ELSEVIER

Teaser This review describes recent advances in the development of nucleic acid-based therapeutic agents for the treatment of dyslipidemia and atherosclerotic cardiovascular disease.

Harnessing nucleic acid-based therapeutics for atherosclerotic cardiovascular disease: state of the art

Fatemeh Vahdat Lasemi[1,‡], Maryam Mahjoubin Tehran[1,‡], Seyed Hamid Aghaee-Bakhtiari[1,2], Amin Jalili[1], Mahmoud Reza Jaafari[3,4] and Amirhossein Sahebkar[4,5,6]

[1] Department of Medical Biotechnology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[2] Bioinformatics Research Group, Mashhad University of Medical Sciences, Mashhad, Iran
[3] Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[4] Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[5] Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[6] School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

Dyslipidemia is one of the major but modifiable risk factors for atherosclerotic cardiovascular disease (ACVD). Despite the accessibility of statins and other lipid-lowering drugs, the burden of ACVD is still high globally, highlighting the need for new therapeutic approaches. Nucleic acid-based technologies, including antisense oligonucleotides (ASOs), small interfering (si)RNAs, miRNAs, and decoys, are emerging therapeutic modalities for the treatment of ACVD. These technologies aim to degrade gene mRNA transcripts to decrease the levels of atherogenic lipoproteins. Using gene-silencing approaches, the levels of atherogenic lipoproteins can be decreased by targeting proteins that have key roles in lipoprotein metabolism. Here, we highlight preclinical and clinical findings using these approaches for the development of novel therapies against ACVD.

Fatemeh Vahdat Lasemi is a PhD candidate in medical biotechnology in the Department of Medical Biotechnology, Mashhad University of Medical Sciences, Mashhad, Iran. Her research focuses on the nanoparticulate delivery of small interfering RNAs for the management of dyslipidemia.

Maryam Mahjoubin-Tehran is a PhD candidate in medical biotechnology in the Department of Medical Biotechnology, Mashhad University of Medical Sciences. Her research focuses on the design of miRNAs for modulating lipid metabolism pathways in atherosclerosis.

Cardiovascular diseases

Noncommunicable diseases (NCDs) are the main cause of mortality globally. According to a 2014 WHO report, NCD causes 38 million deaths of the total 56 million deaths in the world (68%), and 46% of NCD mortality results from CVD. In 2012, 17.5 million people died as a result of CVD, approximately one-third of global deaths [1,2] and it is predicted that, by 2030, 25 million deaths will result from CVD [3]. Thus, the associated costs of CVD around the world, for both society and individuals are considerable [4].

Corresponding author: Sahebkar, A. (sahebkara@mums.ac.ir), (amir_saheb2000@yahoo.com)
‡ Equally contributions as first authors.

https://doi.org/10.1016/j.drudis.2019.04.007

Atherosclerosis is a multifactorial disease that develops as a result of the interplay among several modifiable and non-modifiable risk factors. One of the main modifiable risk factors is dyslipidemia, which arises as a consequence of disturbed cholesterol and lipoprotein metabolism [5]. Proatherogenic lipoproteins, such as low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL) and its remnants, and lipoprotein (a) [Lp(a)], cause the formation of foam cells and ensuing atherosclerotic plaques, whereas high-density lipoprotein (HDL) is an antiatherogenic lipoprotein that can reverse cholesterol transport and exerts anti-inflammatory and antioxidative effects [6]. Increased proatherogenic and decreased antiatherogenic lipoproteins are key features of dyslipidemia, and can eventually lead to atherosclerosis. This has led to the development of drugs for the treatment of atherosclerosis [7,8].

Challenges in traditional therapies for atherogenic dyslipidemia

Numerous studies have aimed at developing drugs for CVD and atherosclerosis. Increased levels of LDL in plasma is an important atherosclerotic risk factor. Therefore, many of the conventional therapeutic agents, such as statins, reduce LDL-cholesterol (LDL-C) levels [9]. Statins are one of the most recommended drugs in patients with CVD. Beyond their well-known LDL-lowering activity, statins also have lipid-independent pleiotropic effects, such as antioxidant, anti-inflammatory, and immunomodulatory activities, which are relevant for the reduction of atherosclerosis risk [10–19,134]. Nevertheless, not all patients can use statins, mainly because of adverse effects. Statins have been reported to elevate liver enzymes, such as transaminase, in some patients [20]. In addition, statins are associated with myopathy disorders, including myalgia, myositis, and, rarely, rhabdomyolysis [21]. Interactions of statins with other drugs is another important concern especially in older patients, who often have other metabolic disorders, including diabetes and hypertension, that require other drugs to be taken alongside statins [22].

Statin therapy can effectively reduce plasma LDL-C levels; however, a considerable 'residual CVD risk' remains after treatment [7]. Even with high doses of statin, some patients do not achieve target LDL-C goals and some are unable to tolerate statins [23]. Under other conditions, in the patients whose LDL-C is controlled, atherogenic dyslipidemia characterized by elevated plasma triglycerides is observed [24].

Lp(a) is another atherogenic lipoprotein that could serve as a promising target for lowering the residual risk of CVD [25]. Despite the similarity of Lp(a) and LDL, Lp(a) cannot be lowered by statins [26]. Elevated Lp(a) can contribute to the residual CVD risk that remains despite intensive statin therapy [23].

Conventional CVD therapies, such as statins, are often not effective in patients with conditions such as familial hypercholesterolemia (FH) [27]. Taken together, conventional therapies for CVDs and atherosclerosis appear to be insufficient, highlighting the need for novel therapeutic approaches with higher efficacy and tolerability. In this context, nucleic acid-based agents have emerged as promising therapeutic options.

Most of the nucleic acid-based drugs and drug candidates that have been developed for the treatment of atherosclerotic CVD target molecules and proteins that regulate lipoprotein metabolism. Although the current trend in atherosclerosis drug discovery focuses on lipids and lipoproteins, lipid-independent pathways (i.e., vascular inflammation) have recently emerged as a causal risk factor for atherosclerosis [28]. Therefore, targeting other nonlipid pathways by nucleic acid-based agents could be a promising avenue for future research.

Nucleic acid-derived therapeutic approaches

Even though protein-based therapeutics (peptides, recombinant proteins, and antibodies) comprise over a quarter of drugs approved by the US Food and Drug Administration (FDA) since 2015 (http://FDA.gov), they have a relatively short shelf life, require cold chains for transport, and rely mainly on living organisms for recombinant production. These problems lead to high production costs, and risks of contamination and variations across batches [29]. Thus, recent research has focused on the development of nucleic acid-based drugs (Table 1), offering the key benefit of chemical manufacturing (Fig. 1). As such, they retain a relatively long half-life, are easily transportable, and are stable from batch to batch [30].

Currently, important therapeutics for atherosclerotic CVD aim to repress targets at the protein level. Antibody-based lipid-lowering drugs, such as PCSK9 inhibitors, block proteins selectively found in plasma in microgram to milligram quantities [31], whereas nucleic acid-based drugs inhibit gene expression in the cell nucleus or cytoplasm to decrease protein production, resulting in decreased plasma levels of the respective target [32]. In addition, antibodies are found in plasma and other extracellular fluids, whereas nucleic acid-based drugs are formulated with the purpose of accumulation in liver cells. Compared with monoclonal antibodies, which decrease the levels of target proteins only in plasma, nucleic acid-based therapies can target proteins both in plasma and inside cells; nevertheless, the clinical importance of such a difference is unknown. Other advantages of nucleic acid-based therapies include the low frequency of drug injection (2–12 times yearly compared with weekly to twice-monthly injections for antibody-based therapies) and low interindividual differences in their lipid-lowering effects. In addition, compared with antibody-based therapies, RNAbased genesilencing therapies target the gene involved in a given disease with more specificity and less risk of off-target effects, leading to better patient compliance [33]. ASOs, RNA interference agents (siRNAs and miRNAs) and decoys are the most important RNAbased genesilencing methods (Figs 2 and 3) for atherosclerotic CVD treatment. Table 2 provides a summary of clinical trial results for nucleic acid-based drugs used for the treatment of atherosclerotic CVD.

Antisense oligonucleotides

ASOs are short, single strands of DNA or RNA [generally 20 nucleotides (nt) in length] that directly bind to complementary sequences of target mRNA. This strong and specific binding occurs via Watson–Crick hybridization [34]. Upon binding, they inhibit gene and protein expression via different mechanisms, including RNase H activation, which leads to degradation of mRNA strand at the DNA–RNA duplex, translation arrest by steric hindrance of ribosomal binding, pre-mRNA destabilization, inhibition of the splicing of pre-mRNA, and intervening with mRNA maturation [35,36].

Generally, these unmodified ASOs are rapidly degraded by biological fluid nucleases. Therefore, first-generation ASOs were devel-

TABLE 1

Development stages of nucleic acid-based drugs for cardiovascular diseases

Target	Drug candidates	Nucleic acid approach	Development stage	Disease
PCSK9	Inclisiran/ ALN-PCSsc/ ALN-60212/ PCSK9si	siRNA	Phase II completed	Hypercholesterolemia
	ALN-PCS/ALN-PCS01/ ALN-PCS02	siRNA	Phase I	Hypercholesterolemia
	SPC 5001	ASO	Phase I terminated	Hypercholesterolemia
	SPC5011	ASO	Phase I terminated	Hypercholesterolemia
	BMS-844421/ BMS- PCSK9Rx-2;	ASO	Phase I terminated	Hypercholesterolemia
	ISIS 394814/Isis141923	ASO	Preclinical	Hyperlipidemia
	SPC4061	ASO	Preclinical	Hypercholesterolemia
	PCS-A2/B2/C2	siRNA	Preclinical	Hypercholesterolemia
ApoA	ISIS-APOARx/ISIS 494372/IONIS-ApoA-RX	ASO	Phase II	Atherosclerosis/high Lp(a)
	ISIS 144367	ASO	Preclinical	Atherosclerosis/high Lp(a)
	ISIS681257 (GalNAc3)-conjugated (ASOs)	ASO	Preclinical	Atherosclerosis/high Lp(a)
ApoB	Mipomersen/ISIS 301012/ISIS/Genzyme/Kynamro	ASO	Approved	Heterozygous FH; atherosclerosis
	ISIS 147764	ASO	Preclinical	Hypercholesterolemia
	ISIS 147483	ASO	Preclinical	Hypercholesterolemia
ApoC-III	ISIS-APOCIIIRx/volanesorsen/ISIS 304801/ IONIS-ApoC IIIRX	ASO	Phase III	Hypertriglyceridemia; familial chylomicronemia
	A301SL	ASO	Preclinical	Hyperlipidemia
	A301S	ASO	Preclinical	Hyperlipidemia
	ALN-AC3	RNAi	Preclinical	Hypertriglyceridemia primary lipodystrophies
ANGPTL3	ISIS ANGPTL3Rx/IONIS- ANGPTL3-LRX	ASO	Phase I	Familial hyperlipidemia; severe hypertriglyceridemia
	ALN-ANG	RNAi	Preclinical	Mixed hyperlipidemia; hypertriglyceridemia
MTP	ISIS 144477	ASO	Preclinical	Hyperlipidemia
DGAT-2	ISIS-DGAT2Rx/ISIS 484137 IONIS-DGAT2Rx /ISIS-217376	ASO	Preclinical	Hypercholesterolemia
miR-33a/b	Anti-miR-33	ASO	Preclinical	Atherosclerosis
E2F-1	Edifoligide/E2F-1 decoy	Decoy ON	Phase III	Atherosclerosis

oped that contain chemical modifications, such as phosphothioate and methylphosphonate backbones [37]. Nevertheless, these modifications can decrease in the affinity of the ASOs for the mRNA target [38]. To enhance the affinity of binding to the target mRNA, second-generation ASOs were developed with modifications of the 2′-alkyl of ribose, such as 2′-*O*-methyl and 2′-*O*-methoxyethyl (MOE), or with an additional bridge that connects the 4′-carbon and 2′-hydroxyl of the ribose to form a locked nucleic acid (LNA). Unexpectedly, these substitutions can lead to loss of the ability to activate RNase H. To solve this problem, gapmer technology, involving chimeric ASOs, was developed in which the central region contains first-generation of ASOs, whereas flanking regions contain the LNA or 2′-MOE modifications [35,39].

RNA interference agents

RNAi is a gene-silencing process at the post-transcriptional level [40] and a natural form of gene regulation that protects the genome against transposable elements and virus replication [41].

siRNAs

Compared with ASOs, siRNAs are double-stranded RNAs with typically 21–23 nt in length and 2 nt 3′-overhangs that are processed by the RNase III enzyme Dicer from longer dsRNAs [36,42]. Subsequently, the RNAs are introduced into the AGO2–RISC enzyme complex [43]. Within the complex, the sense or passenger strand is degraded and the other strand, known as the antisense or guide, binds to complementary or partially complementary sequences of target mRNAs, preventing mRNA translation by its cleavage [44,45].

miRNAs

Similarly, miRNAs are double-strand, short (~18–25 nt), endogenous, noncoding RNAs [46]. RNA polymerase II (Pol II) or rarely Pol III generally transcribes miRNAs in the cell nucleus as primary miRNAs (pri-miRNAs) with a stem-loop structure [47,48]. These long molecules are then processed into 70–100 nt pre-mature miRNAs (pre-miRNAs) by DROSHA/DGCR8 [49,50]. Subsequently, pre-miRNAs are transported to the cytoplasm by exportin 5 [51] and cleaved by Dicer ribonuclease to form a 22-bp mature miRNA [52]. The mature miRNAs are introduced into the RISC complex that binds the 3′-untranslated region (UTR) of target mRNAs in a complementary fashion, thereby preventing translation or promoting degradation of cognate mRNAs [53].

Decoys

Finally, decoys are small, dsDNA molecules matching with the consensus DNA-recognition motif of a target transcription factor in the genome or anchor an identified transcription factor-binding sequence in the promoter of a single gene. The first characteristic means that the sequence is conserved across species and distinguished by the alignment of many identified binding sites in the promoter of many target genes among various species. Thus, decoys can be successfully tested in preclinical studies (i.e., animal models), and then used in clinical trials to assess the efficacy and safety of neutralizing transcription factors that are associated with disease-related genes among species [54].

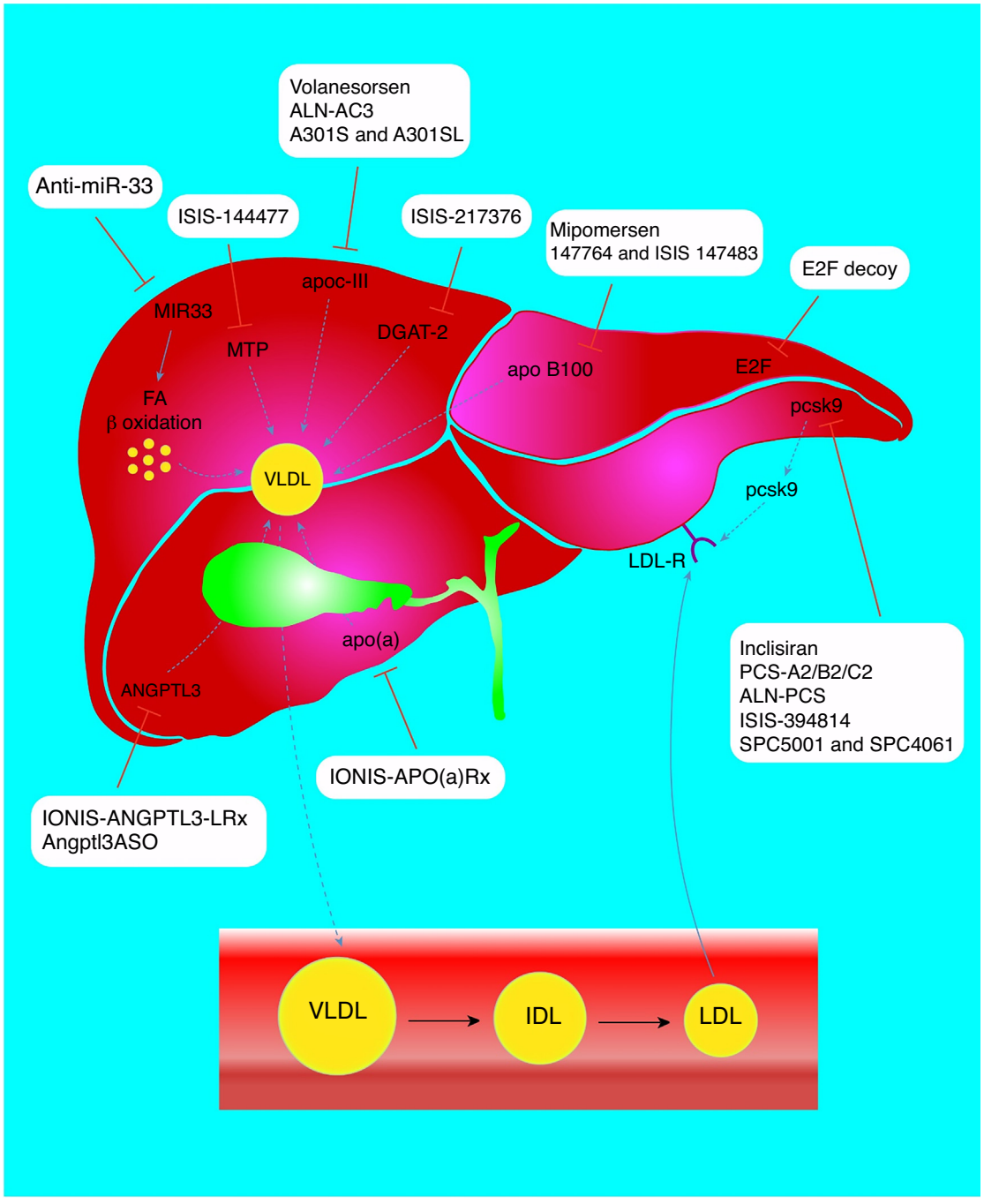

FIGURE 1

Overview of gene silencing approaches for dyslipidemia in the liver. The expression of key genes involved in lipid metabolism could be silenced by several novel drugs that are under development for the treatment of dyslipidemia. Emerging therapeutic agents for lipid lowering will: (i) intervene with lipoprotein synthesis in the liver by silencing the expression of (1) apolipoprotein B (ApoB)100, (2) apo(a), or (3) microsomal triglyceride transfer protein (MTP), by blocking (4) miR33, increasing fatty acid β oxidation and decreasing ApoB lipidation or prohibiting (5) diacylglycerol acyltransferase 1 (DGAT-1); (ii) promote very-low-density-lipoprotein (VLDL) receptor activity by silencing (6) apoC-III; (iii) promote LDL-receptor activity by silencing (7) proprotein convertase subtilisin/kexin type 9 (PCSK9); (iv) increase lipoprotein lipase levels and activity of endothelial lipase and decrease free fatty acids levels in the serum by silencing (8) angiopoietin-like protein 3 (ANGPTL3); (v) prohibit proliferating cell nuclear antigen expression and smooth muscle cell proliferation by trapping (9) E2F.

Delivery of nucleic acid-based drugs

Efficient delivery is a key challenge for the successful development of nucleic acid-based drugs. A successful delivery system should be resistant to enzymatic degradation and allow high cellular uptake into target cells, efficient endosomal escape, and entry to the nucleus (for plasmid-based therapeutics) [55]. Naked RNAs (in the native form) are unstable and have a short half live in vivo. They are often cleared from the body via ribonucleases, renal excretion, and the reticuloendothelial system (RES) [56]. Chemical modifications can enhance the stability of RNAs. Three main

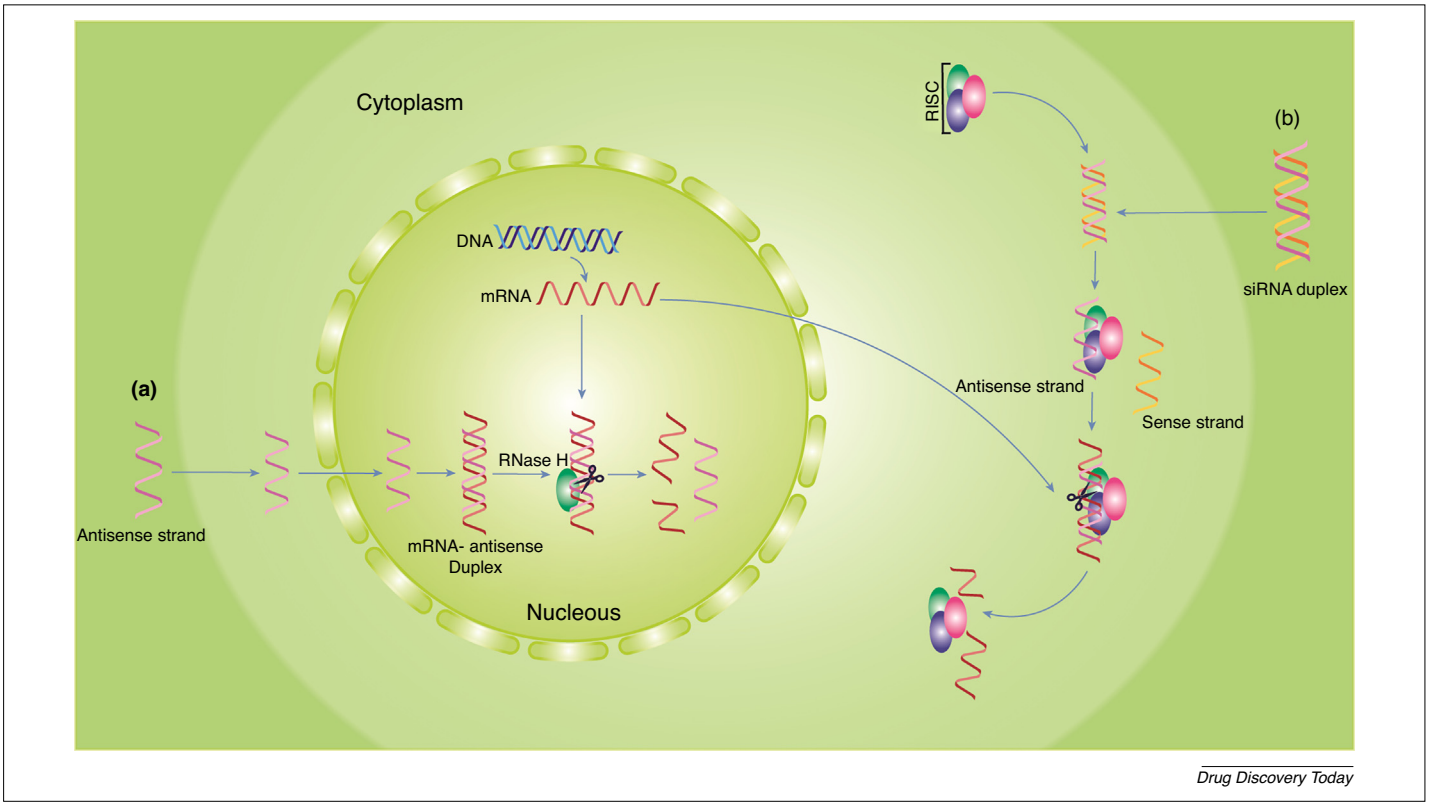

FIGURE 2

Schematic illustration of antisense oligonucleotide-based versus small interfering (si)RNA-based approaches. (a) Antisense oligonucleotide technology utilizes a single-stranded DNA or RNA that specifically hybridizes with their target mRNA and activates RNase H, which consequently degrades the target mRNA; (B) siRNA technology uses a double-stranded RNA incorporated into an RN A-I nduced silencing complex (RISC), leading to cleavage of target mRNA.

categories of chemical modifications are internucleotide linkage (i.e., phosphorothioate), sugar modifications (i.e., 2′-*O*-methyl and 2′-MOE) and nucleobase modifications (6- and 7-positions of purines and the 4- and 5-positions of pyrimidines) [57,58]. A combination of 2′-fluoro nucleotide, phosphorothioate, and 2′-*O*-methyl nucleotide modifications enhance the resistance of siRNAs (e.g., inclisiran) to degradation by nucleotide-modifying enzymes [59]. Furthermore, the conjugation of nucleic acid-based drugs to a receptor ligand can improve their entry into target cells [60]. Considering inclisiran as an example, conjugation to *N*-acetylgalactosamine (GalNAc) moieties facilitates selective uptake into liver hepatocytes by the asialoglycoprotein receptor (ASGPR) [59]. Phosphorothioate nucleotides are another modification that facilitates RNase H-mediated cleavage in drugs such as mipomersen and edifoligide. Mipomersen also contains 2′MOE modifications, which increase target binding affinity, half-life, and resistance to nucleases [61]. Nucleic acid-based drugs in clinical stages of development including inclisiran, mipomersen, ISIS ANGPTL3Rx, and volanesorsen, are administered subcutaneously.

Targets

Apolipoprotein B-100

Lipoproteins comprise a central core of cholesterol esters surrounded by free cholesterol, phospholipids, and apolipoproteins [62]. Lp(a) comprises apolipoprotein(a) bound to ApoB of LDL [63]. Apolipoprotein B-100 (ApoB-100) is the important apolipoprotein of atherogenic lipoproteins [VLDL, intermediate-density lipoprotein (IDL), LDL, and Lp(a)] [64]. ApoB is constitutively express in the liver and is required for the secretion of VLDL from the liver [63,65].

Mipomersen is a 20 nt-long second-generation antisense oligonucleotide designed to inhibit ApoB-100 production in the liver [66]. Mipomersen binds to ApoB-100 mRNA, resulting in inhibition and degradation of the mRNA [66], therefore decreasing the hepatic synthesis of ApoB-100 and reducing the amount of atherogenic lipoproteins that contain ApoB-100 [64].

A meta-analysis of eight randomized controlled trials revealed that mipomersen reduced LDL-C (32.37%), total cholesterol (TC) (24.18%), VLDL-C (21.59%), non-HDL-C (30.83%), and triglycerides (TG; 36.26%) but not HDL-C. ApoB, apolipoprotein A1 (ApoA1) and Lp(a)were also reduced in treatment groups. In a safety analysis, mipomersen increased flu-like symptoms, injection-site reactions (ISRs), alanine aminotransferase (ALT) level, and hepatic steatosis [67].

A dose of 200 mg/week mipomersen for 13 weeks in patients with FH reduced ApoB (19.9%) and LDL-C (22%) levels. There were no increases in ALT or other measures of liver function (prothrombin, bilirubin, or albumin). Approximately 10% of patients in the treatment group developed hepatic steatosis at week 15, but this was reversible following mipomersen termination [68].

In another study, patients with heterozygous FH received mipomersen subcutaneously. The mean reduction in ApoB to week 7 was 23% in the 200 mg/week dose group and 33% in the 300 mg/week dose group, with reductions in LDL-C of 21% and 34%, respectively. In addition, 13 weeks of 300 mg/week mipomersen injection reduced LDL-C (37%), VLDL-C (22%), non-HDL-C(35%),

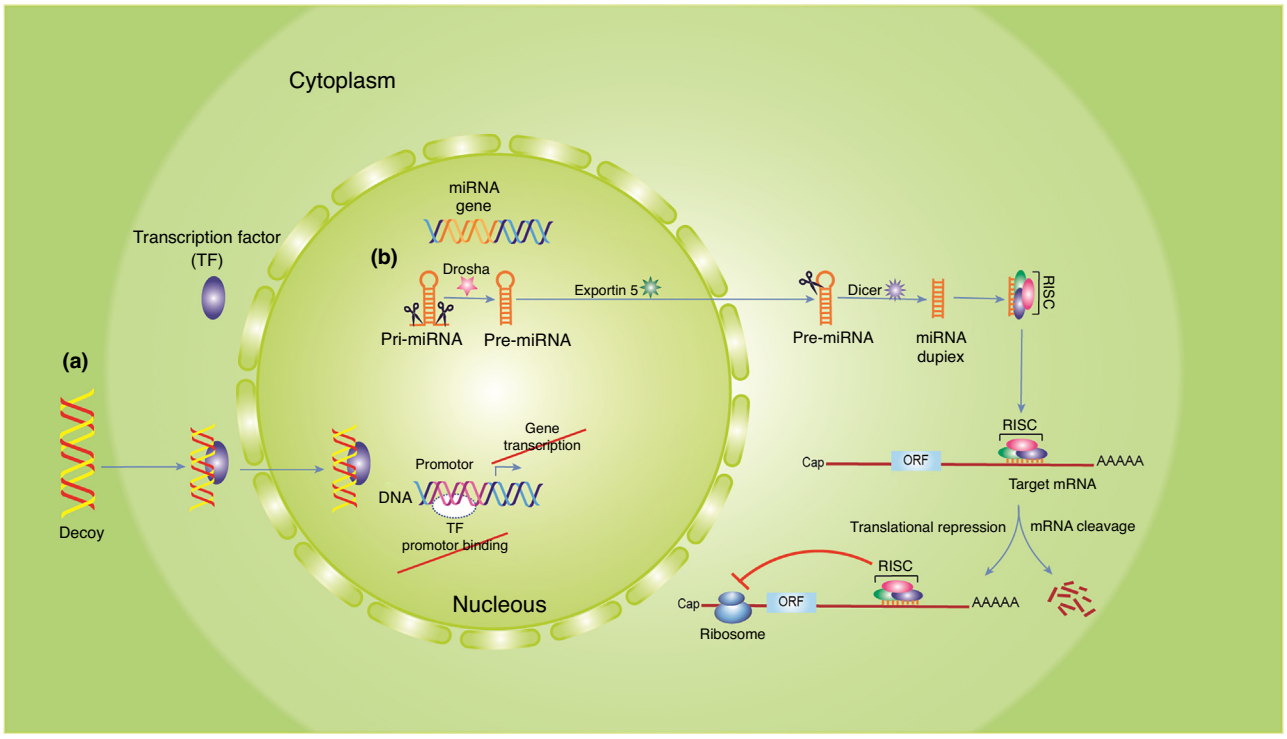

FIGURE 3

Schematic illustration of decoy-based versus miRNA-based approaches. (a) Decoy technology uses double-stranded DNA that blocks the binding of transcription factors (TFs) to promoter regions of target genes, prohibiting gene expression; (b) miRNA technology utilizes a double-stranded RNA, in which one strand of the mature duplex binds to the target 3′-untranslated (UTR) region and results in mRNA degradation or translation inhibition.

TC (29%), TGs (38%), ApoB (37%), and Lp(a) (29%), but increased HDL-C (4%) [69].

In a Phase III study, 200 mg/week mipomersen was administrated subcutaneously for 26 weeks to patients with homozygous FH. Treatment lowered LDL-C concentration (25%), ApoB (27%), TC (21%), non-HDL-C (25%), Lp(a) (31%), TGs (17%), and VLDL-C (17%), but increased HDL-C (15%). If such effects of mipomersen continued, they could decrease the risk of atherosclerotic CVD and improve survival. Mipomersen had no adverse effects on renal function (serum creatinine or estimated glomerular filtration rate), muscle (myalgia or creatine kinase elevation), platelet count, glucose homoeostasis, or blood pressure. No patients developed antibodies to mipomersen. The most common adverse events were ISRs (76% in the mipomersen group versus 24% in the placebo group). Mipomersen also increased ALT by 12% [70].

Administration of 200 mg/week mipomersen for 26 weeks caused a reduction in TGs (25%), LDL-C (37%), non-HDL-C (36%), VLDL-C (25%), Lp(a) (25%), and ApoB (37%), but increased HDL-C (2%). No increases in ALT levels or liver fat content was observed. Liver biopsies showed slight inflammation and little to no fibrosis [65].

In an open-label extension trial, 200 mg/week mipomersen was administrated subcutaneously to patients with FH for up to 104 weeks. Mipomersen therapy reduced LDL-C (28%), ApoB (31%), TGs (12%), TCs (20%), and non-HDL-C (27%), but increased HDL-C (10%). ALT and liver fat increased initially, but trended towards baseline in follow-up. There were no changes in hematology, other biochemistry parameters, or renal function, as shown by serum creatinine levels [71].

ISIS 147764 and ISIS 147483 are ASOs targeting various regions of the coding region in the gene encoding ApoB-100. A concentration of 150 nM of both ISIS 147764 and ISIS 147483 decreased ApoB-100 mRNA (90%) with no effects on cell viability. Administration of 50 mg/kg ISIS 147764 to high-fat-diet (HFD)-fed C57BL/6 mice intraperitoneally twice weekly for 6 weeks reduced liver ApoB-100 mRNA (87%), intestinal ApoB-100 mRNA (54%), TC (55%), LDL-C (88%), and TGs (25%), but not chylomicrons. The number of ApoB-100-containing lipoproteins was suppressed. There were no changes in aspartate transaminase (AST), ALT, or other metabolic parameters [72]. The removal half-life for ISIS 147764 in liver was 20 days. The EC_{50} in treatment HFD-fed mice was 101 mg ISIS 147764/g of liver. Eight weeks after stopping administration, levels of ApoB mRNA returned to pretreatment levels [73].

In another study, ISIS 147764 (25–100 mg/kg/week for 10–12 weeks) was administrated to hypercholesterolemic LDL receptor-deficient ($LDLR^{-/-}$) mice, and resulted in dose-dependent decreases in plasma LDL (60–90%), hepatic ApoB mRNA (60–90%) and sinus atherosclerosis (50–90%) [74].

PCSK9

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease, mainly synthesized in the liver and small intestine, with a key role in the regulation of plasma levels of LDL-C. PCSK9.

TABLE 2

Effects of nucleic acid-based drugs on plasma lipids and lipoproteins in clinical trials

Drug name; company	Phase	NCT number	Population	Time	Dosage mg (no. of doses)	Plasma lipids/lipoproteins change from base line (%)								Refs
						TC	TG	HDL-C	LDL-C	VLDL	Non-HDL-C	Lp(a)	ApoA1/A	
Mipomersen ISIS/ Pharmaceuticals	II	NCT 00231569	N = 62; hypercholesterolemia	5 weeks	30–400 (7)	↓ 2.7, – 38.5	↓ 3.5, –40.5	↑ 2.9, –2.8	↓ 2.3, –51.8	↓ 3, –45.8	↓ 3.2, –52	N	N	[1]
	III	NCT 00231569	*N* = 11; hypercholesterolemia	13 weeks	200 (15)	↓ –22%	15%	↓ –1.1	↓ –36%	↓ –11%	↓ –28.5	N	N	[128]
	II	NCT 00362180	*N* = 21; FC	13 weeks	200 (13)	↓ –16.4	↓ –16.3	↑ +4	↓ –22%	↓ –13.2	↓ –21.3	↓ –19.5	↓ –1.3	[68]
	II	NCT 00281008	*N* = 44; FC	13 weeks	300 (15)	↓ –29	↓ –38	↑ +4	↓ –37	↓ –22	↓ –35	↓ –29	↑ +2	[69]
	III	NCT 00607373	*N* = 51; homozygous FC	26 weeks	200 (26)	↓ –21.2	↓ –17.4	↑ + 15.1	↓ –24.7	↓ –17.4	↓ –24.5	↓ –31.1	↑ +9.3	[70]
	II	NCT 00216463	*N* = 50; good health with mild-to-moderate hyperlipidemia	13 weeks	50–400 (13)	N	↓ –7,–53	N	↓ –7, –71	↓ 4, –61	↓ –12, –66	↓ –42, –49	N	[129]
	III	NCT 00706849	*N* = 124; heterozygous FH and coronary artery disease	26 weeks	200 (26)	↓ –19.4	↓ –14.3	↑ +2.5	↓ –28	↓ –13.8	↓ –25	↓ –21.1	↓ –2.4	[130]
	II	NCT 00707746	*N* = 33; statin-intolerant subjects at high risk for CVD	26 weeks	200 (26)	↓ –36.9	↓ –28%	↑ +8%	↓ –47.3%	↓ –27%	↓ –45.6	↓ –27.1%	0	[131]
	III	NCT 00794664	*N* = 58; severe hypercholesterolemia	26 weeks	200 (26)	↓ –28.3	↓ –8.6	↑ +5.8	↓ –35.9	↓ –9.6	↓ –33.9	↓ –32.7	↓ –3	[132]
	III	NCT 00770146	*N* = 158; hypercholesterolemia with, or at high risk for, CHD	26 weeks	200 (26)	↓ –26.4	↓ –25.2	↑ +2.2	↓ –36.9	↓ –25.3	↓ –35.7	↓ –25.6	↓ –5.6	[65]
	II	?	*N* = 15; atherosclerosis, LDL-C □130 mg/dl	26 weeks	200 (26)	↓ –20.6	↓ –24.7	↓ –3.6	↑ 22.6	↓ –7.3	↓ –24.1	↑ 16.5	N	[133]
	III	NCT 00694109	*N* = 53; familial HC	104 weeks	200 (104)	↓ –20	↓ –12	↑ +10	↓ –28	N	↓ –2	↓ –14	N	[71]
Inclisiran; Medicines Company	II	NCT 02597127	*N* = 501; high risk for CVD and high LDL-C levels	180 days	200–500 −1	↓ (–17.6) to (–26.6)	↓ 1.1 to (–12.8)	↑ 4.4 to 8.8	↓ (–27.9) to (–41.9)	↓ (–11.6) to (–23.8)	↓ (–25.1) to (–36.9)	↓ (–14.3) to (–18.2)	↑ 2.9 to 4.1	[82]
					100–300 (2)	↓ (–22.4) to (–33.2)	↓ (–6.3) to (–14.2)	↑ 7.6 to 10.3	↓ (–35.5) to (–52.6) %	↓ (–16) to (–21.2)	↓ (–31.7) to (–46.0)	↓ (–14.9) to (–25.6)	↑ 5.5 to 8.6	
ALN-PCS; Alnylam Pharmaceuticals	I	NCT 01437059	*N* = 32; healthy adult	28 days	0·015–0·400 mg/kg (1)	↓ (–5.4) to (–23.5)	↓ 37.5 to 86.8	↑ (–21.0) to (–2.0)	↓ (–14.4) to (–36·1)	↓ 4.3 to (–44.8)		↓ (–17.6) to (–30.6)		[83]
ISIS ANGPTL3Rx; Ionis Pharmaceuticals	I	NCT 02709850	*N* = 44; with TG levels of 90–150 mg/dl	6 weeks	10–60 (6)	↓ (–8.7) to (–34.3)	↓ (–33.2) to (–63.1)	↓ (–26.9) to (–3.6)	↓ (–1.3) to (–32.9)	↓ (–27.9) to (–60.0)	↓ (–10.0) to (–36.6)	↓ 19.6 to (–57.4)	↓ (–4.6) to (–23.7)	[105]

Volanesorsen; Isis Pharmaceuticals, Inc	II	NCT 01529424	$N = 57$; hypertriglyceridemia	Once weekly for 13 weeks	200; monotherapy (13)	↓ −1.2	↓ −57.7	↑ 36.2	↓ 79.4	↓−56.1	↓ −6.8	N	[97]
					200; add-on to fibrate (13)	↓ −4.0	↓ −51.0	↑ 50.6	↓ 3.54	↓ −54.30	↓ −15.2	N	
					300; monotherapy (13)	↓ −2.1	↓ −70.9	↑ 45.7	↓ 118.3	↓ −69.2	↓ −11.3	N	
					300; add-on to fibrate (13)	↓ −7.7	↓ −64.0	↑ 51.80	↓ 21.0	↓ −63.2	↓ −18.9	N	
		NCT01647308	$N = 15$; type 2 diabetes mellitus and hypertriglyceridemia	Once weekly for 15 weeks	300 (15)	N	↓ −69.1	↑ 42.5	↓ −0.0	↓ −72.9	↓ −22.1	N	[100]

Following secretion, it binds to the LDLR on the surface of hepatocyte cells, is internalized by endocytosis, and promotes lysosomal degradation of LDLR, thus increasing the levels of plasma LDL-C [75]. Loss-of-function mutations in PCSK9 lead to elevated LDLR expression and lower levels of LDL-C, possibly reducing the risk for CVD [76,77]. ASOs and siRNAs are gene-silencing approaches that can be used to inhibit the expression of PCSK9 protein.

SPC5001, a 14-mer, and SPC4061, a 13-mer, are LNA-modified ASOs targeting PCSK9 mRNA. Preclinical data in nonhuman primates showed that a single dose of SPC5001 (10 mg/kg) resulted in a maximum reduction of 50% in LDL-C levels. This reduction associated well to the reduction in serum TC and ApoB, but not to the reduction in HDL-C.

The potencies of SPC5001 and SPC4061 were examined in a multiple-dose study with a primary dose of 20 mg/kg and four weekly doses of 5 mg/kg. Administration of PC5001 and SPC4061 resulted in reductions of plasma PCSK9 protein concentrations (85% and 55%, respectively), LDL-C levels (70% and 50%, respectively) and circulating ApoB levels (~50% and 35%, respectively), without any effects on HDL-C levels. Therefore, the reduction in TC was secondary to the reduction in LDL-C. In addition, SPC5001 increased liver LDLR protein levels by 67%, whereas SPC4061 had no significant effect [78]. In a Phase I multidose trial [three weekly doses (0.5, 1.5, or 5 mg/kg) on days 1, 8, and 15], SPC5001 was shown to decrease PCSK9 plasma concentration (from 302 ± 80 ng/ml at baseline to 156 ± 85 ng/ml) and LDL-C concentrations (from 3.8 ± 0.8 mmol/l at baseline to 2.9 ± 1.1 mmol/l). In addition, SPC5001 decreased ApoB with a maximal average of nearly 15%, but increased ApoA1 with a mean maximal average of 8%. SPC5001 treatment had no effect on HDL-C, TGs, and VLDL-C concentrations. SPC5001 treatment induced mild to moderate adverse effects relating to ISRs and short-lived renal tubular toxicity. However, clinical trials of SPC 5001 were prematurely terminated for unknown reasons.

Intraperitoneal administration of ISIS 394814, a 20-mer chimeric 2′-O-methoxyethyl phosphorothioate ASO, (50 mg/kg) twice weekly to HFD-fed mice reduced hepatic PCSK9 mRNA levels by 92% following 6 weeks of treatment. The treatment increased hepatic LDLR (twofold) but reduced TG content) ~65%(, LDL (32%), HDL (54%), and TC (52%). The reduction in HDL resulted from increased clearance of apolipoprotein E (ApoE)-containing lipoproteins, simplified by increased hepatic LDLR redundancy. In addition, the effect of ISIS 394814 on ApoBec-1 resulted in a threefold increase in serum ApoB-48 levels and a reduction in ApoB-100 levels (50%), but had no significant effect on Apo A-I levels [79].

PCS-A2/B2/C2 are active, cross-species siRNAs that target murine, rat, nonhuman primate (NHP), and human PCSK9 mRNA. PCSK9 and control siRNAs were formulated into lipidoid-based nanoparticles (LNP) for in vivo studies. Liver-specific siRNA silencing of PCSK9 led to a 50–70% reduction in PCSK9 mRNA in mice and rats. The reduction in PCSK9 mRNA was related to an ~60% reduction in plasma TC concentrations. These effects were demonstrated to be mediated by rapid amplification of cDNA ends (5′-RACE), an RNAi-mediated mechanism. In transgenic mice expressing human PCSK9, siRNAs reduced the human PCSK9 mRNA by >70% and dramatically lowered PCSK9 plasma protein levels. In this study, a single administration of 5 mg/kg of

LNP-PCS-A2 or LNP-PCS-B2 to NHP led to a reduction in LDL-C beginning at Day 3 post-dose and returning to baseline levels over ~14 days (for LNP-PCS-A2) and ~21 days (for LNPPCS-B2). LNP-PCS-B2 led to an average 56% reduction in LDL-C. Furthermore, this LDL-C reduction was associated with a tendency toward lower circulating ApoB levels [80].

Inclisiran (ALN-PCSsc) is a long-acting, synthetic siRNA that is conjugated to triantennary N-acetylgalactosamine carbohydrates. It is administrated subcutaneously and directed against PCSK9. In a Phase I trial, participants received either single-dose (25, 100, 300, 500, or 800 mg) or multiple-dose (125 mg weekly for four doses, 250 mg every other week for two doses, or 300 or 500 mg monthly for two doses) inclisiran versus placebo. Some participants also were given inclisiran in combination with statins in the multiple-dose study. Inclisiran resulted in a dose-dependent reduction in plasma PCSK9 levels with a peak reduction of ≈74% at a dose of ≥300 mg at Day 84 after single-dose administration and remained significantly lower (>50% reduction) at Day 180. Multiple-dose inclisiran administration with or without statins led to a peak reduction of ≈83% at 500 mg, and lasted up to Day 196 after the first dose. In addition, LDL-C levels were decreased in both the single-dose and multiple-dose groups, with a peak reduction of ≈50% and ≈60%, respectively at Day 84. Most importantly, the marked decrease in LDL-C after the first dose persisted for ≤180 days, paralleling the decrease in plasma PCSK9 levels. Importantly, no serious adverse effects were observed [81].

In a Phase II double-blind, placebo-controlled, multiple ascending-dose clinical trial, inclisiran was administered subcutaneously to 501 patients. The patients were randomly selected to receive a single dose of placebo or 200, 300, or 500 mg of inclisiran or two doses (at Days 1 and 90) of placebo or 100, 200, or 300 mg of inclisiran, and showed dose-dependent reductions in PCSK9 and LDL-C. In this group, all patients responded with a significant LDL-C-lowering profile. The least-squares mean reductions in LDL-C levels at Day 180 were 27.9–41.9% after a single dose of inclisiran and 35.5–52.6% after two doses ($P < 0.001$ for all comparisons versus. placebo). In addition, the largest reduction in LDL-C was shown in the two-dose 300-mg regimen: in 48% of the patients who received this regimen, LDL-C level decreased to <50 mg per deciliter (1.3 mmol/l) at Day 180. In association with all inclisiran regimens, PCSK9 and LDL-C levels remained significantly lower than at baseline by Day 240. In 11% of patients who received inclisiran and in 8% of patients who received placebo, serious adverse effects occurred. In addition, ISRs occurred in 5% of patients who received injections of inclisiran [82].

ALN-PCS is a PCSK9 siRNA that was investigated in a randomized, single-blind, placebo-controlled, Phase I clinical trial. In this dose-escalation study in 32 healthy participants with LDL-C >3 mmol/l (116 mg/dl), ALN-PCS was given intravenously and a single dose (0.015–0.400 mg/kg) produced dose-dependent reductions in plasma PCSK9 and LDL-C levels, with a mean reduction of 70% in PCSK9 and of 40% in LDL-C at the highest dose, an effect that was sustained for 2–3 weeks after administration. In addition, ALN-PCS was well tolerated, and the occurrence of treatment-related adverse effects was similar among active treatment and placebo groups (ALN-PCS 79% versus placebo 88%). Although the maximum tolerable dose of ALN-PCS and its efficacy remain to be determined in statin-resistant and high-risk patient populations, this study holds promise for therapeutic approaches that prohibit the synthesis of PCSK9 in the liver [83]. Alnylam recently initiated another Phase I study testing subcutaneously administered ALN-PCS (www.clinicaltrials.gov; NCT02314442).

ApoC-III

Apolipoprotein C-III (ApoC-III) is a 79-amino acid glycoprotein that binds to the surface of HDL, VLDL and chylomicrons. It plays a key role in the regulation of plasma triglyceride levels [84]. ApoC-III promotes the hepatic secretion of VLDL, and reduces plasma TG clearance via prohibition of lipoprotein lipase (LPL) function and uptake of VLDL and its remnants [84–86]. Studies indicate that ApoC-III affects the metabolism of plasma TG, and gain-of-function mutations in ApoC-III eventually lead to insulin resistance, nonalcoholic fatty liver disease (NAFLD) and a twofold increase in plasma TG levels [87–89]. In addition, hyperglycemia and insulin resistance that can cause ApoB hepatic secretion enhance the expression of ApoC-III. Furthermore, nonsense and missense mutations in ApoC-III are associated with low plasma ApoC-III, TG, and LDL-C levels and high HDL levels. These mutations also are associated with reduced indices of subclinical atherosclerosis, intrahepatic TG [90–92], and increased life span [93].

A301S and A301SL, ApoC-III-specific LNA-based ASOs, were administered via intraperitoneal injection (10 mg/kg and 20 mg/kg) to C57Bl/6J mice five times over 2 weeks. A301SL produced dose-dependent reductions in hepatic ApoC-III mRNA by ~29% and ~72% on average at doses of 10 mg/kg and 20 mg/kg, respectively, whereas A301S had no reduction effect on ApoC-III mRNA even at the higher dose.

In addition, A301SL (20 mg/kg) resulted in removal of approximately half the plasma ApoC-III protein on Day 16, whereas A301S had no significant reduction effect on ApoC-III protein levels, which is associated with changes in levels of hepatic ApoC-III mRNA expression. A301SL treatment at 20 mg/kg reduced total serum TG levels by ~56% and ~87% over time (at days 8 and 16, respectively), whereas a ~54% reduction in total serum TG levels was seen with A301S on Day 16. A301SL also resulted in remarkable reductions in HDL-C and VLDL-C and a milder but similar trend was observed with A301S, associated with a slight but not significant reduction in ApoC-III mRNA and protein levels [94].

ALN-AC3, an ApoC-III RNAi, has been investigated in mouse models matching human genetics. Preclinical data showed that a single dose (3 mg/kg) administered subcutaneously of a GalNAc-conjugated siRNA targeting ApoC3 knocked down ApoC3 levels by up to 94% and the durability of the knockdown effect lasted for at least 30 days. Furthermore, results of a multiple-dose study showed that dosing of 3 mg/kg every other week knocked down 96% of ApoC3 through to Day 35, which was the last time point in the study [95].

Volanesorsen (formally ISIS-APOCIIIRx and ISIS-304801) is a second-generation ASO that binds to ApoC-III mRNA and blocks protein synthesis as well as limiting mRNA availability because of enhanced ribonuclease H1-mediated destruction of the target mRNA [89]. It is a promising new agent for lowering ApoC-III levels and TG concentrations, as shown by Phase I, II and III studies [96]. Preclinical studies, including multiple rodent models and NHPs, demonstrated that ASO led to suppression of ApoC-III and robust lowering of TG and VLDL levels. Importantly, ApoC-III

ASO was well tolerated and safe in all preclinical studies [89]. A Phase I double-blind, placebo-controlled, dose-escalation clinical study in healthy human volunteers with normal TG levels showed comparable results with substantial (parenteral) doses and a time-dependent reduction in plasma levels of ApoC-III and TG, without transaminase elevation or hepatic steatosis [89]. Several Phase II trials were implemented in patients with severe or uncontrolled hypertriglyceridemia [97], in patients with the familial chylomicronemia syndrome (FCS) [98,99], and in patients with type 2 diabetes mellitus (T2DM) [100]. A randomized, double-blind, placebo-controlled, dose-ranging Phase II study of volanesorsen was conducted with and without the addition of fibrate therapy. In monotherapy, volanesorsen led to decreases in plasma ApoC-III of 40–80%, and plasma TG levels declined by 30–71% in a dose-dependent manner. As an add-on to fibrates, it led to in decrease in ApoC-III levels by 60–71% and decrease in TG levels by 51–64% [97]. In another Phase II study of volanesorsen, monotherapy with 100, 200, and 300 mg/dose in patients with hypertriglyceridemia, including FCS, led to a decrease in ApoB–ApoC-III, ApoC-III–ApoAI, and ApoC-III–LP(a) complex levels by 80%, whereas the add-on to the fibrate group led to a decrease in ApoC-III–ApoB, ApoC-III–ApoA1, and ApoC-III–Lp(a) complex levels by 75%. A similar decrease in the complex levels was observed in the FCS group with a 300-mg dose [98]. Notably, ApoC-III-ApoB levels coordinated well with total ApoC-III, TG, VLDL–ApoC-III, VLDL-C, chylomicron-ApoC-III, chylomicron-TG, chylomicron-cholesterol, and LDL-C. In a pilot Phase II study, three patients with FCS received weekly subcutaneous injections of 300 mg volanesorsen for 13 weeks [99]. Upon treatment, plasma ApoC-III levels in all three patients decreased by 70–90% and TG levels by 56–86%.

Another randomized double-blind placebo-controlled trial with volanesorsen was reported in 15 adult patients with T2DM [HbA_1c >7.5% (58 mmol/mol)] and hypertriglyceridemia (TG > 200 and <500 mg/dl). The results of demonstrated that volanesorsen (300 mg/week subcutaneous injection for 15 weeks) reduced ApoC-III (−88%), VLDL-ApoC-III (−90%), and TG (−69%), and increased HDL-C (1 43%) compared with placebo. Furthermore, these changes were accompanied by improved insulin sensitivity [100].

Phase III clinical trials include APPROACH [101] (a study of ISIS-APOCIIIRx in patients with FCS, NCT02211209), COMPASS [102] (a study of volanesorsen in patients with hypertriglyceridemia, NCT02300233), Approach Open Label Study (a study of volanesorsen in patients with FCS, NCT02658175), and BROADEN (a study of volanesorsen in patients with partial lipodystrophy, NCT02527343). These studies investigated whether volanesorsen is capable of decreasing plasma TG levels in patients with FCS, severe hypertriglyceridemia, and partial lipodystrophy compared with placebo. Both the APPROACH and the COMPASS trials successfully met their primary endpoints, whereas the BROADEN trial is ongoing [101,102]. In 2016, the results of the COMPASS trial were released and showed a statistically significant mean decrease in TG levels in patients treated with volanesorsen compared with placebo (71.2% versus 0.9% reduction, $P < 0.0001$), which was sustained during the 26 weeks of treatment. This randomized controlled trial included 113 patients with severe hypertriglyceridemia; the mean baseline level of TG was 1261 mg/dl. Most patients (82%) were treated with volanesorsen and, including three patients with FCS, achieved TG levels of <500 mg/dl after 13 weeks of treatment ($P < 0.0001$) compared with 14% of patients in the placebo group. In the treatment group, ISRs were the most common adverse effects. They were often mild but adequate enough to lead to discontinuation of the treatment in 13% of patients. No serious platelet-related events and no deaths were reported [102]. In addition, the APPROACH study met its primary endpoint, consistent with the results of the COMPASS trial. This study was conducted in 66 patients with FCS and a mean TG level of 2209 mg/dl. The average decrease in TG levels was 77% in the volanesorsen group compared with an average increase in 18% ($P < 0.0001$) in the placebo group. A decrease was also observed in the frequency of pancreatitis attacks and abdominal pain in the treatment arm. ISR was the most common adverse reaction along with thrombocytopenia, which combined resulted in a discontinuation rate of treatment of 30% (ten out of 33 patients). Although the COMPASS and APPROACH studies both reached their endpoints, these results are primary, and safety remains an issue [101].

ANGPTL3

Angiopoietin-like protein 3 (ANGPTL3) is a member of a secretory protein family that affects LPL and endothelial lipase-mediated hydrolysis of TGs and phospholipids and, thus, regulates levels of plasma lipids [103]. ANGPTL3 deficiency resulting from loss-of-function mutations in the ANGPTL3 gene increases LPL levels and endothelial lipase activity, enhances insulin sensitivity, and decreases free fatty acid (FFA) levels in the serum [104]. Therefore, ANGPTL3 is a potential therapeutic target for combined hyperlipidemia therapy, a main risk factor for coronary artery disease (CAD; atherosclerosis) [103].

ANGPTL3ASO, a mouse-specific second-generation ASO, was administered to a variety of mouse models fed either a normal chow or a Western diet, including wild-type C57BL/6 mice, $LDLR^{-/-}$ mice, $ApoC3^{-/-}$ mice, and mice overexpressing human ApoC-III. The ASO resulted in significant and selective decreases in hepatic Angptl3 mRNA expression (by 69–91%) and plasma levels of ANGPTL3 protein (50–90%) in each mouse model. Administration of ANGPTL3 ASO also reduced levels of TGs (by 35–85%), LDL-C (7–64%), and HDL-C (3–23%). In some of the mouse models, including in $LDLR^{-/-}$ mice, the TG content was also decreased within VLDL, IDL, and LDL particles, indicating an LDLR-independent process. In C57BL/6 mice that received Angptl3 ASO, a significant reduction in liver TG secretion was observed. In addition, TG clearance was increased, independently of whole lipoprotein uptake, given that no change was observed in the clearance rate of VLDL. Human APOC3-overexpressing mice treated with ANGPTL3 ASO had significantly lowered plasma TG and LDL-C levels. ANGPTL3 ASO treatment of mice with diet-induced obesity also led to higher insulin sensitivity compared with control obese mice. Likewise, in mice receiving the ASO, accumulation of hepatic TG was 81% lower compared with the control group. Treatment of $LDLR^{-/-}$ mice with ANGPTL3 ASO retarded the progression of atherosclerosis, relative to that observed in mice that received the control ASO (by 52% or 37% depending on the weekly dose) [105].

IONIS-ANGPTL3-L_{Rx} (hereinafter referred to as ANGPTL3-L_{Rx}), a 20-mer second-generation ligand-conjugated antisense drug,

contains three GalNAc residues covalently bound to its 5′-end. In a Phase I trial, 12 participants were randomly assigned to single-dose groups (nine active, three placebo) and 32 were randomly assigned to multiple-dose groups (24 active, eight placebo). Results of subcutaneous injections of placebo or ANGPTL3-L_{Rx} showed dose-dependent reductions in the levels of ANGPTL3 protein. Levels of TG were reduced from baseline by a maximum of 63.1%, ApoB by a maximum of 25.7%, ApoC-III by a maximum of 58.8% and non-HDL-C by a maximum of 36.6%. Plasma levels slowly returned to normal between the final dose (Day 36) and the end of follow-up (Day 127). No serious adverse effects were reported [105].

ALN-ANG is an active siRNA molecule developed to target the ANGPTL3 gene. A single dose of 1 mg/kg ALN-ANG in hyperlipidemia ob/ob mouse models reduced serum ANGPTL3 protein by <95%, triglyceride by <95%, LDL-C by <85%, and TC by <60%. A subcutaneous dose of 3 mg/kg ALN-ANG reduced TG by >595%, LDL-C by >585%, and TC by >560% [106].

DGAT2

Acyl-coenzyme A:diacylglycerol acyltransferase (DGAT) is an enzyme involved in the synthesis of TGs. DGAT catalyzes the last step in TG synthesis by covalently binding the acyl moiety to diacylglycerol. DGAT has high expression in liver, fat, and small intestine. Two different gene families encode two DGATs: DGAT1 and DGAT2. Studies show that both DGAT1 and DGAT2 have key roles in TG synthesis [107].

Genetically obese and diabetic db/db mice were fed a methionine and choline (MCD) diet for 4 or 8 weeks. A mouse model of nonalcoholic steatohepatitis (NASH) induced by a diet deficient in MCD, was administrated with 25 mg/kg DGAT2 ASO intraperitoneally twice weekly. DGAT2 ASO decreased hepatic steatosis, necroinflammation, and fibrosis, but increased hepatic FFAs, markers of lipid peroxidation/oxidant stress, cytochrome P4502E1, lobular fibrosis, and necroinflammation. Liver damage progressed despite a reduction in the hepatic expression of TNF-alpha, increasing serum adiponectin, and improvement in systemic insulin sensitivity. Treatment with DGAT2 ASO also reduced DGAT2 mRNA expression by 90% and had no effect on expression of DGAT1 [108].

ISIS-217376 is a DGAT2 ASO that targets a binding site in the coding region of DGAT2 mRNA. Administration of DGAT2 ASO to diet-induced obese (DIO) mice decreased DGAT2 mRNA by >75% in liver and fat tissues. Treatment resulted in reduction of total DGAT activity in the liver. The mice were treated with DGAT2 ASO at a dose of 25 mg/kg or 37.5 mg/kg twice a week. DGAT2 ASO treatment decreased DGAT2 mRNA by 75%, decreased TG by 62%, improved hepatic steatosis and decreased blood lipid levels, TC, FFAs, and diacylglycerol. The reduction in DGAT2 mRNA was associated with a 55–75% inhibition of TG synthesis, indicating an important role of DGAT2 in hepatic TG synthesis. DGAT2 ASO administration caused an increase in fatty acid oxidation of 30% and a reduction in hepatic triglyceride secretion of 20%. The decrease in DGAT2 expression can improve hepatic steatosis and reduce hyperlipidemia [107].

MTP

Microsomal triglyceride transfer protein (MTP) has a role in lipoprotein metabolism and promotes the lipidation of newly translated ApoB with phospholipid, TGs, and cholesteryl esters. Lipid transfer to emerging ApoB results in the production and secretion of VLDL [62]. In the absence of functional MTP, ApoB fails in lipidation and undergoes ubiquitination and subsequent degradation [109].

ISIS 144477 is a second-generation ASO targeting MTP mRNA. ASOs were injected 50 mg/kg/week intraperitoneally for 6 weeks to male $LDLR^{-/-}$ mice (fed either a chow or Western diet). ASO treatment reduced MTP mRNA expression by 70–90%, consistent with hepatic MTP protein suppression and decreased protein activity by 70%. MTP ASO treatment also reduced plasma TG (75%), HDL-C (30%), VLDL (85%), and LDL-C (42%), and elevated liver TGs and cholesteryl esters. Treatment also led to increases in plasma ALT and AST concentrations of 2.8-fold and 1.4-fold, respectively. Consequently, the animals developed more hepatic steatosis and liver injury, as supported by elevated transaminases [109].

miRNA-33

The miR-33 miRNA family (miR-33a and miR-33b) is a well-characterized miRNA family that mediates regulation of lipid metabolism [110]. Numerous groups have described the crucial role of miR-33a and miR-33b, located within the genes encoding sterol response element binding protein 2 and 1 (SREBP2 and SREBP1), respectively. SREBP1 and SREBP2 are two of the most essential transcription factors in the regulation of fatty acid and cholesterol metabolism, respectively [110]. Furthermore, miR-33 controls the expression of ABCA1 and ABCG1. As a result, miR-33 regulates HDL biogenesis and cellular cholesterol efflux [111]. Research has suggested that miR-33 represses lipid droplet catabolism via lysosomal cholesterol efflux [112].

$LDLR^{-/-}$ mice were fed a western diet for 14 weeks after which they were fed a chow diet to block atherosclerosis progression and then injected subcutaneously with 10 mg/kg 2′F/MOE anti-miR33 or control anti-miR oligonucleotides for 4 weeks. Anti-miR33 treatment decreased miR-33 by > 60%, increasing circulating HDL and HDL-C by 25–30% and 35%, respectively, with no effect in total circulating cholesterol. Both ABCA1 and ABCG1 proteins were increased in the livers of anti-miR33-treated mice. No elevation in the serum hepatotoxicity markers AST and ALT was detected. The lesion area in the aortic sinus was reduced by 35% and lesional macrophage Abca1 expression was increased by ~66%. Anti-miR33 treatment also increased the flux of cholesterol from cholesterol-loaded macrophages and resulted in an 82% increase in fecal 3H-sterol excretion. Although there were no significant changes in genes lacking miR-33 target sites (i.e., INSIG1, HMGCR, and SREBF2), the expression of miR-33 target genes involved in fatty acid metabolism was significantly increased with anti-miR33 treatment (i.e., CROT, HADHB, and CPT1A). Anti-miR33 increased circulating HDL and, therefore, enhanced the reverse cholesterol transport (RCT) pathway in which cholesterol is removed from atherosclerotic lesions [113].

$LDLR^{-/-}$ mice received 7 mg/kg anti-miR-33 (5′-TGCAACTACAATGCA)-locked nucleic acid oligonucleotides once a week for 14 weeks. After 2 weeks on chow, mice were switched to a Western diet containing 21% fat and 1.25% cholesterol for 12 weeks. Anti-miR-33 increased circulating cholesterol levels, plasma TGs, and HDL-C during the first 2 weeks of treatment, but HDL-C was not

sustained until the end of the experiment. Treatment decreased the miR-33 level by 75%. The size or composition of lesions between the groups was changed [114].

$LDLR^{-/-}$ mice were injected subcutaneously with 10 mg/kg/week anti-miR-33 oligonucleotides for 12 weeks. Hepatic expression of several miR-33 target genes, including ABCA1, CPT1A, IRS2, AMPK, and HADHB, was increased. Anti-miR-33 therapy increased plasma HDL-C and reduced plaque size and macrophage [111]. Other studies showed that treating atherosclerotic $LDLR^{-/-}$ mice with anti-miR-33 restored defective autophagy in macrophage foam cells and plaques and promoted apoptotic cell clearance to reduce plaque necrosis [112].

In another study, C57BL/6 mice received 5 mg/kg/week subcutaneously for 12 weeks. Hepatic miR-33 expression was reduced, whereas TC, circulating HDL-C, and TG increased [115].

Apolipoprotein(a)

Lp(a) is a modified LDL particle comprising ApoA and LDL. It is an important risk factor for CVD [116], but available therapies often do not decrease Lp(a) levels and might even increase it [117]. ApoA is produced in the liver and subsequently bound to ApoB-100 to form Lp(a) [118].

ISIS 681257 is a 2′-*O*-methoxyethyl-modified ASO (20-mer) conjugated to triantennary N-acetylgalactosamine carbohydrates and ISIS 494372, its unconjugated congener, have been developed for targeting human ApoA in human LPA transgenic mice. Administration of ISIS 681257 at doses of 0.3, 1, 3, and 10 mg/kg, or ISIS 494372 (unconjugated) at doses of 3, 10, and 30 mg/kg once weekly for 6 weeks resulted in dose-dependent reductions in target mRNA expression. An inhibitory effect sigmoidal Emax model was fitted to the data and ED_{50} (50% of maximum drug-induced inhibitory effect) values were estimated. The ED_{50} for ISIS 681257 for liver ApoA mRNA and plasma ApoA protein levels was 0.32 and 0.54 mg/kg/week, respectively, whereas that for ISIS 494372 was 6.38 and 11.0 mg/kg/week, respectively. Thus, ISIS 681257 improved the potency of the ApoA ASO by over 20-fold as measured by direct target knockdown in the liver ApoA mRNA and plasma ApoA protein levels [119].

ISIS 144367 is an ASO (5-10-5 MOE gapmer) that targets the Kringle (K) IV-2 repeats (KIV-2) in the ApoA transcript. The effect of ISIS 144367 was investigated in three transgenic mouse models: (i) 8 K-ApoA, expressing 8 Kringle IV (KIV) repeats with a single copy of KIV-2; (ii) 8 K-Lp(a), expressing both the 8 K ApoA plus human ApoB-100; and (iii) 12 K-ApoA, expressing a 12 K ApoA. ISIS 144367 was injected intraperitoneally for 4–6 weeks. Treatment with ISIS 144367 reduced hepatic ApoA mRNA by 52% in 8 K-ApoA mice and 8 K-Lp(a) mice. Treatment reduced Lp(a) by 24.8% in 8 K-Lp(a) mice. It also decreased ApoA by 19.2% in the 8 KLp(a) model, 30.0% in the 8 K-ApoA model, and 86% in the 12 K-ApoA model. OxPL/ApoB was also reduced by 22.4% in the 8 K-Lp(a) model. ISIS 144367 reduced OxPL/ApoA in the 8 K-Lp(a) model (19.9%), 8 K-ApoA model (22.1%), and 12 K-ApoA model (92.5%). Treatment with ISIS 144367 did not change the weight of the body, liver, kidney, or spleen in 12 K-ApoA mice [118].

IONIS-ApoARx is a second-generation ASO that targets ApoA [120]. A single dose of 50–400 mg ISIS-ApoARx did not reduce the Lp(a) level by Day 30. By contrast, administration of six doses (100–300 mg) ISIS-ApoARx caused a dose-dependent decrease in Lp(a) level (396–778%) but TC, ApoB, LDL-C, VLDL-C, and HDL-C did not change [121]. In other studies, (two randomized, double-blind, placebo-controlled, dose-ranging trials) IONIS-ApoARx 125-438 nmol/L/week for 12 weeks reduced Lp(a) by 66·8–71·6% [116].

E2F-1

E2F-1 is an important cell cycle transcription factor that is released from a regulatory complex involving the retinoblastoma gene product during late G1 [122]. E2F-1 is associated with the upregulated expression of genes involved in DNA synthesis and cell cycle progression. Thus, it could be an ideal target for cell cycle inhibition [123]. The E2F-1 decoy oligodeoxynucleotide (edifoligide) inhibited proliferating cell nuclear antigen (PCNA) expression and smooth muscle cell proliferation. Moreover, this inhibition of gene expression can inhibit vascular proliferative disorders, such as atherosclerosis [122].

In an animal study, a E2F-1 decoy was delivered to graft vascular smooth muscle cells (VSMCs) of New Zealand white rabbits by nondistending pressure-mediated transfection. Six months after operation, rabbits were fed with cholesterol for 6 weeks. The E2F-1 decoy inhibited PCNA upregulation, smooth muscle cell proliferation and medial VSMC proliferation. Treatment also led to a reduction in neointimal thickness. At 6 months after grafting, extensive plaque was seen in the control group, whereas, in the E2F-1 decoy group, grafts remained free of macroscopic plaque [122].

In a prospective, randomized, double-blind trial, 41 patients with primary graft failure undergoing peripheral bypass grafting were studied. The E2F-1 decoy solution, double-stranded phosphorothioate oligodeoxynucleotide, was delivered via ex vivo pressure-mediated DNA transfection into human vein grafts. Results showed that the E2F-1 decoy reduced PCNA, c-myc mRNA, and bromodeoxyuridine incorporation by 73%, 70%, and 74%, respectively. There was no difference in postoperative complication rates and adverse events between the E2F-1 decoy group and the control group. Graft occlusion revisions and critical stenosis were lower in the E2F-1 decoy group compared with the control group. Thus, the E2F-1 decoy could be safe and feasible for use, inhibiting cell cycle gene expression and DNA replication in a sequence-specific manner [123].

In a single-center randomized Phase II trial, 200 patients underwent coronary bypass grafting and were followed up for 1 year. Postoperative complication rates and adverse events were not changed in the E2F-1 decoy group and the treatment caused a 30% reduction in critical stenosis and a 32% decrease in neointimal volume compared with the control group [124].

A prospective, randomized, double-blinded multicenter Phase III trial was carried out to prevent vein graft failure in patients undergoing infrainguinal revascularization for critical limb ischemia (CLI). In total, 1404 patients with CLI undergoing vein grafting and treated with either edifoligide or placebo were followed for 1 year. No differences in primary graft patency and limb salvage were observed between the groups. The primary end point (defined as the time to graft reintervention or amputation because of index graft failure) and secondary end point (including graft failure and stenosis) were no different between the groups. There was more improvement seen in secondary graft patency in the E2F-1 decoy group compared with the control group (83% and 78%,

respectively). Thus, treatment of vein grafts with E2F-1 decoy did not confer protection against vein graft failure [125].

In another multicenter, randomized, double-blind Phase III trial, 3014 patients undergoing vein grafting were treated ex vivo with either edifoligide or placebo and were followed from 12 to 18 months. Delivery was carried out by a pressure-mediated system. The E2F-1 decoy had no effect on the primary end point (death or vein graft stenosis) or on any secondary end point (incidence of major adverse cardiac events) [126]. Follow-up of 2865 of the 3014 patients [126] for 5 years suggested that the E2F-1 decoy had the same effects (rates of death, myocardial infarction, revascularization, and rehospitalization) as the placebo [127].

Concluding remarks

Limitations of conventional therapies for atherosclerosis have led to extensive investigations to find novel drugs. Nucleic acid-derived therapeutic approaches, including ASOs, siRNAs, miRNAs, and decoys, have been developed in recent years. Anti-miR-33, ISIS 144477, ALN-ANG, ISIS-217376, ISIS-217376, ISIS 394814, SPC4061, PCS-A2/B2/C2, ISIS 147764, ISIS 147483, ALN-AC3, A301SL, A301S, ISIS 144367, ALN-ANG, and ISIS681257 are in preclinical studies. The candidates currently in clinical trials include ISIS ANGPTL3Rx (Phase I), ISIS-APOARx (Phase II), and inclisiran, volanesorsen, and edifoligide (Phase III). Mipomersen is the only approved ASO-based drug.

Therapeutic targets for atherosclerosis include PCSK9, ApoB, ApoC-III, ApoA, ANGPTL3, E2F, MTP, DGAT and miR-33a/b. Inclisiran, SPC 5001, ISIS 394814, SPC4061, and PCS-A2/B2/C2 act by inhibition of PCSK9 expression, increasing the hepatic LDLR density, which ultimately reduces LDL-C. Mipomersen, ISIS 147764, and ISIS 147483 target ApoB. Thus, they reduce atherogenic ApoB-containing lipoproteins, which are important causal agents of atherosclerosis. ApoCIII-targeting drugs, including volanesorsen, ALN-AC3, A301SL, and A301S, increase hepatic uptake of triglyceride-rich lipoproteins, such as chylomicrons and VLDL. ISIS-APOARx, ISIS 144367, and ISIS681257 reduce LDL-C by inhibition of ApoA. ISIS-217376 is a DGAT-2 inhibitor. ALN-ANG and ISIS ANGPTL3Rx can target ANGPTL3. Anti-miR-33 increases circulating HDL-C by targeting miR-33a/b. Edifoligide inhibits E2F-1, whereas ISIS 144477 is an ASO targeting MTP mRNA.

The most common adverse effect of nucleic acid-derived therapeutic agents used for CVD treatment is ISRs, which could be eliminated by further modifications. In general, these novel agents have shown promise for the treatment of atherosclerotic CVD, although several limitations and gaps need to be taken into consideration. Statins, in addition to lowering serum cholesterol levels, exert many pleiotropic effects; however, in the case of nucleic acid-derived agents, the lack of 'pleiotropic' effects could be considered a limitation. By contrast, given the high risk of new-onset diabetes in patients taking statins, nucleic acid-derived agents have the advantage of target-specific action and do not appear to accelerate new-onset diabetes. It is important to demonstrate the long-term safety of nucleic acid-derived agents because of the potential application of these agents as lifelong therapies. Notably, any neurocognitive effect that might arise with nucleic acid-derived agents as a result of aggressive LDL-C reduction should be monitored in future long-term studies. Given that the mechanism of action of agents derived from nucleic acids is via reducing the expression of the gene within the cell and because many of the intracellular effects of these proteins have not been clearly identified, their intracellular inhibition could prevent many of the functions that have not yet been discovered and, thus, could cause adverse effects. Hence, their long-term safety compared with monoclonal antibodies needs to be more accurately assessed.

Supportive evidence from Phase III trials is still needed before the introduction of nucleic acid-based therapeutics to the clinic. The paucity of studies targeting lipid-independent pathways by nucleic acid-derived agents for the treatment of atherosclerotic CVD also requires consideration. In this context, vascular inflammation recently emerged as a causal risk factor for atherosclerosis. Reduction of inflammation via blocking interleukin-1β using a monoclonal antibody called canakinumab was shown to significantly reduce CVD outcomes by ~15% in a large-scale Phase III trial (33). Similar to inflammation, targeting other nonlipid pathways by nucleic acid-based agents in the context of atherosclerotic CVD is largely unexplored and deserves further investigation. Finally, combining nucleic acid-derived agents with a statin or a monoclonal antibody might reduce the required dose and increase the tolerance and cost-effectiveness of treatment in patients, although this needs to be investigated further in future trials.

References

1 Mendis, S. *et al.* (2015) Organizational update: the world health organization global status report on noncommunicable diseases 2014; one more landmark step in the combat against stroke and vascular disease. *Stroke* 46, e121–e122

2 Saiz, L.C. *et al.* (2017) Blood pressure targets for the treatment of people with hypertension and cardiovascular disease. *Cochrane Database Syst. Rev.* 10, Cd010315

3 Okwuosa, I.S. *et al.* (2016) Worldwide disparities in cardiovascular disease: challenges and solutions. *Int. J. Cardiol.* 202, 433–440

4 Walker, I.F. *et al.* (2018) The economic costs of cardiovascular disease, diabetes mellitus, and associated complications in south asia: a systematic review. *Value Health Reg. Issues* 15, 12–26

5 Rader, D.J. and Daugherty, A. (2008) Translating molecular discoveries into new therapies for atherosclerosis. *Nature* 451, 904–913

6 Koba, S. and Hirano, T. (2011) [Dyslipidemia and atherosclerosis]. *Nippon Rinsho. Jpn. Clin. Med.* 69, 138–143

7 Kypreos, K.E. *et al.* (2018) Pharmacological management of dyslipidemia in atherosclerosis: limitations, challenges, and new therapeutic opportunities. *Angiology* 70, 197–209

8 Sahebkar, A. and Watts, G.F. (2013) New therapies targeting apoB metabolism for high-risk patients with inherited dyslipidaemias: what can the clinician expect? *Cardiovasc. Drugs Ther.* 27, 559–567

9 Granik, V.G. (2012) The search for new drugs: some chemical and biochemical aspects of the problem of atherosclerosis. *Pharm. Chem. J.* 46, 139–153

10 Chruściel, P. *et al.* (2016) Impact of statin therapy on plasma adiponectin concentrations: a systematic review and meta-analysis of 43 randomized controlled trial arms. *Atherosclerosis* 253, 194–208

11 Derosa, G. *et al.* (2016) Impact of statin therapy on plasma uric acid concentrations: a systematic review and meta-analysis. *Drugs* 76, 947–956

12 Parizadeh, S.M.R. *et al.* (2011) Simvastatin therapy reduces prooxidant-antioxidant balance: results of a placebo-controlled cross-over trial. *Lipids* 46, 333–340

13 Sahebkar, A. *et al.* (2015) Statin therapy reduces plasma endothelin-1 concentrations: a meta-analysis of 15 randomized controlled trials. *Atherosclerosis* 241, 433–442

14 Sahebkar, A. *et al.* (2016) Effects of statin therapy on augmentation index as a measure of arterial stiffness: a systematic review and meta-analysis. *Int. J. Cardiol.* 212, 160–168

15 Sahebkar, A. *et al.* (2015) Does statin therapy reduce plasma VEGF levels in humans? A systematic review and meta-analysis of randomized controlled trials. *Metab. Clin. Exp.* 64, 1466–1476
16 Sahebkar, A. *et al.* (2016) Statin therapy and plasma cortisol concentrations: a systematic review and meta-analysis of randomized placebo-controlled trials. *Pharmacol. Res.* 103, 17–25
17 Sahebkar, A. *et al.* (2015) Association between statin use and plasma d-dimer levels: a systematic review and meta-analysis of randomised controlled trials. *Thromb. Haemost.* 114, 546–557
18 Sahebkar, A. *et al.* (2016) The impact of statin therapy on plasma levels of von Willebrand factor antigen: systematic review and meta-analysis of randomised placebo-controlled trials. *Thromb. Haemost.* 115, 520–532
19 Serban, C. *et al.* (2015) A systematic review and meta-analysis of the effect of statins on plasma asymmetric dimethylarginine concentrations. *Sci. Rep.* 5, 9902
20 Naci, H. *et al.* (2013) Comparative tolerability and harms of individual statins: a study-level network meta-analysis of 246 955 participants from 135 randomized, controlled trials. *Circ. Cardiovasc. Qual. Outcomes* 6, 390–399
21 Bellosta, S. and Corsini, A. (2012) Statin drug interactions and related adverse reactions. *Expert Opin. Drug Saf.* 11, 933–946
22 Bellosta, S. and Corsini, A. (2018) Statin drug interactions and related adverse reactions: an update. *Expert Opin. Drug Saf.* 17, 25–37
23 Moss, J.W.E. *et al.* (2018) Nutraceuticals as therapeutic agents for atherosclerosis. *BBA Mol. Basis Dis.* 1864, 1562–1572
24 Zhao, W. *et al.* (2017) Risk factors associated with atherogenic dyslipidemia in the presence of optimal statin therapy. *Int. J. Cardiol.* 248, 355–360
25 Dai, W. *et al.* (2018) Elevated plasma lipoprotein(a) levels were associated with increased risk of cardiovascular events in Chinese patients with stable coronary artery disease. *Sci. Rep.* 8, 7726
26 Tsimikas, S. *et al.* (2018) NHLBI Working Group recommendations to reduce lipoprotein(a)-mediated risk of cardiovascular disease and aortic stenosis. *J. Am. Coll. Cardiol.* 71, 177–192
27 Ito, M.K. and Watts, G.F. (2015) Challenges in the diagnosis and treatment of homozygous familial hypercholesterolemia. *Drugs* 75, 1715–1724
28 Ridker, P.M. *et al.* (2017) Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N. Engl. J. Med.* 377, 1119–1131
29 Ahirwar, R. *et al.* (2016) In silico selection of an aptamer to estrogen receptor alpha using computational docking employing estrogen response elements as aptamer-alike molecules. *Sci. Rep.* 6, 21285
30 Zumrut, H.E. *et al.* (2016) Ligand-guided selection of target-specific aptamers: a screening technology for identifying specific aptamers against cell-surface proteins. *Nucleic Acid Ther.* 26, 190–198
31 Geyer, P.E. *et al.* (2017) Revisiting biomarker discovery by plasma proteomics. *Mol. Syst. Biol.* 13, 942
32 Nordestgaard, B.G. *et al.* (2016) Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points-a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. *Eur. Heart. J.* 37, 1944–1958
33 Nordestgaard, B.G. *et al.* (2018) Advances in lipid-lowering therapy through gene-silencing technologies. *Nat. Rev. Cardiol.* 15, 261–272
34 Crooke, S.T. (2004) Progress in antisense technology. *Annu. Rev. Med.* 55, 61–95
35 Chan, J.H. *et al.* (2006) Antisense oligonucleotides: from design to therapeutic application. *Clin. Exp. Pharmacol. Physiol.* 33, 533–540
36 Koenig, O. *et al.* (2013) New aspects of gene-silencing for the treatment of cardiovascular diseases. *Pharmaceuticals* 6, 881–914
37 Ito, M.K. (2007) ISIS 301012 gene therapy for hypercholesterolemia: sense, antisense, or nonsense? *Ann. Pharmacother.* 41, 1669–1678
38 Crooke, S.T. (2000) Progress in antisense technology: the end of the beginning. *Methods Enzymol.* 313, 3–45
39 Lee, R.G. *et al.* (2013) Antisense technology: an emerging platform for cardiovascular disease therapeutics. *J. Cardiovasc. Transl. Res.* 6, 969–980
40 Ozcan, G. *et al.* (2015) Preclinical and clinical development of siRNA-based therapeutics. *Adv. Drug Deliv. Rev.* 87, 108–119
41 Davidson, B.L. and McCray, P.B., Jr (2011) Current prospects for RNA interference-based therapies. *Nat. Rev. Genet.* 12, 329–340
42 Lares, M.R. *et al.* (2010) RNAi and small interfering RNAs in human disease therapeutic applications. *Trends Biotechnol.* 28, 570–579
43 Hammond, S.M. *et al.* (2001) Argonaute2, a link between genetic and biochemical analyses of RNAi. *Science* 293, 1146–1150
44 de Fougerolles, A. *et al.* (2007) Interfering with disease: a progress report on siRNA-based therapeutics. *Nat. Rev. Drug Discov.* 6, 443–453
45 Kim, D.H. and Rossi, J.J. (2007) Strategies for silencing human disease using RNA interference. *Nat. Rev. Genet.* 8, 173–184
46 Simonson, B. and Das, S. (2015) MicroRNA therapeutics: the next magic bullet? Mini Rev. *Med. Chem.* 15, 467–474
47 Lee, Y. *et al.* (2004) *MicroRNA genes are transcribed by RNA polymerase II. EMBO J.* 23, 4051–4060
48 Borchert, G.M. *et al.* (2006) RNA polymerase III transcribes human microRNAs. *Nat. Struct. Mol. Biol.* 13, 1097–1101
49 Denli, A.M. *et al.* (2004) Processing of primary microRNAs by the Microprocessor complex. *Nature* 432, 231–235
50 Han, J. *et al.* (2004) The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev.* 18, 3016–3027
51 Bohnsack, M.T. *et al.* (2004) Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. *RNA* 10, 185–191
52 Bernstein, E. *et al.* (2001) Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* 409, 363–366
53 Ono, K. (2016) Functions of microRNA-33a/b and microRNA therapeutics. *J. Cardiol.* 67, 28–33
54 Mann, M.J. (2005) Transcription factor decoys: a new model for disease intervention. *Ann. N.Y. Acad. Sci.* 1058, 128–139
55 Kapoor, M. and Burgess, D.J. (2015) Targeted delivery of nucleic acid therapeutics via nonviral vectors. In *Targeted Drug Delivery: Concepts and Design. Advances in Delivery Science and Technology* (Devarajan, P. and Jain, S., eds), Springer, Cham
56 Whitehead, K.A. *et al.* (2009) Knocking down barriers: advances in siRNA delivery. *Nat. Rev. Drug Discov.* 8, 129–138
57 Deleavey, G.F. and Damha, M.J. (2012) Designing chemically modified oligonucleotides for targeted gene silencing. *Chem. Biol.* 19, 937–954
58 Sharma, V.K. *et al.* (2014) Antisense oligonucleotides: modifications and clinical trials. *MedChemComm* 5, 1454–1471
59 Wang, N. and Tall, A.R. (2017) A new approach to PCSK9 therapeutics. *Circ. Res.* 120, 1063–1065
60 Tanowitz, M. *et al.* (2017) Asialoglycoprotein receptor 1 mediates productive uptake of N-Acetylgalactosamine-conjugated and unconjugated phosphorothioate antisense oligonucleotides into liver hepatocytes. *Nucleic Acids Res.* 45, 12388–12400
61 Gebhard, C. *et al.* (2013) Apolipoprotein B antisense inhibition - update on mipomersen. *Curr. Pharm. Des.* 19, 3132–3142
62 Toth, P.P. (2016) Novel therapies for low-density lipoprotein cholesterol reduction. *Am. J. Cardiol.* 118 (6), 19A–32A
63 Santos, R.D. *et al.* (2015) Mipomersen, an antisense oligonucleotide to apolipoprotein B-100, reduces lipoprotein(a) in various populations with hypercholesterolemia: results of 4 Phase III trials. *Arterioscler. Thromb. Vasc. Biol.* 35, 689–699
64 Duell, P.B. *et al.* (2016) Long-term mipomersen treatment is associated with a reduction in cardiovascular events in patients with familial hypercholesterolemia. *J. Clin. Lipidol.* 10, 1011–1021
65 Thomas, G.S. *et al.* (2013) Mipomersen, an apolipoprotein b synthesis inhibitor, reduces atherogenic lipoproteins in patients with severe hypercholesterolemia at high cardiovascular risk: a randomized, double-blind, placebo-controlled trial. *J. Am. Coll. Cardiol.* 62, 2178–2184
66 Santos, R.D. *et al.* (2015) Mipomersen preferentially reduces small low-density lipoprotein particle number in patients with hypercholesterolemia. *J. Clin. Lipidol.* 9, 201–209
67 Panta, R. *et al.* (2015) Efficacy and safety of mipomersen in treatment of dyslipidemia: a meta-analysis of randomized controlled trials. *J. Clin. Lipidol.* 9, 217–225
68 Visser, M.E. *et al.* (2010) Effect of apolipoprotein-B synthesis inhibition on liver triglyceride content in patients with familial hypercholesterolemia. *J. Lipid Res.* 51, 1057–1062
69 Akdim, F. *et al.* (2010) Effect of mipomersen, an apolipoprotein B synthesis inhibitor, on low-density lipoprotein cholesterol in patients with familial hypercholesterolemia. *Am. J. Cardiol.* 105, 1413–1419
70 Raal, F.J. *et al.* (2010) Mipomersen, an apolipoprotein B synthesis inhibitor, for lowering of LDL cholesterol concentrations in patients with homozygous familial hypercholesterolaemia: a randomised, double-blind, placebo-controlled trial. *Lancet* 375, 998–1006
71 Santos, R.D. *et al.* (2015) Long-term efficacy and safety of mipomersen in patients with familial hypercholesterolaemia: 2-year interim results of an open-label extension. *Eur. Heart J.* 36, 566–575
72 Crooke, R.M. *et al.* (2005) An apolipoprotein B antisense oligonucleotide lowers LDL cholesterol in hyperlipidemic mice without causing hepatic steatosis. *J. Lipid Res.* 46, 872–884
73 Yu, R.Z. *et al.* (2009) Cross-species comparison of in vivo PK/PD relationships for second-generation antisense oligonucleotides targeting apolipoprotein B-100. *Biochem. Pharmacol.* 77, 910–919

74 Mullick, A.E. *et al.* (2011) Antisense oligonucleotide reduction of apoB-ameliorated atherosclerosis in LDL receptor-defi cient mice. *J. Lipid Res.* 52, 885–896

75 Shen, L. *et al.* (2013) The next generation of novel low-density lipoprotein cholesterol-lowering agents: proprotein convertase subtilisin/kexin 9 inhibitors. *Pharmacol. Res.* 73, 27–34

76 Horton, J.D. *et al.* (2009) PCSK9: a convertase that coordinates LDL catabolism. *J. Lipid Res.* 50 (Suppl), S172–177

77 Zhao, Z. *et al.* (2006) Molecular characterization of loss-of-function mutations in PCSK9 and identification of a compound heterozygote. *Am. J. Hum. Genet.* 79, 514–523

78 Lindholm, M.W. *et al.* (2012) PCSK9 LNA antisense oligonucleotides induce sustained reduction of LDL cholesterol in nonhuman primates. *Mol. Ther.* 20, 376–381

79 Graham, M.J. *et al.* (2007) Antisense inhibition of proprotein convertase subtilisin/kexin type 9 reduces serum LDL in hyperlipidemic mice. *J. Lipid. Res.* 48, 763–767

80 Frank-Kamenetsky, M. *et al.* (2008) Therapeutic RNAi targeting PCSK9 acutely lowers plasma cholesterol in rodents and LDL cholesterol in nonhuman primates. *Proc. Natl. Acad. Sci. U. S. A.* 105, 11915–11920

81 Fitzgerald, K. *et al.* (2017) A highly durable RNAi therapeutic inhibitor of PCSK9. *N. Engl. J. Med.* 376, 41–51

82 Ray, K.K. *et al.* (2017) Inclisiran in patients at high cardiovascular risk with elevated LDL cholesterol. *N. Engl. J. Med.* 376, 1430–1440

83 Fitzgerald, K. *et al.* (2014) Effect of an RNA interference drug on the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: a randomised, single-blind, placebo-controlled, Phase I trial. *Lancet* 383, 60–68

84 Adiels, M. *et al.* (2006) Overproduction of large VLDL particles is driven by increased liver fat content in man. *Diabetologia* 49, 755–765

85 Chan, D.C. *et al.* (2010) Effect of ezetimibe on hepatic fat, inflammatory markers, and apolipoprotein B-100 kinetics in insulin-resistant obese subjects on a weight loss diet. *Diabetes Care* 33, 1134–1139

86 Sanwald-Ducray, P. *et al.* (2010) Pharmacokinetics, pharmacodynamics, and tolerability of aleglitazar in patients with type 2 diabetes: results from a randomized, placebo-controlled clinical study. *Clin. Pharmacol. Ther.* 88, 197–203

87 Sundaram, M. *et al.* (2010) Functional analysis of the missense APOC3 mutation Ala23Thr associated with human hypotriglyceridemia. *J. Lipid. Res.* 51, 1524–1534

88 Atzmon, G. *et al.* (2006) Lipoprotein genotype and conserved pathway for exceptional longevity in humans. *PLoS Biol.* 4, e113

89 Graham, M.J. *et al.* (2013) Antisense oligonucleotide inhibition of apolipoprotein C-III reduces plasma triglycerides in rodents, nonhuman primates, and humans. *Circ. Res.* 112, 1479–1490

90 Sundaram, M. *et al.* (2010) Expression of apolipoprotein C-III in McA-RH7777 cells enhances VLDL assembly and secretion under lipid-rich conditions. *J. Lipid. Res.* 51, 150–161

91 Getz, G.S. *et al.* (2010) HDL apolipoprotein-related peptides in the treatment of atherosclerosis and other inflammatory disorders. *Curr. Pharm. Des.* 16, 3173–3184

92 Sharifov, O.F. *et al.* (2011) Apolipoprotein E mimetics and cholesterol-lowering properties. *Am. J. Cardiovasc. Drugs.* 11, 371–381

93 Nayyar, G. *et al.* (2012) Apolipoprotein E mimetic is more effective than apolipoprotein A-I mimetic in reducing lesion formation in older female apo E null mice. *Atherosclerosis* 224, 326–331

94 Yamamoto, T. *et al.* (2014) Locked nucleic acid antisense inhibitor targeting apolipoprotein C-III efficiently and preferentially removes triglyceride from large very low-density lipoprotein particles in murine plasma. *Eur. J. Pharmacol.* 723, 353–359

95 Alnylam (2014) *Alnylam Presents New Pre-Clinical Data on RNAi Therapeutic Programs for CardioMetabolic Diseases at American Heart Association Scientific Sessions 2014.* Alnylam

96 Norata, G.D. *et al.* (2015) Apolipoprotein C-III: from pathophysiology to pharmacology. *Trends Pharmacol. Sci.* 36, 675–687

97 Gaudet, D. *et al.* (2015) Antisense inhibition of apolipoprotein C-III in patients with hypertriglyceridemia. *N. Engl. J. Med.* 373, 438–447

98 Yang, X. *et al.* (2016) Reduction in lipoprotein-associated apoC-III levels following volanesorsen therapy: Phase 2 randomized trial results. *J. Lipid. Res.* 57, 706–713

99 Gaudet, D. *et al.* (2014) Targeting APOC3 in the familial chylomicronemia syndrome. *N. Engl. J. Med.* 371, 2200–2206

100 Digenio, A. *et al.* (2016) Antisense-mediated lowering of plasma apolipoprotein C-III by volanesorsen improves dyslipidemia and insulin sensitivity in type 2 diabetes. *Diabetes Care* 39, 1408–1415

101 Gaudet, D. *et al.* (2017) The approach study: a randomized, double-blind, placebo–controlled, Phase III study of volanesorsen administered subcutaneously to patients with familial chylomicronemia syndrome (FCS). *Atherosclerosis* 263, e10

102 Gouni-Berthold, I. *et al.* (2017) Apolipoprotein C-III inhibition with volanesorsen in patients with hypertriglyceridemia (COMPASS): A randomized, double-blind, placebo-controlled trial. *Atheroscler. Suppl.* 28, e1–e2

103 Tikka, A. and Jauhiainen, M. (2016) The role of ANGPTL3 in controlling lipoprotein metabolism. *Endocrine* 52, 187–193

104 Robciuc, M.R. *et al.* (2013) Angptl3 deficiency is associated with increased insulin sensitivity, lipoprotein lipase activity, and decreased serum free fatty acids. *Arterioscler. Thromb. Vasc. Biol.* 33, 1706–1713

105 Graham, M. *et al.* (2017) Cardiovascular and metabolic effects of ANGPTL3 antisense oligonucleotides. *N. Engl. J. Med.* 377, 222–232

106 Borodovsky, A. *et al.* (2013) A Subcutaneous Platform for RNAi Therapeutics Targeting Metabolic Diseases: PCSK9 and ANGPTL3. *Alnylam Pharmaceuticals*

107 Yu, X.X. *et al.* (2005) Antisense oligonucleotide reduction of DGAT2 expression improves hepatic steatosis and hyperlipidemia in obese mice. *Hepatology* 42, 362–371

108 Yamaguchi, K. *et al.* (2007) Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. *Hepatology* 45, 1366–1374

109 Lee, R.G. *et al.* (2013) Comparison of the pharmacological profiles of murine antisense oligonucleotides targeting apolipoprotein B and microsomal triglyceride transfer protein. *J. Lipid Res.* 54, 602–614

110 Price, N.L. *et al.* (2017) Genetic dissection of the impact of miR-33a and miR-33b during the progression of atherosclerosis. *Cell Rep* 21, 1317–1330

111 Rotllan, N. *et al.* (2013) Therapeutic silencing of MicroRNA-33 inhibits the progression of atherosclerosis in Ldlr–/– mice - Brief report. *Arterioscler. Thromb. Vasc. Biol.* 33, 1973–1977

112 Ouimet, M. *et al.* (2017) MicroRNA-33 regulates macrophage autophagy in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 37, 1058–1067

113 Rayner, K.J. *et al.* (2011) Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. *J. Clin. Invest.* 121, 2921–2931

114 Marquart, T.J. *et al.* (2013) Anti-miR-33 therapy does not alter the progression of atherosclerosis in low-density lipoprotein receptor-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* 33, 455–458

115 Goedeke, L. *et al.* (2014) Long-term therapeutic silencing of miR-33 increases circulating triglyceride levels and hepatic lipid accumulation in mice. *EMBO Mol. Med.* 6, 1133–1141

116 Viney, N.J. *et al.* (2016) Antisense oligonucleotides targeting apolipoprotein(a) in people with raised lipoprotein(a): two randomised, double-blind, placebo-controlled, dose-ranging trials. *Lancet* 388, 2239–2253

117 Yeang, C. *et al.* (2016) Effect of therapeutic interventions on oxidized phospholipids on apolipoprotein B100 and lipoprotein(a). *J. Clin. Lipidol.* 10, 594–603

118 Merki, E. *et al.* (2011) Antisense oligonucleotide lowers plasma levels of apolipoprotein (a) and lipoprotein (a) in transgenic mice. *J. Am. Coll. Cardiol.* 57, 1611–1621

119 Yu, R.Z. *et al.* (2016) Disposition and pharmacology of a GalNAc3-conjugated ASO targeting human lipoprotein (a) in mice. *Mol. Ther. Nucleic Acids* 5, e317

120 Marcovina, S.M. *et al.* (2017) Temporal variability in lipoprotein(a) levels in patients enrolled in the placebo arms of IONIS-ApoARx and IONIS-ApoA-LRx antisense oligonucleotide clinical trials. *J. Clin. Lipidol.* 12, 122–129

121 Tsimikas, S. *et al.* (2015) Antisense therapy targeting apolipoprotein(a): a randomised, double-blind, placebo-controlled Phase I study. *Lancet* 386, 1472–1483

122 Ehsan, A. *et al.* (2001) Long-term stabilization of vein graft wall architecture and prolonged resistance to experimental atherosclerosis after E2F decoy oligonucleotide gene therapy. *J. Thorac. Cardiovasc. Surg.* 121, 714–722

123 Mann, M.J. *et al.* (1999) Ex-vivo gene therapy of human vascular bypass grafts with E2F decoy: the PREVENT single-centre, randomised, controlled trial. *Lancet* 354, 1493–1498

124 Grube, E. *et al.* (2001) Phase II trial of the E2F decoy in coronary bypass grafting. *American Heart Association Annual Meeting (Late Breaking Clinical Trials)*, Anaheim, Calif

125 Conte, M.S. *et al.* (2006) Results of PREVENT III: a multicenter, randomized trial of edifoligide for the prevention of vein graft failure in lower extremity bypass surgery. *J. Vasc. Surg.* 43, 742–751

126 Alexander, J.H. *et al.* (2005) Efficacy and safety of edifoligide, an E2F transcription factor decoy, for prevention of vein graft failure following coronary artery bypass graft surgery: PREVENT IV: a randomized controlled trial. *JAMA* 294, 2446–2454

127 Lopes, R.D. *et al.* (2012) Edifoligide and long-term outcomes after coronary artery bypass grafting: PRoject of Ex-vivo Vein graft ENgineering via Transfection IV (PREVENT IV) 5-year results. *Am. Heart J.* 164, 379–386

128 Akdim, F. *et al.* (2010) Efficacy and Safety of mipomersen, an antisense inhibitor of apolipoprotein B, in hypercholesterolemic subjects receiving stable statin therapy. *J. Am. Coll. Cardiol.* 55, 1611–1618
129 Akdim, F. *et al.* (2011) Efficacy of apolipoprotein B synthesis inhibition in subjects with mild-to-moderate hyperlipidaemia. *Eur. Heart J.* 32, 2650–2659
130 Stein, E.A. *et al.* (2012) Apolipoprotein B synthesis inhibition with mipomersen in heterozygous familial hypercholesterolemia: results of a randomized, double-blind, placebo-controlled trial to assess efficacy and safety as add-on therapy in patients with coronary artery disease. *Circulation* 126, 2283–2292
131 Visser, M.E. *et al.* (2012) Mipomersen, an apolipoprotein B synthesis inhibitor, lowers low-density lipoprotein cholesterol in high-risk statin-intolerant patients: A randomized, double-blind, placebo-controlled trial. *Eur. Heart J.* 33, 1142–1149
132 McGowan, M.P. *et al.* (2012) Randomized, placebo-controlled trial of mipomersen in patients with severe hypercholesterolemia receiving maximally tolerated lipid-lowering therapy. *PLoS ONE* 7, e49006
133 Waldmann, E. *et al.* (2017) Effect of mipomersen on LDL-cholesterol in patients with severe LDL-hypercholesterolaemia and atherosclerosis treated by lipoprotein apheresis (The MICA-Study). *Atherosclerosis* 259, 20–25
134 (2018) MicroRNAs: novel molecular targets and response modulators of statin therapy. *Trends Pharmacol. Sci.* 39, 967–981