[39] See later in text for further details of these ASOs.

Short interfering RNAs (siRNAs)

Short interfering RNAs are short (20–25 nucleotides), non-coding, double-stranded RNA molecules that use the RNA interference pathway to degrade a target mRNA.[29, 30] SiRNA is entirely complementary to the target transcript and therefore is highly specific.[29] Once delivered into the cell, siRNA interacts with the endogenous RNA-induced silencing complex (RISC) to elicit RNA interference.[22, 29] The endonuclease argonaute 2 (AGO2) component of the RISC cleaves the sense strand, leaving intact the antisense strand.[29, 30] The antisense strand then guides the RISC to the target mRNA which is recognised and cleaved.[29, 30] The RISC-bound siRNA complex can be recycled and therefore drives the cleavage of multiple mRNA molecules.[22] Inclisiran is an example of a synthetic chemically modified siRNA that is covalently conjugated to an N-Acetylgalactosamine (GalNAc) ligand.[43, 44] Inclisiran targets proprotein convertase subtilisin/kexin type 9 (PCSK) mRNA (see later in text for further details).[43, 44]

MicroRNA mimics/inhibitors

MicroRNAs (miRNAs) are small, non-coding RNA molecules that bind to mRNA in a complementary way, and cause gene silencing through the inhibition of translation and/or degradation of mRNA.[29, 30, 45] MiRNA mimics are double-stranded RNA molecules that mimic miRNAs, miRNA inhibitors are single-stranded RNA oligos designed to interfere with miRNAs. Various miRNAs are currently in clinical trials for use in different indications, including cancer.[30] MiRNAs play a role in cardiac development and regeneration, and they are involved in cardiovascular pathophysiology.[45] Their expression is altered in various cardiovascular diseases.[45] miRNA-based therapeutics have produced beneficial outcomes in animal models of heart failure, cardiac hypertrophy, fibrosis, and hyperlipidaemias, and a few miRNA-based therapeutics have reached early clinical trials.[45-47]

Table 1. **Lipid-lowering therapies**

Therapy	Mechanism of action	Comments
Statins	Inhibition of 3-hydroxy3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase)	The degree of LDL-C reduction is dose dependent and varies between the available statins[3, 23] Long-term statin therapy has been associated with rare serious adverse effects including myopathy, and new-onset diabetes mellitus[3, 23]
Fibrates	Agonists of PPAR-α, acting via transcription factors regulating various steps in lipid and lipoprotein metabolism[3]	Lower fasting TG levels, as well as post-prandial TGs and TG-rich lipoprotein remnant particles[3, 14] Reduce LDL-C levels (but a paradoxical small LDL-C increase may be observed with high TG levels) [3, 14] Increase HDL-C levels[3, 14] Generally well tolerated, but may be associated with myopathy (mainly gemfibrozil[24])[3, 14]
Bile acid sequestrants	Binding the bile acids and prevent the reabsorption of cholesterol into the blood, and thereby remove a large portion of the bile acids from the enterohepatic circulation	Reduce LDL-C levels but to a less extent than statins The adverse event profile (including gastrointestinal side effects) often limits their practical use[3, 14]
Nicotinic acid	Nicotinic acid primarily raises HDL-C and ApoA1 by stimulating ApoA1 production in the liver[3,25]	Used in addition to statin therapy where insufficient lipid control has been achieved[14]
Cholesterol absorption inhibitor (ezetimibe)	Inhibits the intestinal absorption of cholesterol and related plant sterols Molecular target is the sterol transporter, Niemann-Pick C1-Like 1 (NPC1L1)	Used as add-on therapy when treatment goals have not been achieved with statins or as monotherapy when statins are not tolerated or contraindicated[3, 14]
PCSK9 inhibitors	PCSK9 is involved in the control of the low-density lipoprotein receptor (LDLR).[3, 26] Increased levels or functioning of PCSK9 reduces LDLR expression by promoting LDLR lysosomal catabolism and a subsequent increase in plasma LDL concentrations. Lower levels or functioning of PCSK9 lowers plasma LDL-C levels[26]	Used as add-on therapy to maximum statin treatment when treatment goals have not been achieved with statins, or when statins are not tolerated or contraindicated[3] Currently, approved PCSK9 inhibitors included two fully human monoclonal antibodies, alirocumab[27] and evolocumab[3, 28]
RNA therapeutics	Regulation of gene transcription and the enhancement or inhibition of protein activity (see text for further details)	Two main approaches used:[21, 29] • the delivery of exogenous mRNA into a cell; and • the inhibition of translation of specifically targeted endogenous mRNA (gene silencing)

HMG-CoA = 3-hydroxy-3-methylglutaryl-CoA; **PCSK9** = proprotein convertase subtilisin/kexin type 9; **PPAR-α** = peroxisome proliferator-activated receptor α.

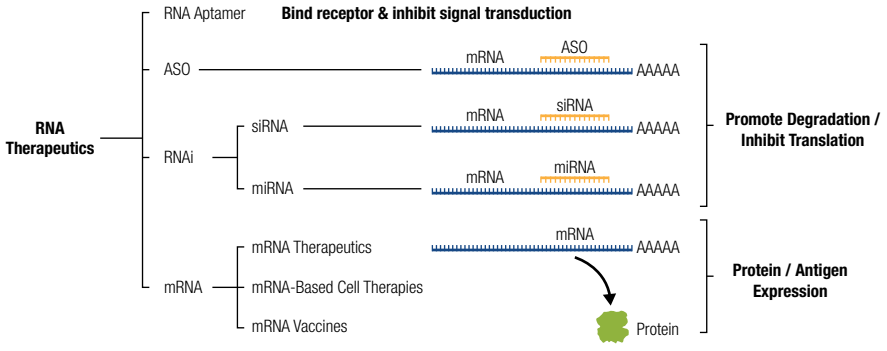

Figure 1. Classes of RNA therapeutics.[29] Adapted from Damase TR et al. Front Bioeng Biotechnol. 2021;9:628137.
ASO = antisense oligonucleotide; **RNA** = ribonucleic acid; **RNAi** = RNA interference; **siRNA** = small interfering RNA; **miRNA** = microRNA; **mRNA** = messenger RNA; **A** = adenosine molecule.

mRNA

mRNAs can be used as a therapeutic to produce proteins or peptides, and are considered to be a safer alternative to DNA when used therapeutically.[29, 30] Exogenous mRNA is translated into protein in the cytoplasm, and degrades within the cytoplasm (typically within minutes to hours) posing no risk of integration into the genome or from long-lasting expression.[29, 30]

mRNA therapeutics have been investigated for use as a protein replacement therapy (e.g. vascular endothelial growth factor [VEGF] delivery after myocardial infarction), vaccines for infectious diseases (e.g. Covid-19 vaccines), or for *in vivo* production of monoclonal antibodies.[29, 30] For example, the Covid-19 vaccines which deliver mRNA encode the spike protein of the SARS-CoV-2 virus to which the body produces an immune response.[31, 48]

Currently, an mRNA therapeutic encoding VEGF (AZD8601) is undergoing clinical trials to determine if it can restore ischaemic, but viable myocardial regions, in patients with coronary artery disease in the EPICCURE trial.[49]

Aptamers

Aptamers are short, single-stranded nucleic acids that bind to targets, such as proteins, peptides, carbohydrates, and other molecules.[29-31] RNA aptamers behave like a nucleic acid antibody or chemical inhibitor to modulate protein function.[31] To date, only pegaptanib has been approved for use (in the US) for the treatment of age-related macular degeneration. Aptamers have potential for use in a variety of cardiovascular therapeutic applications, most prominently as anti-thrombotics and anti-coagulants.[50-51] However, the field of aptamer therapeutics is still in its infancy.[50, 51]

Comment from expert David Sullivan

The review concisely summarises the emergence of RNA therapies by highlighting structural features in relation to mechanisms of action. ASOs and siRNAs represent completely new pathways for the development of therapeutic agents. They reflect the application of the principles of Mendelian randomisation, which assert that a biomarker (such as LDL-C) associated with a clinical outcome (such as coronary heart disease) is likely to be causative for that outcome if genetic polymorphisms (such as PCSK9 variants) affecting the biomarker have the anticipated associations with both the biomarker and the outcome.[53] Furthermore, this implies that the gene in question could be a possible target for therapy using strategies such as PCSK9 siRNA, as illustrated by inclisiran.[43]

Cross-sectional data, such as that provided by UK biobank and other genetic epidemiological studies, are sufficient because the genetic traits are randomised at conception. Analysis permits anticipation of both the benefits and the detrimental consequences of manipulation of the particular gene product. This foreshadows the likely outcome of formal randomised controlled trials (RCTs). In retrospect, the RCT success of statins and ezetimibe could have been deduced from Mendelian randomisation, whilst the success of PCSK9 inhibitors was accurately predicted.[53, 54]

Any loss-of-function genetic variant identified as a possible therapeutic target can be considered as a candidate for ASO or siRNA therapy. The sequence of the wild-type gene is known, so specific RNA therapies can be designed to decrease translation and mimic the benefits of loss-of-function variants. Inclisiran is an example of a siRNA targeting PCSK9, with the anticipated benefits of decreased LDL-C, decreased CHD, and little in the way of side-effects.[43]

RNA therapies have commenced RCTs within a short-time frame of discovery of the target molecule.[52] This represents a major reduction in development time and associated costs. Efficiency is likely to improve as these techniques are refined. Accordingly, ASO and siRNA represent novel forms of treatment based on Mendelian randomisation with anticipation of treatment benefits and possible side effects resulting in accelerated development.[22, 71] They are distinct from the final class of RNA therapies, i.e. unmodified RNAs which are designed to elicit the synthesis of specific proteins, often for inoculation, such as the Pfizer and Moderna Covid vaccines.[34-36]

Strategies for delivery of RNA-based therapeutics

Manufacturing of RNA therapeutics is relatively simple, and involves either chemical synthesis (ASOs, siRNA, miRNA) or enzymatic transcription *in vitro* from the DNA template (mRNA).[21]

However, several major hurdles need to be overcome before RNA molecules can be used clinically.[29, 55] The major hurdles of delivering exogenous RNA include (1) the rapid degradation of exogenous RNA by endogenous RNAses; (2) the delivery of large and negatively-charged RNA across the hydrophobic cytoplasmic membrane; and (3) the strong immunogenicity of exogenous RNA.[29]

Various strategies have, and continue to be, developed to enable the safe and efficient delivery of RNA therapeutics, including chemically modifying RNA, employing synthetic carriers such as lipid nanoparticle or polymer-based nanoparticle systems, or by conjugating RNA with other cell/tissue-targeting moieties (e.g. GalNAc).[56] Circularising mRNA also reduces RNA fragility, and is being investigated as a method of avoiding degradation by RNA exonucleases.[57, 58]

Chemical modifications

RNA molecules are inherently unstable, due to the presence of the 2' hydroxyl (OH) group.[59] Chemical modifications of oligonucleotide molecules can greatly improve their stability and pharmacokinetic properties.[30, 31] Modifications have been introduced into the phosphate backbone, the ribose group, the RNA termini, or the nucleobases themselves.[30, 60] Examples of chemical modifications include phosphate backbone modifications in which the phosphodiester (PO) link is changed to phosphorothioate (PS).[31, 61]

PS linkages confer increased stability against nucleases and improve serum protein binding, thus facilitating tissue distribution.[61] Modifying the ribose on the 2'-OH position with methyl (2'-methoxy) or methoxyethyl (2'-MOE), or direct substitution with fluorine (2'-fluoro) also dramatically increases the stability of the oligonucleotide molecules.[30, 60] Connection of the 2′-O position with the 4′-C position of the ribosome with a methylene bridge (i.e. locked nucleic acids) is another means of stabilising oligonucleotides.[59]

Nanoparticles

An early approach to delivering RNA focused on lipid nanoparticles and synthetic nanoparticles. The advantages of nanoparticles are that they can mask the RNA charge, protect it from degradation by RNAses, and protect the RNA from renal clearance.[30] mRNA vaccines developed by Pfizer–BioNtech and Moderna for the treatment of Covid-19 use a lipid-based nanoparticle carrier system that prevents the rapid enzymatic degradation of mRNA and facilitates *in vivo* delivery. This lipid-based nanoparticle carrier system is further stabilised by a polyethylene glycol (PEG) lipid conjugate.[29, 62] Rare anaphylactic reactions to these RNA vaccines have been attributed to the PEG component of the liponanoparticle.[62]

Conjugation of RNA to targeting moieties

The attachment of a targeting moiety to RNA can aid in the targeting of a tissue/cell and in the internalisation of the RNA/conjugate into target cells.[30] Important characteristics of the targeting moiety are the presence of active groups to enable conjugation, good binding affinity, and reduced immunogenicity.[30]

Given the central role for the liver in lipid metabolism, GalNAc-conjugation presents a valuable opportunity for treating dyslipidaemia.[63] The conjugation of an siRNA or ASO molecule to GalNAc has successfully enabled the delivery of the conjugate to the liver.[64] GalNAc is a high-affinity ligand for the asialoglycoprotein receptors, which are predominantly expressed on liver hepatocytes.[64] Conjugation of an RNAi drug molecule to GalNAc therefore directs the conjugate to the hepatocytes where it can exert its gene-silencing activity.[64] This specificity reduces the potential for off-target adverse effects.[22]

Inclisiran is a double-stranded siRNA molecule, in which the sense strand is conjugated with GalNAc, which facilitates the uptake of inclisiran by hepatocytes (**Figure 2**, page 4).[65] Other GalNAc–siRNA conjugates that have reached clinical trials in indications other than CVD include givosiran which has been investigated in patients with acute hepatic porphyria[66] and fitusiran for treatment of haemophilia A and B.[67]

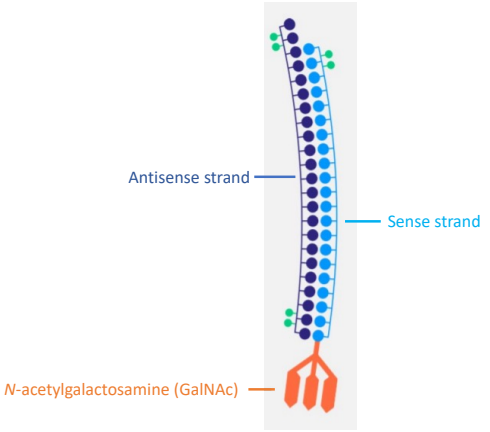

Figure 2. Image of inclisiran molecule

Comment from expert David Sullivan

ASO and siRNA therapies typically mimic the effect of favourable loss-of-function genetic variants identified by Mendelian randomisation. These therapies have the greatest effect when targeted towards the main sites of synthesis. An early example was the LDL-C lowering agent mipomersen, which targeted apo B-associated transport of cholesterol.[68] In addition to predictable side-effects such as fatty liver disturbance, its use was associated with persistent 'flu-like' symptoms and severe, recurrent injection site reactions. [68] Rare cases of severe thrombocytopaenia have been reported with the use of other ASOs.[69, 70]

These early problems have been largely overcome by improvements in the targeting of RNA therapy towards sites of synthesis such as the liver.[22] RNA therapies to reduce the hepatic synthesis of PCSK9 and lipoprotein (a) have successfully employed the inclusion of GalNAc component to mediate specific uptake by the asialoglycoprotein receptor in hepatocytes.[64] This has permitted substantial reductions in dosage, thereby drastically reducing or eliminating the previously mentioned side-effects.[22] Despite dose reduction, therapeutic half-life remains impressive resulting in a dosing interval of 6 months for inclisiran.[43]

Successful techniques are broadly applicable across the range of RNA therapies because the only point of difference is the nucleotide sequence. Lipoprotein metabolism is particularly amenable to down-regulation of hepatic synthesis of many enzymes, cofactors, and apolipoproteins. As a result, RNA therapies targeting lipoprotein (a) are under investigation for prevention of CHD (and possibly aortic stenosis),[71] whilst others targeting apolipoprotein C3 and ANGPTL3 are under assessment for triglyceride reduction for the prevention of acute pancreatitis and eventual CHD.[42, 72]

Focus on gene silencing in cardiovascular disease

Gene-silencing therapy targets investigated for the management of CVD include PCSK9, Lp(a), ApoB, and ApoCIII.[10, 22, 63] Other targets are also being explored in preclinical and clinical trials.

Targeting PCSK9

Patients with FH are characterised by having markedly elevated levels of LDL-C, which are associated with premature atherosclerosis and CVD.[73, 74] In 2003, studies demonstrated that a gene variation of PCSK9 (gain of function) resulted in inherited high levels of cholesterol, which was clinically manifested as FH.[75]

PCSK9 is involved in the control of the low-density lipoprotein receptor (LDLR).[26] Increased levels or functioning of PCSK9 reduces LDLR expression by promoting LDLR lysosomal catabolism and a subsequent increase in plasma LDL concentrations. The inhibition of PCSK9 activity lowers plasma LDL-C levels.[26]

The concept of inhibiting PCSK9 as a means of lowering lipid levels has been explored, with an siRNA, inclisiran, progressing through to clinical trials.[20, 43, 76, 77]

Inclisiran is a double-stranded siRNA molecule, the antisense strand of which is modelled to specifically correspond to human PCSK9 mRNA.[20, 43, 76, 77] The sense strand is conjugated with GalNAc, which facilitates the uptake of inclisiran by hepatocytes.[65] Other chemical modifications which have been made to the mRNA include the introduction of phosphorothioate into the phosphate backbone and modification of the ribose molecules (with 2′-deoxy, 2′-flu-oro, and 2′-O-methyl groups introduced).[78] Following uptake into hepatocytes, the antisense strand of inclisiran (which specifically corresponds to human PCSK9 mRNA) is integrated into the RISC, and directs the catalytic breakdown of PCSK9.[20, 76, 77] By halting the transcription of PCSK9, inclisiran increases the numbers of LDL receptors in the hepatocyte membranes, which increases LDL-C uptake and lowers LDL-C levels in the circulation (**Figure 3**).[20, 43, 76, 77]

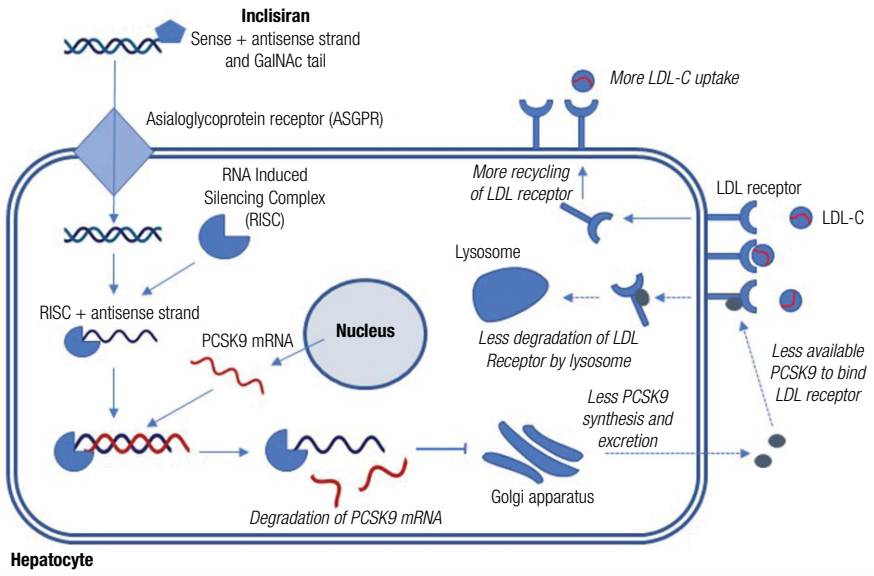

Figure 3. Mechanism of action of inclisiran in the hepatocyte[77]
Image from Cupido and Kastelein. Cardiovasc Res. 2020;116(11):e136-e9.

The efficacy and tolerability of inclisiran was assessed in the multicentre, multinational ORION programme.[43, 80, 81] Follow-up data from a phase 2 trial indicated that inclisiran resulted in durable reductions in LDL-C over a 1-year period in patients with elevated LDL-C despite maximally tolerated statin therapy.[79] Three phase 3 ORION studies further evaluated inclisiran in individuals with ASCVD (ORION-10[80]); ASCVD or ASCVD risk equivalents (type 2 diabetes mellitus, FH, or 10-year risk of 20% or greater of having a cardiovascular event assessed by Framingham Risk Score or equivalent; ORION-11[80]); or FH (ORION-9[81]).

In each of these studies, patients were randomised to subcutaneous injections of inclisiran sodium 300 mg (equivalent to 284 mg of inclisiran) or placebo and received treatment on day 1, day 90, and then every 6 months thereafter over a period of 540 days.[80, 81] The co-primary efficacy endpoints were the placebo-corrected percentage change in LDL-C from baseline to day 510 and the time-adjusted percentage change in LDL-C from baseline after day 90 and up to day 540.

ASCVD and ASCVD risk equivalents: ORION-10 and –11 trials focused on patients with ASCVD (ORION-10, ORION-11) or ASCVD risk equivalents (ORION-11) who had elevated LDL-C levels despite taking maximally tolerated statins.[80] More than 3,000 patients were enrolled (1,561 in ORION-10 and 1,617 in ORION-11).[80] At baseline, mean LDL-C levels were 2.71±0.99 mmol/L and 2.73±1.01 mmol/L, respectively. At day 510, the time-adjusted reductions (from baseline after day 90 and up to day 540) in LDL-C were 53.8% in the ORION-10 trial and 49.2% in the ORION-11 trial ($p<0.001$ vs placebo; **Figure 4A and B**, page 5).

Adverse events were generally similar in the inclisiran and placebo groups in both trials.[80] Injection-site reactions were more common with inclisiran than placebo (2.6% vs 0.9% in the ORION-10 trial and 4.7% vs 0.5% in the ORION-11 trial).[80] These reactions were generally mild and none were serious or persistent.[80]

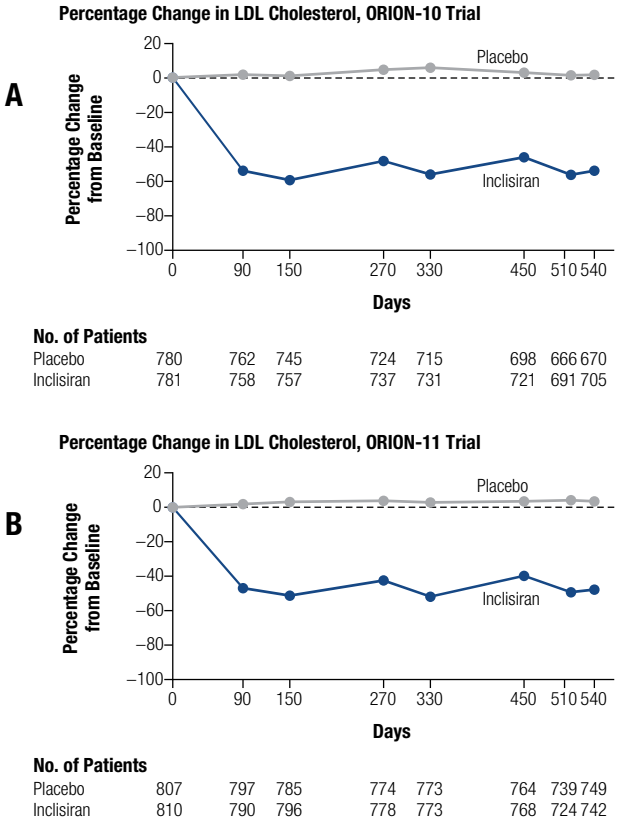

Figure 4. Efficacy of inclisiran versus placebo in lowering LDL cholesterol over the 540-day trial period (intention-to-treat population) in the A) ORION-10 trial and the B) ORION-11 trial[80]
Adapted from Ray et al. N Engl J Med. 2020;382(16):1507-19.

Familial hypercholesterolaemia: The ORION-9 trial assessed the use of inclisiran in 482 adult patients with heterozygous FH who had been treated with a maximally tolerated dose of statin therapy.[81] At baseline, the mean LDL-C was 4.0 mmol/L (153 mg/dL).[81]

Inclisiran reduced LDL-C levels to a greater extent than placebo across all FH genotypes.[81] In the intention-to-treat population:

- At day 510, the mean percent change in the LDL-C level (co-primary endpoint) was a reduction of 39.7% in the inclisiran group versus an increase of 8.2% in the placebo group; the between-group difference was−47.9 percentage points (95% CI −53.5, −42.3; p<0.001).[81]
- The time-averaged percent change in LDL-C between day 90 and day 540 (co-primary endpoint) was a decrease of 38.1% in the inclisiran group and an increase of 6.2% in the placebo group; the between-group difference was−44.3 percentage points (95% CI −48.5, −40.1; p<0.001).[81]

The incidence of adverse events and serious adverse events was similar between the two groups, and most (94.6% in the inclisiran group and 91.9% in the placebo group) were reported as mild to moderate.[81]

Targeting lipoprotein (a)/apolipoprotein (a)

Epidemiological and genetic studies indicate that Lp(a) is an independent risk factor for cardiovascular diseases.[8,9] To date, a pharmacological approach that effectively lowers Lp(a) levels in patients with progressive ASCVD and high plasma Lp(a) has been missing.[10] The Lp(a) particle is an ApoB-containing lipoprotein similar to LDL, but with apolipoprotein(a) covalently bound to the apoB moiety on the surface of the particle.[71,82,83] Apolipoprotein (a) is encoded by the *LPA* gene.[71] Thus, inhibiting apolipoprotein(a) production in the hepatocytes with RNA therapeutics has the potential to reduce plasma Lp(a) levels.

Pelacarsen (AKCEA-APO(a)-L_{Rx}; TQJ230) is a $GalNAc_3$-conjugated 2′-methoxyethyl chimeric second-generation ASO drug which is targeted at mRNA transcribed from the *LPA* gene.[84] A randomised, double-blind, placebo-controlled, dose-ranging phase 2 trial involving 286 patients with established CVD and screening Lp (a) levels ≥150 nmol/L (≥60 mg/dL) investigated the effect of pelacarsen on Lp(a) levels.[84] Pelacarsen reduced mean Lp(a) levels in a dose-dependent manner (35%-80%) compared with placebo (6%; p=0.003 to <0.001).[84] There were no significant differences between the pelacarsen groups and placebo with regards to effects on platelets, or renal or liver function, or in the risk of influenza-like symptoms.[84] The most common adverse events were injection site reactions, which were generally mild.[84]

The phase 3 Lp(a) HORIZON trial is currently being conducted and plans to enrol approximately 7,680 patients with established ASCVD with Lp(a) levels >70 mg/dL. Patients will be randomised to pelacarsen 80 mg once every 4 weeks or to placebo, both administered as subcutaneous injections.[85] The primary outcome measures are the time to the first occurrence of expanded major adverse cardiovascular events (cardiovascular death, non-fatal MI, non-fatal stroke and urgent coronary re-vascularization requiring hospitalisation) in patients with Lp(a) >70 mg/dL or >90 mg/dL.[85]

AMG 890, which is an siRNA, was designed to reduce the production of Lp(a) by targeting mRNA transcribed from the *LPA* gene. Phase 1 and 2 clinical trials have indicated its potential for use in patients with elevated Lp(a).[86]

Targeting apolipoprotein CIII

ApoCIII is a multifunctional protein which exerts various atherogenic effects, either by intervening in the function and catabolism of many lipoproteins, or by inducing endothelial inflammation and smooth muscle cell proliferation.[87] The levels of triglycerides can be lowered using an ASO directed against ApoCIII mRNA, and this approach resulted in the development of volanesorsen to treat familial chylomicronemia syndrome (FCS), hypertriglyceridemia and familial partial lipodystrophy (FPL).[37] Volanesorsen has been approved in the EU for the treatment of adult patients with FCS based on positive results from the multinational, phase III APPROACH and COMPASS studies.[37]

Targeting angiopoietin-like 3 protein

Angiopoietin-like 3 protein has genetic validation as a novel target for cardiovascular disease.[88,89] ARO-ANG3 is an RNA interference therapeutic currently in clinical trials in patients with mixed dyslipidaemia.[90]

Comment from expert John Amerena

The advent of RNA therapies to target proteins at an intracellular level rather than block or enhance their actions peripherally is an extremely promising area of research that is now making its way into the clinical domain. The monoclonal antibodies evolocumab[28] and alirocumab[27] which inhibit PCSK9 and lower LDL markedly, have a well-characterised safety profile, as well as being effective, but there were unexpected off-target effects of lipid modulation with the CETP inhibitor torcetrapib which resulted in increased CV death.[91] There are several ways to target RNA (see above), with different techniques shown to be effective in reducing the production of specific proteins at an intracellular level e.g. inclisiran reducing PCSK9 production by hepatic cells. There has been concern that there could be off-target effects of interfering with RNA-mediated synthesis of proteins, with bystander inhibition of mRNA in other cells/organs resulting in adverse effects. This would not appear to be the case with the siRNA incliseran which specifically targets hepatic cells to reduce PCSK9 by reducing transcription of mRNA for PCSK9, and to date, there have been no safety signals or off-target effects documented in the ongoing phase 3 clinical trials. The PCSK9 inhibitors are extremely effective in reducing LDL and are well-tolerated, but may be superseded by inclisiran which has approximately the same efficacy but with less frequent injections (biannually).[43,44] Other research is ongoing with siRNAs to reduce Lp(a) which is postulated to be a residual risk factor in patients with LDL at target who have recurrent CV events. It is yet to be proven that reduction of Lp(a) improves outcomes.[92,93] but RNA-based strategies are being tested in this area and we await the outcomes of the clinical trial with these new agents.

Take-home messages

- RNA therapeutics represent an emerging approach in CVD management.
- Different types of RNA therapeutics have reached clinical trials including ASOs, and siRNA that result in the “silencing” of endogenous mRNA, as well as mRNA therapeutics, in which exogenous mRNA is delivered to cells to effect the translation of a protein or peptide.

- Various strategies have enabled the delivery of RNA therapeutics, including chemically modifying RNA, employing synthetic carriers such as lipid nanoparticle or polymer-based nanoparticle systems, or by conjugating RNA with other cell/tissue-targeting moieties (e.g. GalNAc).
- Specific targeting of PCSK9, ApoCIII, and Lp(a) by RNA therapeutics for the management of CVD has been explored in clinical trials.
- Inclisiran is a double-stranded siRNA molecule, the antisense strand of which is modelled to specifically correspond to human PCSK9 mRNA. By halting the transcription of PCSK9, inclisiran increases the numbers of LDL receptors in the hepatocyte membranes, which increases LDL-C uptake and lowers LDL-C levels in the circulation (as demonstrated in the ORION clinical trial programme).

Concluding comments from expert John Amerena

Interfering with RNA-mediated production of proteins is an exciting new area of drug development that will have major implications for disease management in the future. These therapies are most advanced in management of hyperlipidaemia, and we will soon have the ability to modify many components of the lipid profile including LDL-C, Lp(a), and triglycerides with new agents, which if shown to be safe as well as effective, will change the landscape of treating hyperlipidaemia in the future.

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