



Review article

Antisense therapy in the treatment of hypercholesterolemia

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ABSTRACT

Cardiovascular disease, the leading causes of death worldwide, is a “preventable” pathology, so that accessible and affordable interventions should be established to target the leading risk factors, including hypercholesterolemia. Although statin based therapy is commonplace in primary and secondary prevention, several economical, clinical and safety issues have been raised, so that there is ongoing research into new, safer and more effective agents to be used alone or in combination with existing cardiovascular drugs. Antisense oligonucleotides (ASOs) are a class of short, single-stranded synthetic analogs of nucleic acids that bind to a target mRNA, preventing its translation and thereby inhibiting protein synthesis. Apolipoprotein B-100 (apoB-100) is the major protein moiety of the atherogenic lipoproteins LDL and Lp(a), thus representing the ideal target for antisense therapy. Two anti-apoB100 (i.e., ISIS 301012 and ISIS 147764) and one anti-apolipoprotein(a) (i.e., ASO 144367) have already been developed and tested in some animal and human trials, providing promising results in terms of significant reduction of both LDL and Lp(a). Nevertheless, some safety issues – especially injection-site reactions and potential hepatotoxicity – have also emerged, thereby slowing down the large clinical diffusion of these agents. The present article provides an update on clinical data regarding antisense therapy targeting human apolipoproteins, highlighting the benefits and the potential risks of this innovative therapeutic approach for hypercholesterolemia and hyperlipoproteinemia(a).

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1. Epidemiology of cardiovascular disease

Cardiovascular disease typically encompasses a variety of vascular (arterial) occlusive disorders such as coronary heart disease (CHD), cerebrovascular disease (stroke) and peripheral artery disease (PAD). According to the World Health Organization (WHO) statistics, out of an estimated 58 million worldwide deaths from all causes in 2005, an estimated 17.1 million people die from CVD i.e., ~29% of all worldwide deaths (7.2 million from CHD and 5.7 million from stroke). Even more striking, the burden of CVD is expected to grow exponentially over the next 20 years, since nearly 23.6 million people are expected to die from CVD in the year 2030 [1].

Similar data are reported in the most recent statistics released by the American Heart Association (year 2007), where more than 2200 Americans are reported to die of CVD each day, reflecting one death every 39 s. The statistics for stroke are rather similar, in that 795,000 persons experience a new or recurrent stroke each year (610,000 first episode and 185,000 recurrent) [2]. As specifically regards blood lipid abnormalities, an estimated 33.6 million US adults ≥ 20 years of age

have total serum cholesterol levels ≥ 240 mg/dL, with an overall prevalence of 15.0% in the year 2008. Similarly, the prevalence of low density lipoprotein-cholesterol (LDL-C) ≥ 130 mg/dL was 31.9%, whereas that of high density lipoprotein-cholesterol (HDL-C) < 40 mg/dL was 18.9% [2]. Among the various disorders of lipoprotein metabolism, familial hypercholesterolemia (FH) is the most prevalent in the general population, heterozygous and homozygous individuals occurring any 1:500 and 1:1,000,000 individuals, respectively. This lipoprotein disorder follows an autosomal codominant pattern of inheritance and is mainly caused by mutations in the gene encoding the LDL receptor (LDLR). The LDLR binds apolipoprotein B-100 (apo B-100), the main protein moiety of LDL and Lipoprotein(a) (Lp(a)), thereby mediating endocytosis and removing of both lipoproteins from the circulation [3]. An additional cause of genetically determined hypercholesterolemia, with a frequency of ~1 in 1000 in Central Europe, is familial defective apoB-100 (FDB), which is due to mutations in the LDLR-binding domain of apoB-100 (especially the Arg3500Gln missense polymorphism) [3]. Besides FH and FDB, there are additional but less common forms of genetic hypercholesterolemia, such as mutations in the PCSK9 gene, autosomal recessive hypercholesterolemia (ARH), sitosterolemia (due to a defect in sterol efflux from cells), cholesterol 7 α -hydroxylase deficiency and polygenic hypercholesterolemia, this last condition being caused by the association of a susceptible genotype and one or more acquired conditions (e.g., atherogenic diet, obesity, sedentary lifestyle, etc.) [3].

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2. Statins in primary and secondary prevention of CVD

CVD is typically considered a “preventable” pathology, so that cost-effective (i.e., accessible and affordable) interventions for both people with established disease and those at higher risk of such disease should be established through population-wide interventions targeting the leading risk factors. According to the WHO “Guidelines for assessment and management of cardiovascular risk”, based on existing guidelines retrieved from the Cochrane Library, Embase Medline, the trials register of the International Society for Hypertension (ISH), and the British Medical Journal clinical effectiveness reviews, the leading interventions should be tailored to counteract the major biological cardiovascular risk factors, and thereby include reduction of cigarette smoking, body weight, blood pressure, hypercholesterolemia, hypertriglyceridemia and blood glucose [1]. As specifically concerns hypercholesterolemia, the effectiveness of cholesterol lowering agents such as acyl coenzyme A: cholesterol acyltransferase (HMG-CoA) reductase inhibitors (statins) is now well recognized in patients with established atherosclerotic disease and the overall benefit depends on the initial level of cardiovascular risk (i.e., the higher the risk, the greater the benefit) as well as on the overall achievable reduction in LDL-C. Although information on primary and secondary outcomes of statin use trials in primary prevention seems more limited so far, there is a general consensus that cholesterol lowering agents would be effective also in this setting. Since the benefits in primary prevention are highly dependent on the absolute cardiovascular risk, treatment should be specifically targeted at patients with highest total risk rather than at those with hypercholesterolemia only [1]. Recently, additional reasons have been provided to support a larger use of statins, attributable to the so-called “pleiotropic effect” as anti-inflammatory, immunomodulatory and antithrombotic agents [4].

Although statins represent a mainstay of primary and secondary prevention of CVD (and potentially venous thromboembolism) along with antihypertensive (e.g., in subjects with blood pressure at or above 160/100 mmHg) and hypoglycemic drugs (e.g., in individuals with persistent hyperglycemia or diabetes), several economical, clinical and safety issues have been raised, including the number of people needed to be treated in order to reduce one event or save one life, patient compliance or adherence to therapy, the evidence that a large number of high-risk patients do not reach the lipid goals required to provide personal benefits, especially those with genetic disorders of lipoprotein metabolism, and the potential side effects of these drugs [5]. In particular, primary prevention trials have provided reliable evidence that statin therapy might only be cost-effective in subjects with a high risk of developing cardiovascular disease ($\geq 20\%$ over 10 years). In contrast, despite the widespread held clinical and patient belief that statins are safe, adverse effects such as myalgia, and muscle weakness up to frank rhabdomyolysis have also been reported, especially in subjects with genetic predisposition [6]. As such, there is ongoing research to develop novel, safer and more effective agents to target selected categories of hypercholesterolemic patients (especially those carrying inherited disorders of lipoprotein metabolism), which could be used alone or in combination with existing cardiovascular drugs such as HMG-CoA reductase inhibitors, ezetimibe, bile acid transport inhibitors, squalene synthase inhibitors and other inhibitors of intestinal cholesterol absorption (e.g., disodium ascorbyl phytostanol phosphate) [4].

3. Antisense therapy against human apolipoproteins

The fats (i.e., unesterified cholesterol and triglycerides) are insoluble molecules in water and hence also in plasma. Accordingly, they are transported within the circulation incorporated within macromolecules called lipoproteins. The general structure of a lipoprotein consists in a “lipid” core containing triacylglycerols

and/or cholesteryl esters and a surface monolayer of phospholipids, unesterified cholesterol and specific proteins (i.e., apolipoproteins). The various lipoproteins differ mainly in composition, ratio of protein to lipids and in the type of apolipoprotein contained. They are basically classified according to their relative density, as follows: chylomicron, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), LDL, HDL and Lp(a). The major atherogenic lipoprotein particles are LDL and Lp(a), whereas HDL exerts a protective action, i.e., the traditional “reverse cholesterol transport” from peripheral tissues (e.g., arteries) to the liver. Conversely, the main function of both LDL and Lp(a) is to deliver cholesterol from the liver to the peripheral tissue. When the concentration of these two lipoproteins is particularly elevated, large amounts of cholesterol accumulate within the artery walls, which represent the hallmark of the atherosclerotic process. LDL and Lp(a) share a common protein (i.e., apoB-100) but mainly differ for the presence of the unique apolipoprotein(a) (apo(a)), which confers distinctive biological and highly atherogenic properties to Lp(a) (Fig. 1) [7–9]. Increased levels of apoB-100 and consequently of LDL-C – either due to inherited diseases or to abnormalities of apoB-100 metabolism (e.g., those characterizing diabetes mellitus and obesity) – are strongly associated with atherosclerosis and its occlusive complications such as CHD, stroke and PAD [10].

Antisense oligonucleotides (ASOs) are a class of short, single-stranded synthetic analogs of nucleic acids that bind to a target messenger RNA (mRNA), thereby preventing its translation into the corresponding protein. Upon ASO binding of mRNA and formation a hybrid, the consequent abnormal conformation leads to degradation of the mRNA by RNase H, a cellular nuclease with hybrid nucleic acid as substrates. As regards cardiovascular disease, apoB (i.e., apoB-100) appears an appealing target for pharmacological intervention on the assumption that suppression of apoB mRNA would also reduce total cholesterol, LDL-C and Lp(a) [11].

3.1. ISIS 301012

The ISIS 301012 (mipomersen-sodium), which specifically targets apoB-100 synthesis, has been the first ASO of its class to be developed and the most widely investigated in the treatment of dyslipidemia so far. This 20-nucleotide phosphorothioate oligonucleotide (5'-GCCTCAGTCTGCTTCGCACC-3') is a “second-generation” ASO, where nucleotides are linked with phosphorothioate linkages rather than the phosphodiester linkages and the sugar parts are represented by deoxyribose in the middle part of the molecule and

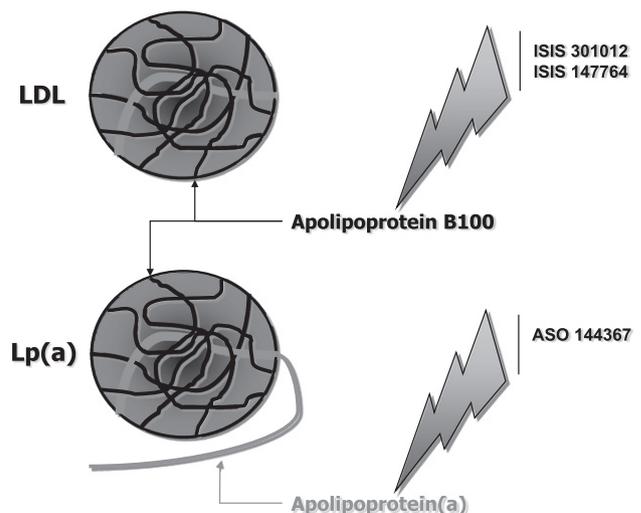


Fig. 1. Structure of low density lipoprotein (LDL) and Lipoprotein(a) (Lp(a)) and the leading targets of anti-apolipoprotein antisense oligonucleotides.

2'-O-methoxyethyl-modified ribose at the two ends, which prevents its degradation by nucleases thereby permitting an extended life and facilitating the need for less regular (i.e., weekly) administration [12]. The binding of ISIS 301012 to apoB mRNA triggers cellular ribonuclease H to hydrolyze RNA phosphodiester bonds, thereby inhibiting translation and synthesis of protein apoB [13].

The cross-species pharmacokinetics of this ASO has been originally assessed by Yu et al. in mouse, rat, monkey, and human [14]. Basically, the plasma pharmacokinetics after parental (i.v.) administration was rather similar among the different species, displaying a rapid distribution phase of several hours and a prolonged elimination phase of days, due to slow elimination from tissues. After subcutaneous (s.c.) administration, absorption was nearly complete with 80 to 100% bioavailability and modest interspecies variation attributable to body weight. The ASO circulated in plasma highly bound to plasma proteins, thereby limiting immediate renal clearance (i.e., short term excretion in urine was <3%). Only at higher doses (e.g., 25 mg/kg s.c. and 5-mg/kg i.v. in animals), was urinary excretion raised to $\geq 16\%$ within the first 24 h (due to overcoming of protein binding capacity). Nevertheless, renal clearance was confirmed as the leading catabolic pathway in the long term, as attested by 32% of dose recovered in urine by 14 days [14].

Kastelein et al. first conducted a double-blind, placebo, dose-escalation investigation in subjects with mild dyslipidemia [15]. An initial single dose of 50 to 200 mg of ISIS 301012 was administered (an additional 400 mg dose was only tested in two subjects), followed by a 4-week multiple-dosing regimen with the same dose. Pharmacokinetics analysis revealed that the maximum plasma concentration of ISIS 301012 after s.c. administration was dose-dependent, with a terminal elimination tissue half-life ranging from 23 to 31 days. The ISIS 301012 200 mg dose produced a remarkable decrease in the concentration of total cholesterol, LDL-C and apoB of 27%, 35% and 50%, 72 h after the last dose. The effect persisted up to 90 days after treatment in 75% of subjects. No significant changes were observed in HDL-C. No severe drug-related side effects were reported other than injection-site reactions (i.e., mild, painless erythema appearing within 24 h of s.c. injection and resolving spontaneously after ~5 days) occurring in 72% of patients. Although no abnormal changes in vital signs, electrocardiogram or urinalysis were recorded, 14% of patients showed an asymptomatic elevation of liver enzymes (only one patient >3 times the upper limit of the reference range [URL]) during the study period, which however was not associated with increase of bilirubin or prolongation of prothrombin time [15]. Merki et al. generated transgenic mice overexpressing human apoB (h-apoB-100 [h-apoB mice]) or h-apoB-100 plus human apo(a) to generate Lp(a) particles [Lp(a) mice], which were treated with ISIS 301012 for 4 to 11 weeks by intraperitoneal injection and then monitored for additional 10 weeks after the end of the therapy [16]. After 4 weeks of treatment, ISIS 301012 already reduced the plasma levels of total cholesterol and Lp(a) by 38% and 61%, respectively. In contrast, there was no effect on apo(a) levels or hepatic apo(a) mRNA expression, suggesting that the remarkable reduction obtained in plasma Lp(a) was entirely due to a reduction in apoB. Ten weeks after discontinuation of ISIS 301012 treatment both h-apoB-100 and Lp(a) levels had returned to baseline levels. Interestingly, the levels of proatherogenic oxidized phospholipids present on apoB (OxPL-apoB) also decreased significantly [16]. Interestingly, Akdim et al. assessed the efficacy and safety of ISIS 301012 in hypercholesterolemic subjects receiving stable statin therapy, carrying out a randomized, placebo-controlled, dose-escalation Phase 2 study [17]. The patients were administered ISIS 301012 at either 7 doses of 30 to 400 mg for 5 weeks, or 15 doses of 200 mg for 13 weeks. In the former group, total cholesterol, LDL-C and apoB levels were reduced by respectively 15%, 21% and 19% with doses of 100 mg/week; 15%, 27% and 24% with doses of 200 mg/week; 38%, 52% and 54% with doses of 300 mg/week, and 28%, 37% and 44% with doses of 400 mg/week, respectively. In the latter group total

cholesterol, LDL-C and apoB levels were reduced by 22%, 36% and 36%, respectively. In all groups HDL-C levels remained substantially unchanged. The most common side effects were injection site reactions accompanied by mild to moderate erythema (88 to 100% of patients), influenza-like illness (6 to 50% of patients), hepatic enzyme increase (6 to 50% of patients), fatigue (13 to 44% of patients), headache (20 to 38% of patients) and urinary tract infection (0 to 38% of patients) [17]. In a further randomized, double-blind, placebo-controlled, dose-escalation study, 8 subcutaneous doses of ISIS 301012 (ranging from 50 to 300 mg) were administered for 6 weeks to heterozygous FH patients undergoing conventional lipid-lowering therapy [18]. In patients receiving the 300-mg dose, the treatment was prolonged for an additional 7 weeks with once-per-week administration. After 6 weeks of therapy, total cholesterol declined from 10% to 28%, LDL-C from 11% to 34%, apoB from 8% to 33% and Lp(a) from 3% to 24%, respectively. After 13 weeks of ISIS 301012 300 mg weekly, total cholesterol, LDL-C, apoB and Lp(a) declined by 29%, 37%, 37% and 29%, respectively. In both cases HDL-C levels were unmodified throughout both study periods. Analogously to the previous study, the most common adverse effects included injection site reactions (97% of patients), headache (22% of patients), nasopharyngitis (19% of patients), myalgia and nausea (17% of patients). Liver aminotransferases levels significantly increased (≥ 3 times the URL) in 11% of patients, remained elevated 2 to 19 weeks after therapy and were accompanied with signs of steatosis on abdominal computed tomography [18].

Visser et al. carried out a double-blind study in patients with FH on continuing conventional lipid lowering therapy, who were randomized to receive a weekly s.c. dose of 200 mg ISIS 301012 or placebo [19]. After 13 weeks of therapy the patients receiving ISIS 301012 displayed a significant reduction of total cholesterol TC, LDL-C, apoB and Lp(a) by 16%, 22%, 20% and 20%, respectively. The concentration of HDL-C remained substantially unmodified at the end of the therapy. Although no serious side effects occurred in this study (e.g., no clinically significant elevations of liver enzymes, electrocardiogram changes or urinalysis abnormalities), injection site reactions following s.c. administration and erythema occurring 24 h after the injection were regularly observed in the vast majority of patients. Additional symptoms included influenza-like illness (70% of patients) and – in 20 to 30% of patients – also influenza (30% of patients), nasopharyngitis, headache, fatigue, myalgia, back pain, abdominal pain, nausea, and cough. ISIS 301012 administration was also associated with a non significant trend toward increase in intra-hepatic triglyceride content (1.2% at baseline versus 2.1% at week 15, respectively), with one out of the 13 subjects studied (i.e., 10%) progressing into new-onset hepatic steatosis [19].

Recently, a randomized, double-blind, placebo-controlled phase 3 study has been undertaken in nine lipid clinics in seven countries [20]. Fifty-one homozygous FH patients aged ≥ 12 years and already receiving lipid-lowering drugs were randomly assigned to ISIS 301012 200 mg s.c. every week ($n=34$) or placebo ($n=17$) for 26 weeks. The effect of ISIS 301012 on lipid endpoints was as follows: total cholesterol declined by 21%, LDL-C by 25%, apoB by 27% and Lp(a) by 31%, all changes being highly significant. Notably, the value of HDL-C also showed a significant trend toward increase (15.1%) whereas highly sensitive C Reactive Protein (hsCRP) was substantially unaffected by ISIS 301012 therapy. The most common adverse events included injection-site reactions (76% of patients) accompanied with erythema (56% of patients), hematoma at the injection site (35% of patients), pain (35% of patients), pruritus (29% of patients), discoloration (29% of patients), macule (15% of patients), papule (12% of patients), and swelling (12% of patients). Modest elevations of liver enzymes (<3 URL) occurred in 47% of the patients, whereas more clinically meaningful increases (≥ 3 URL) were recorded in 12% of patients [20].

While the FDA granted ISIS 301012 orphan drug status to treat FH in June 2006, this ASO is also in phase III clinical evaluation for the treatment

of a form of type IIa hyperlipoproteinemia, and hypercholesterolemia in patients with severely high cholesterol levels or at high risk for CHD [12].

3.2. ISIS 147764

ISIS 147764 is another 20-nucleotide phosphorothioate apoB-100 antisense compound (5'-GTCCTGAAGATGTCAATGC-3') which has been produced and tested in mouse models of hyperlipidemia [21]. In the first animal study carried out by Crooke et al., this ASO was proven effective to reduce liver apoB mRNA and serum apoB levels in a dose- and time-dependent fashion [21]. In particular, significant amounts of ISIS 147764 were detected in the liver 48 h after the first administration, increased steadily and reached a maximum level after 6 weeks. Forty eight hours after the first dose, both hepatic apoB-100 mRNA and liver protein were reduced by 50%. Accordingly, a reduction of 25–55% and 40–88% was recorded in total cholesterol and LDL-C, reaching the maximum decrease 4 to 6 weeks after treatment. These pharmacological effects disappeared 8 weeks after cessation of treatment, concomitantly with the gradual decrease of hepatic ASO levels. The elimination half-life was reported to vary between 11 and 19 days, mostly depending on the initial dose. Interestingly, ISIS 147764 was not associated with hepatic injury or intestinal steatosis and did not modify significantly the dietary fat absorption [21]. The anti-atherogenic effects of ISIS 147764 administered weekly at 25–100 mg/kg for 10–12 weeks were also assessed in hypercholesterolemic LDLR deficient (LDLR^{-/-}) mice [22]. In agreement with previous studies, this ASO produced a dose-dependent reduction of hepatic apoB mRNA as well as plasma LDL-C by 60–90% but, even more importantly, it reduced atherosclerotic disease severity within the aortic sinus (valve) and aorta in a dose-dependent manner, from 50 to 90% [22]. Straarup et al. also designed a series of locked nucleic acid (LNA) antisense oligonucleotides of different length (10- to 20-mers) with 100% sequence identity to the cynomolgus monkey and human apoB mRNA sequences, which were further administered to mice and non-human primates [23]. Although the 15-, 14-, 13- and 12-mer LNA oligonucleotides all potentially reduced apoB mRNA expression in a dose-dependent manner and with nearly identical IC50 values, the most effective was the 13-mer, since it was associated with a powerful and long lasting reduction in all the parameters studied in mice. Basically, the apoB mRNA expression was maximally reduced (up to 83%) at doses comprised between 10 and 15 mg/kg, at 24 and 48 h. Furthermore, the decline in hepatic apoB mRNA levels at the end of the 7-week study were 70%, 80% and 90% for lower doses of 1.0, 2.5 and 5.0 mg/kg, regardless of weekly or biweekly administration. Nevertheless, all these doses also affected HDL-C, producing a 40 to 60% decrease at the end of the study [23].

3.3. ASO 144367

At variance with other lipoproteins, the optimal therapeutic management of Lp(a) excess is still debated, since the plasma concentration of the protein is mostly genetically determined (up to 90%) and no safe and effective treatment exists so far other than nicotinic acid [24]. As such, antisense therapy targeted to the genetically polymorph apo(a) is an appealing perspective, which might even produce better lipid status endpoints than those already observed with ISIS 301012. Accordingly, ASO 144367 has been recently developed, and comprises 20-nucleotide phosphorothioate oligonucleotides with 5' 2'-O-(2-methoxyethyl) modified ribonucleosides at the 3' and 5' ends and 10 2'-deoxynucleosides in-between, targeted to position 174 within kringle (K) IV type 2 of the apo(a) transcript [25]. The compound was administered to 8K-apo(a) mice expressing 8 KIV repeats with a single copy of KIV-2, 8K-Lp(a) mice expressing both the 8 K apo(a) plus human apoB-100 and 12K-apo(a) mice expressing a 12K apo(a) with 3 KIV-2 repeats. After 6 weeks of therapy the compound determined a significant reduction of total cholesterol (–22%) and Lp(a) (–27%) in 8K-Lp(a) mice.

Lp(a) returned to baseline values after cessation of therapy (i.e., after 9 weeks), whereas total cholesterol remained decreased by 14% at week 12 off therapy. OxPL-apoB levels were also reduced by 17% at 3 weeks and 22% at 6 weeks of therapy, but returned to baseline by 9 to 12 weeks. In 12K-apo(a) mice the values of OxPL-apoB, OxPL-apo(a) and apo(a)-apoB were all significantly decreased by 64%, 82% and 93%, respectively [25].

4. Conclusions

Several clinical trials recently reviewed by Poli and Corsini [26] have confirmed that statins reduce CHD and CVD events in a broad spectrum of clinical conditions. Nevertheless, it has also been highlighted that some patients might not benefit from lipid-lowering treatment for the so-called “non-reversibility” of some cardiovascular risk factors, so that alternative therapeutic approaches against hypercholesterolemia might be established.

Antisense drugs are currently being investigated in a variety of diseases including solid and blood malignancies (e.g., renal cell carcinoma, acute myeloid leukemia, prostate, breast and non-small cell lung cancer), diabetes, amyotrophic lateral sclerosis, Duchenne muscular dystrophy and other pathologies such as asthma, arthritis and infectious disease such as chronic HIV infection, hepatitis C and West Nile virus [11]. Nevertheless, there are several aspects supporting the use of second- and third-generation ASOs instead of, or in combination with, traditional lipid-lowering therapy in CVD and hypercholesterolemic patients, especially those carrying inherited disorders of lipoproteins metabolism such as FH, FDB, ARH, mutations in the PCSK9 gene, sitosterolemia, cholesterol 7 α -hydroxylase deficiency and polygenic hypercholesterolemia. These include a superior efficacy over traditional statins especially in patients with inherited lipoproteins disorders, a longer therapeutic effect, fewer occurrences of side effects, friendlier means of delivery (e.g., by inhalation), their potential usefulness in statin-intolerant patients as well as the possibility to include multiple ASOs within one product to target multiple disease pathways [11]. Moreover, the evidence that ISIS 301012 does not exert clinically relevant pharmacokinetic interactions with the disposition, metabolism and clearance of other traditional lipid-lowering drugs such as simvastatin or ezetimibe would not hamper its use in combination statins as a potential adjunctive therapy for hypercholesterolemia [27] (Table 1).

As regards safety issues, the major concerns of anti-apoB OSA include injection-site reactions (observed in the vast majority of patients) and – even more importantly – the potential hepatotoxicity. According to reliable evidence it seems reasonable to conclude that the increase of liver enzymes would reflect an overload of hepatic triglycerides but no severe or irreversible parenchymal injury. In fact the increase of aminotransferases levels, observed in 15 to 20% of the patients treated with ISIS 301012, was not accompanied by elevation of other conventional markers of liver injury such as bilirubin or prothrombin time. It is also noteworthy that this side effect seems to be limited to anti-apoB ASO therapy, since the nearly 5000 patients who have been previously treated with ASOs against other targets had no evidence of high levels of liver enzymes [28]. At variance with ISIS 301012, the first two clinical studies with ISIS 147764 demonstrated that this ASO is equally effective in lowering LDL-C in hyperlipidemic mice but it did not cause hepatic steatosis. Although murine cholesterol homeostasis varies greatly from that of humans, these promising results make ISIS 147764 highly appealing for further phase 2 and 3 studies in humans. Only one study has been carried out with the anti-apo(a) ASO 144367 in mice. Overall, the outcomes in terms of total cholesterol and Lp(a) reduction are comparable to those observed with both ISIS 301012 and ISIS 147764, but the efficacy and safety of this compound in lowering hyperLp(a) in humans needs to be fully tested. Nevertheless, a suggestive approach in patients with a very high risk cardiovascular risk (e.g., those with extremely elevated

Table 1

Clinical efficacy of antisense oligonucleotides (ASOs) in lowering total (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), apolipoprotein B (apoB) and Lipoprotein(a) (Lp(a)).

Compound	Target	Experimental model	Outcome	Reference
ISIS 301012	apoB-100	Humans, mildly dyslipidemic	<ul style="list-style-type: none"> ■ TC reduction by 27% ■ LDL-C reduction by 35% ■ ApoB reduction by 50% ■ No change in HDL-C ■ TC reduction by 38% 	[15]
ISIS 301012	apoB-100	Mice	<ul style="list-style-type: none"> ■ Lp(a) reduction by 61% ■ No change in apo(a) and HDL-C ■ TC reduction by 15 to 38% 	[16]
ISIS 301012	apoB-100	Humans, hypercholesterolemic receiving stable statin therapy	<ul style="list-style-type: none"> ■ LDL-C reduction by 21 to 52% ■ ApoB reduction by 19 to 54% ■ No change in HDL-C ■ TC reduction by 10 to 29% 	[17]
ISIS 301012	apoB-100	Humans, hypercholesterolemic receiving stable statin therapy	<ul style="list-style-type: none"> ■ LDL-C reduction by 11 to 37% ■ ApoB reduction by 8 to 37% ■ Lp(a) reduction by 3 to 29% ■ No change in HDL-C ■ TC reduction by 16% 	[18]
ISIS 301012	apoB-100	Humans, familial hypercholesterolemia	<ul style="list-style-type: none"> ■ LDL-C reduction by 22% ■ ApoB reduction by 20% ■ Lp(a) reduction by 16% ■ No change in HDL-C ■ TC reduction by 21% 	[19]
ISIS 301012	apoB-100	Humans, homozygous familial hypercholesterolemia under lipid-lowering drugs	<ul style="list-style-type: none"> ■ LDL-C reduction by 25% ■ ApoB reduction by 27% ■ Lp(a) reduction by 27% ■ TC reduction up to 55% 	[20]
ISIS 147764	apoB-100	Mice	<ul style="list-style-type: none"> ■ LDL-C reduction up to 88% ■ TC reduction up to 78% 	[21]
ISIS 147764	apoB-100	Mice	<ul style="list-style-type: none"> ■ LDL-C reduction up to 87% ■ Atherosclerotic disease severity reduction up to 90% ■ LDL-C reduction up to 90% 	[22]
Locked nucleic acid antisense oligonucleotides	apoB-100	Mice	<ul style="list-style-type: none"> ■ Total cholesterol reduction by 22% ■ Lp(a) reduction by 27% 	[23]
ASO 144367	apo(a)	Mice	<ul style="list-style-type: none"> ■ Total cholesterol reduction by 22% ■ Lp(a) reduction by 27% 	[25]

Lp(a) levels and severe hypercholesterolemia) would be the combination of both anti-apoB and anti-apo(a) ASOs within one product, to target both Lp(a) apolipoproteins, since these patients are less likely to benefit from conventional hypocholesterolemic drugs.

5. Learning points

- Cardiovascular disease is a “preventable” pathology, so that accessible and affordable interventions should be established.
- Although statin-based therapy is widely used in primary and secondary prevention of cardiovascular disease, economical, clinical and safety issues have been raised.
- Antisense oligonucleotides (ASOs) are a class of short, single-stranded synthetic analogs of nucleic acids that bind to a target mRNA, preventing its translation and thereby inhibiting protein synthesis.
- Two anti-apolipoprotein B (i.e., ISIS 301012 and ISIS 147764) and one antiapolipoprotein(a) ASOs have already been developed and tested in some animal and human trials, providing promising results in terms of significant reduction of both low density lipoprotein-cholesterol and Lipoprotein(a).
- Some safety issues, including injection-site reactions and potential hepatotoxicity have emerged, slowing down the large clinical diffusion of these agents.

Conflict of interest

All authors have no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

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