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Review Articles

Antisense therapy and emerging applications for the management of dyslipidemia

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KEYWORDS:
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Mipomersen;
Safety

BACKGROUND: Because a significant percentage of patients who require high-dose statin therapy for dyslipidemia experience treatment-related muscle symptoms and an inconsistent clinical response, alternative or adjunctive approaches to the management of dyslipidemia are needed. One alternative approach, antisense therapy, may offer an effective and well-tolerated option for patients not satisfactorily responsive to or intolerant to standard pharmacologic dyslipidemia therapies.

OBJECTIVE: This review provides an overview of antisense technology and its potential role in the management of dyslipidemia.

METHODS: Source material was obtained primarily from the published literature identified through a search of the PubMed database.

RESULTS: Antisense technology is an evolving approach to therapy that has gone through a series of refinements to enhance molecular stability, potency, and tolerability. Mipomersen is an antisense molecule capable of producing clinically meaningful reductions in low-density lipoprotein cholesterol in patients with severe familial hypercholesterolemia. Further long-term clinical studies are required to more clearly quantify its impact on risk for cardiovascular events and establish whether it increases risk for hepatosteatosis.

CONCLUSION: Antisense therapy represents a potentially effective and well-tolerated emerging treatment modality for numerous diseases. In the treatment of hypercholesterolemia, the antisense therapy mipomersen may provide a possible treatment option for patients with treatment-resistant dyslipidemia.

Elevations in serum levels of low-density lipoprotein (LDL) cholesterol and apolipoprotein B (ApoB) are major modifiable risk factors in the pathogenesis of atherosclerotic cardiovascular disease.[1,2] A subgroup of patients with dyslipidemia—patients with familial hypercholesterolemia (FH)—experience pronounced elevations in LDL cholesterol and are at risk for accelerated atherosclerosis.[3] FH represents a genetic disorder attributable to a great number of mutations in the LDL receptor gene resulting in marked reductions in LDL receptor binding and LDL clearance and elevations in serum LDL cholesterol levels.[3,4]

Recent clinical studies highlight the role of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors or statins in achieving Adult Treatment Panel (ATP) III lipid goals for the management of dyslipidemia and in reducing the risk of cardiovascular disease.[5] Moreover, high-dose statin treatment remains a cornerstone of the multidrug therapy required to manage FH.[3,5] However, for many patients,

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Table 1 Selected antisense compounds in development

Compound	Target	Indication	Phase
Fomivirsen	CMV	CMV retinitis	Approved
Oblimersen sodium	Bcl-2	Chronic lymphocytic leukemia	3
Alicaforsen/ISIS 2302	ICAM-1	Ulcerative colitis	3
Mipomersen/ISIS 301012	ApoB-100	Cardiovascular	3
GTI-2040	RNR R2	Renal cell carcinoma	2
ISIS 113715	PTP-1B	Diabetes	2
OGX-011	Clusterin	Prostate/breast/non-small-cell lung cancer	2
AVI-4020	C-myc	Cardiovascular restenosis	1/2
Cand5	VEGF	Wet AMD	2
		Diabetic neuropathy	Preclinical
SPC2996	Bcl-2	Chronic leukemia	1/2

AMD, age-related macular degeneration; ApoB-100, apolipoprotein B 100; CMV, cytomegalovirus; PTB, protein tyrosine phosphatase 1B; RNR, ribonucleotide reductase; VEGF, vascular endothelial growth factor.

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high-dose statin therapy is unacceptable because of intolerable adverse events, especially myalgia or motor weakness.[6,7] The prediction of muscular risk in observational conditions (PRIMO) study, the first large-scale study to examine the risk of moderate-to-severe muscle symptoms in patients taking high-dose statin therapy in clinical practice, demonstrated that muscle symptoms occurred in 10.5% of patients, typically within 1 month of starting therapy.[8] Muscle pain was so severe in 38% of patients that it prevented even moderate physical exertion, whereas 4% were confined to bed. In addition, for high-risk patients, such as those with FH, ATP III guidelines advocate intense lipid-lowering therapy to achieve at least a 30% to 40% reduction in LDL cholesterol.[5]

Despite high-dose statin therapy, up to 60% of patients with FH do not achieve the ATP III goal.[9] These findings underscore the need for an alternative or an adjunct to maximal-tolerated doses of lipid-lowering therapies for patients with potentially intractable conventional approaches to the management of dyslipidemia, especially FH. One emerging approach to dyslipidemia management is antisense therapy. Antisense therapy may offer a potentially safe and effective option for high-risk patients not satisfactorily responsive to or intolerant to standard therapy for dyslipidemia.

Antisense technology and concept

Antisense therapy has provided a targeted and generally well-tolerated mechanism for the management of a number of disorders.[10,11] Fomivirsen is an antisense agent that was approved in 1998 for the treatment of cytomegalovirus-induced retinitis in patients with AIDS. Since that time, the list of antisense compounds in development (Table 1) and the number of potential therapeutic applications have grown.

Antisense therapy represents a conceptually straightforward approach to disease treatment.[12] With the mapping of the human genome, it is now possible to specifically target proteins independently, an approach that is potentially more safe and predictable for treating complex metabolic diseases than conventional, less-specific pharmacologic approaches.[7] If the genetic sequence encoding a specific disease-related protein is known, molecules can be designed to bind the corresponding messenger RNA (mRNA), hindering translation and protein biosynthesis (Fig. 1).[13] The nucleotide sequence of DNA or RNA containing the amino acid sequence of the target protein is called the sense strand, whereas the complementary nucleotide sequence is the antisense strand.[13] Essentially, antisense therapy uses an oligonucleotide sequence that is complementary to, and thus binds with, a specific mRNA sequence. Antisense oligonucleotides (ASOs) control gene expression at the translational level and are metabolized by cellular exonucleases and endonucleases, not by the hepatic CYP450 system.[14] As a consequence, ASOs often manifest a reduced potential for drug–drug interactions, an especially important feature for patients who typically receive multidrug therapy, as is the case for most patients with FH.[3]

Other oligonucleotides that act at the transcriptional level form triplexes with genomic DNA called triplex-forming oligonucleotides.[15] A third approach, RNA interference, has also been established as a highly efficient method for suppressing gene expression through the use of small, interfering RNA molecules.[16]

Pharmacokinetic and drug-delivery challenges

The stability of ASO remains a primary challenge. Rapid ASO degradation by serum and cellular nucleases results in a relatively short plasma half-life, the formation of potentially cytotoxic byproducts, and the need for frequent dosing of the ASO.[15] Another persistent and daunting technical challenge has been to consistently deliver specific ASOs to target cells

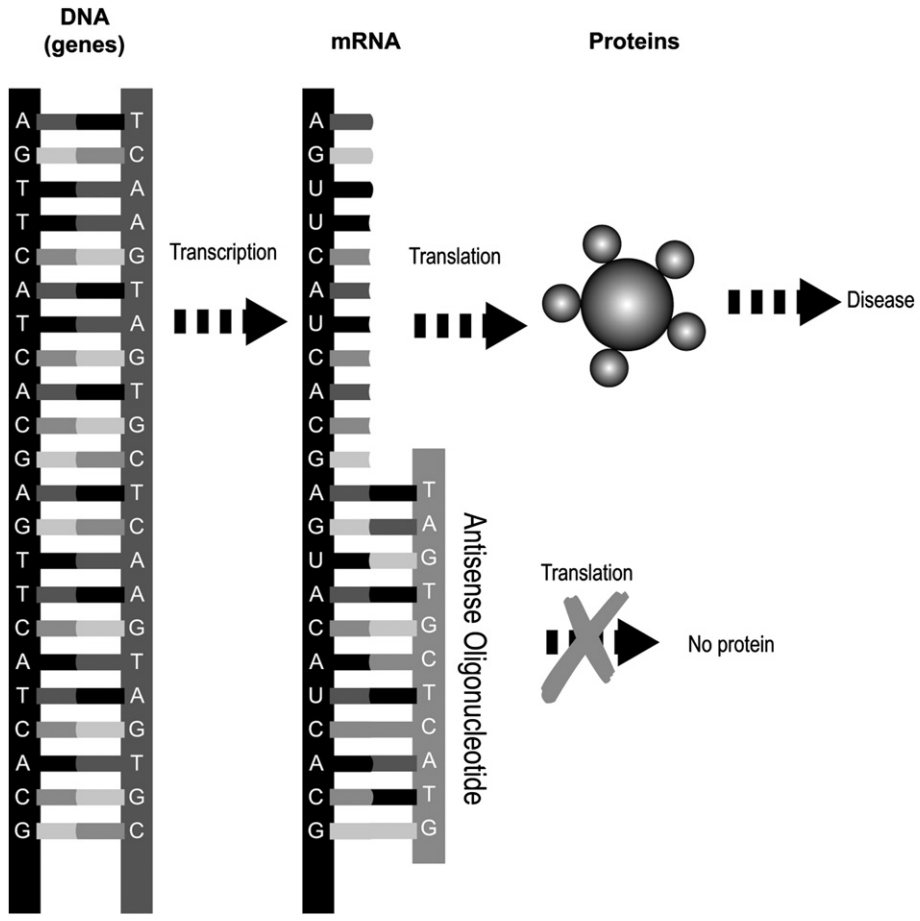

Figure 1 Antisense concept. With antisense therapy, oligonucleotides control gene expression at the translational level by binding to mRNA. (Reprinted with permission from Antisense Therapeutics Limited © Copyright 2011. Technology overview. antisense.com.au.)

in various organs and tissues. Uptake of free ASOs into the cell is inefficient because charged ASOs must cross a hydrophobic cell membrane, a process that thermodynamically hinders transport.[16]

Several macromolar delivery systems have been developed that can promote efficient cellular uptake and protect the bound ASOs from degradation in biological fluids. Examples include dendrimers that are characterized by highly branched three-dimensional structures, biodegradable polymers, and ASO-binding nanoparticles.[16] In addition, receptor-mediated endocytosis has been used as a strategy for effectively targeting ASOs. In this process, ASOs are conjugated to antibodies specifically recognized by certain receptors that mediate their cellular uptake.[16]

ASOs' mechanism of action

ASOs comprise relatively short (13–25 nucleotides), chemically modified, single-stranded DNA molecules that bind via Watson-Crick hybridization with a unique RNA sequence.[12,13] The minimum antisense ASO length required to recognize a specific gene is 12 to 15 base pairs, with a minimum of 12 base pairs required for stable hybridization under physiologic conditions.[15]

ASOs act via either one of two principal mechanisms to achieve hybridization (Fig. 2). Most ASOs activate RNase H, which cleaves the RNA moiety of the DNA-RNA heteroduplex, degrading the target mRNA but leaving the ASO intact.[7,16] ASOs that do not induce RNase H can inhibit translation by steric ribosomal blockade. Unlike steric blocking ASOs, RNase H–dependent ASO can target any region of the mRNA.[17] ASOs also can prevent the maturation of mRNA by splicing the RNA or destabilizing pre-mRNA.[7] All of these mechanisms reduce protein biosynthesis.

ASO evolution: structural modifications

Unmodified ASOs are rapidly degraded; consequently, stabilization of the ASO molecule remains a principal goal in improving the efficacy of antisense therapy.[16] Approaches to enhance ASO stability involve modifications to the base, ribose sugar (especially at the 2′ ribose position), and to the phosphate backbone (Fig. 3).[14,16] The chemical backbone of ASOs has been modified in recent years to bolster nuclease resistance, and, consequently, the antisense effect.[11] Nuclease resistance does not, however, automatically confer efficient delivery of the ASO

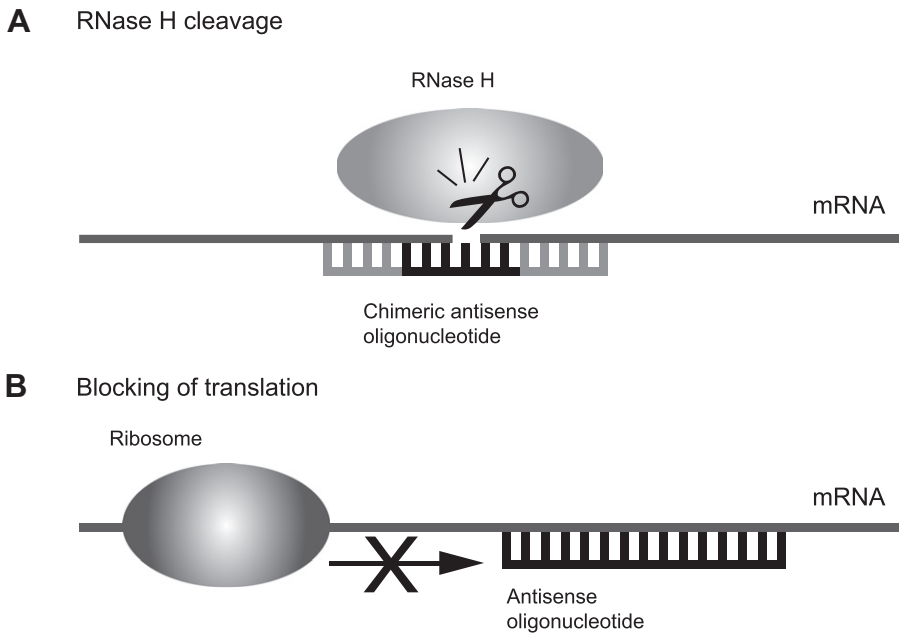

Figure 2 Antisense mechanism of action. A, RNase H cleavage induced by (chimeric) antisense oligonucleotides. B, Translational arrest by blocking the ribosome. (Reprinted with permission of Blackwell Publishing Ltd. from Kurreck.[16])

molecule to the target site. For antisense therapy to be effective, ASOs must distribute to the target organ and be taken up by the target cells. After intravenous administration, the greatest accumulation of ASOs occurs in the liver and kidneys, suggesting that these organs may represent natural targets for this form of therapy.[11] In the synthesis of ASO molecules, structural modifications have been made with the goal of preserving biological activity while enhancing nuclease resistance, cell penetration, and tolerability.[18]

First-generation ASOs

First-generation ASOs—chiefly represented by the phosphorothioate (PS)-ASOs—have been the most widely studied.[16] PS-ASOs were designed to render the internucleotide linkages more resistant to nuclease attack by replacing one of the nonbridging oxygen atoms in the phosphate group (phosphodiester bond) with sulfur (Fig. 4A).[15] However, the PS backbone of PS-ASOs binds to certain proteins, particularly those that interact with polyanions, such as heparin-binding proteins, including platelet-derived growth factor and vascular endothelial growth factor, yielding cellular toxicity at high doses. This dose-limiting cellular toxicity has led to cardiovascular collapse and death in nonhuman primates.[12,16] In addition, first-generation ASOs have been linked to transient inhibition of the clotting cascade, resulting in prolongation of clotting times in nonhuman primates.[19] Moreover, compared with their corresponding phosphodiester ASOs, PS-ASOs display a lower affinity for their complementary RNA molecules and, thus, reduced potency.[20]

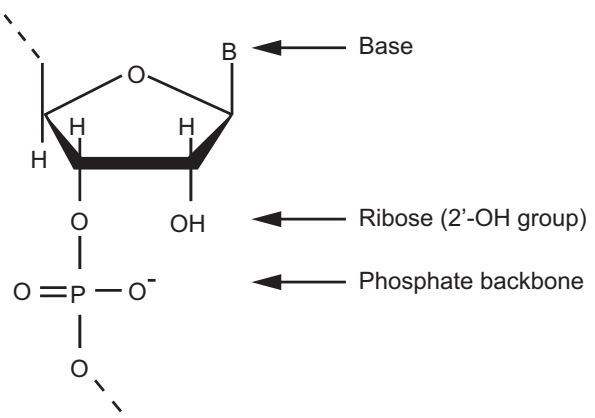

Figure 3 Targets for oligonucleotide modification. (Reprinted with permission of Blackwell Publishing Ltd. from Kurreck.[16])

Second-generation ASOs

Second-generation ASOs have overcome some of the limitations associated with PS-ASOs. These ASOs contain nucleotides with alkyl modifications at the 2′ position of the ribose, represented chiefly by 2′-*O*-methyl (2′ OMe) and 2′-*O*-methoxyethyl (2′ MOE) modifications (Fig. 4B). Second-generation ASOs manifest less toxicity than PS-ASOs and a greater affinity and specificity for their complementary RNA sequence.[7,15,21] In vitro assays suggest that these modifications yield a 3- to 10-fold increase in potency compared with first-generation ASOs.[19] Because the 2′ alkyl-modified ASOs do not recruit RNase H, their antisense effect can be conveyed only by steric blockade of translation.[16]

For most antisense therapies, however, targeted RNA cleavage by RNase H remains desirable to maintain antisense potency.[16] Gapmer or gap-based technology has been used to retain RNase H activity. With this approach, 2′ OMe or 2′ MOE is inserted on the flanking 3′ and 5′ "wings" of

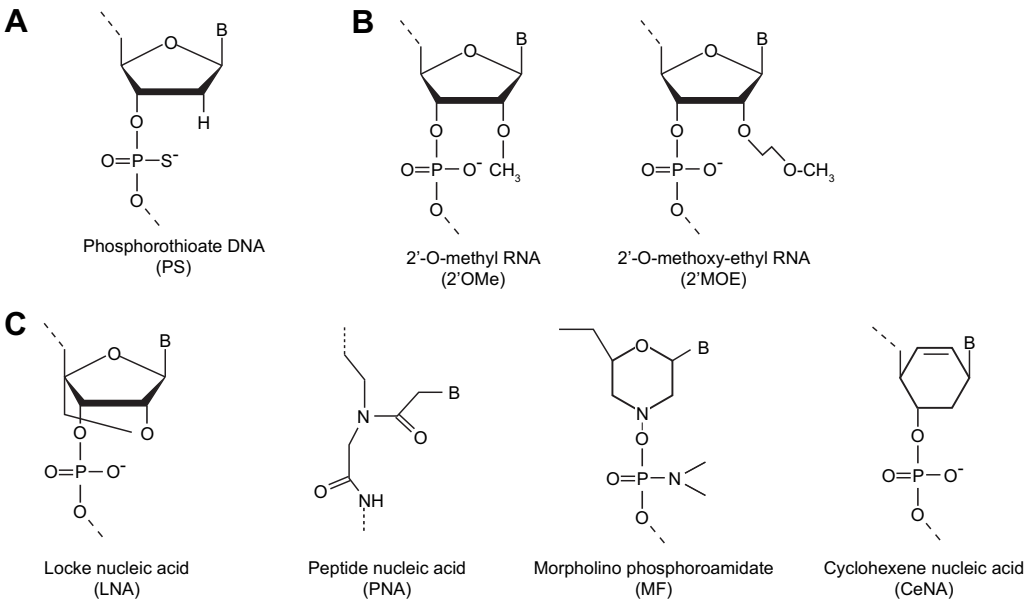

Figure 4 (A) First-generation oligonucleotide. (B) Second-generation oligonucleotides. (C) Selected third-generation oligonucleotides. (Reprinted with permission of Blackwell Publishing Ltd. from Kurreck.[16])

the ASO molecule, a configuration in which the internal nucleotides retain RNase H activity; the modified wings also diminish exonuclease activity.[7,11]

Mixed-backbone ASO (MBO) modifications, which combine 2′ OMe PS-ASOs and methylphosphonate central linkages, have been shown to attenuate polyanionic activity and enzymatic degradation and to enhance both affinity to RNA and RNase activity.[22,23] In addition to central modifications, various sugar modifications have been introduced to increase binding affinity and nuclease resistance.[15] For instance, altering the stereochemistry of the molecule, that is, changing the ribose glycosidic linkage configuration to the α-isomer, can boost nuclease stability, although it also undermines hybridization stability and ability to activate RNase H.[15]

Third-generation ASOs

Numerous classes of third-generation ASOs have emerged in recent years that display improved target affinity, nuclease resistance, and pharmacokinetics.[18] For these ASOs, the concept of conformational restriction has been widely used to bolster binding affinity and stability.[24] Several promising third-generation ASOs are discussed in this section.

Locked nucleic acid (LNA), a ribonucleotide containing a methylene bridge that connects the 2′-oxygen of the ribose with the 4′-carbon, represents one of the most promising approaches to modifying ASOs (Fig. 4C).[25] LNAs display increased complementary DNA and RNA affinities, RNase H activation, and stability against nucleases.[25] Recently, investigators have shown that LNA ASOs can be taken up by cells without the use of delivery technology such as liposome-based delivery through a process dubbed gymnosis, that is, by simply adding LNA ASOs without transfection reagents to the medium.[2] When LNA ASOs are delivered via gymnosis in cell cultures, a shorter LNA ASO sequence (12 base pairs) acts more potently than a longer sequence (16 base pairs), with a shorter sequence yielding a 2.5-fold greater decrease in ApoB mRNA expression in vitro.[2] The absence of toxic lipid reagents in this process suggests that it may provide an attractive alternative to standard liposome-based ASO delivery.

Peptide nucleic acids (PNAs), one of most intensively studied DNA analogs, display high sequence specificity for the recognition of single- or double-stranded DNA.[17] With PNAs, the deoxyribose phosphate backbones are replaced by polyamide linkages. PNA ASOs store sequence information in a manner similar to that of DNA so that the same nucleotide bases form specific G:C and A:T base pairs with a complementary DNA strand.[26] PNA-DNA or PNA-RNA strands form stable duplex structures with higher thermal stability and fewer mismatched base pairs. Because PNA ASOs contain a nonnatural polyamide backbone, these molecules are resistant to degradation by nucleases and proteases.[26] PNAs display favorable hybridization kinetics and a high degree of biological stability, with robust specificity for PNA/DNA duplex formation, but they do not elicit target RNA cleavage by RNase H.[16,17]

Morpholino oligomers represent nonionic DNA analogs in which the ribose phosphodiester bond is replaced by a morpholino moiety and phosphoramidite linkage.[15,16] The backbone alteration renders morpholino ASOs resistant to nucleases, and the absence of a negative charge renders the molecule less likely to interact nonselectively with cellular proteins. Despite their altered backbone, morpholino ASOs bind to complementary nucleic acid sequences no more tightly than the binding of the analogous DNA and

RNA oligomers, thus requiring the use of relatively long, 25-base pair ASOs to achieve effective antisense inhibition.[27] In addition, because of limited morpholino ASO cellular uptake, higher dosing is required.[16]

Cyclohexane nucleic acids, which include a 6-membered unsaturated ring, form a duplex with RNA that is more stable than the DNA-RNA duplex.[28] These ASOs are characterized by a high degree of conformational rigidity and stability that protect against enzymatic degradation.[16] Cyclohexane nucleic acid- RNAs only weakly activate RNase H, however, with a catalytic rate 600-fold less than that of the natural DNA-RNA duplex.[28]

Antisense therapy and dyslipidemia

Antisense therapy in lipid management is an approach that chiefly focuses on lowering LDL cholesterol by blocking the formation and secretion of hepatic very low–density lipoprotein (VLDL) particles by targeting apolipoprotein B-100 (ApoB-100), a protein necessary for VLDL secretion.[29] Indeed, ApoB is contained within each atherogenic particle in plasma—VLDL, LDL, intermediate-density lipoprotein, and lipoprotein (a)—and, thus, represents a high-value therapeutic target in the treatment of dyslipidemia.[7,30] An ApoB paradigm may even replace the LDL receptor paradigm since ApoB is involved with both aspects of cholesterol homeostasis, plasma LDL secretion and clearance where the LDL paradigm reflects LDL clearance only.[31] Accordingly, Sniderman et al suggest that the information from non-HDL-C and ApoB should be combined for a more accurate cardiovascular risk assessment.[32] Because the liver is one of the main sites of ASO distribution, these antisense molecules may be ideal candidates for reducing ApoB production and hepatic VLDL secretion. ASO therapy may be a particularly useful approach in patients with FH. These patients respond inconsistently to lipid-lowering therapies that act by up-regulating hepatic LDL receptors, such as statins.[33] However, in FH, the LDL receptor gene itself has undergone mutation, yielding a receptor that no longer effectively removes LDL particles.

Microsomal triglyceride transfer protein is essential for the assembly of VLDL and, in animal models, its inhibition has been shown to diminish plasma levels of triglycerides, cholesterol, and ApoB-containing lipoproteins[34]; however, ASO inhibition of microsomal triglyceride transfer protein in a murine model yielded hepatic and intestinal steatosis.[35]

A second-generation 2′ MOE-modified mouse-specific ASO inhibited ApoB-100 protein formation in a time-dependent and dose-responsive fashion, without inducing hepatic or intestinal steatosis.[35] Other investigators also have shown that short LNA ASOs (12–13 base pairs) display high target affinity against ApoB with no liver toxicity in mice and nonhuman primates.[36] Furthermore, in transgenic mice, 2′ MOE-modified ASO (mipomersen sodium) directed against human ApoB-100 mRNA reduced ApoB to near undetectable levels.[37] Together, these findings support the concept that antisense inhibition of ApoB may be an effective and well-tolerated strategy for the management of dyslipidemia in humans.

Mipomersen

Efficacy findings

Mipomersen is a second-generation 2′ MOE-modified 20-nucleotide ASO directed towards the coding region of human ApoB-100 RNA. Essentially, mipomersen inhibits the hepatic synthesis of ApoB-100. The mipomersen clinical trial program is summarized in Table 2. Human studies of mipomersen have yielded encouraging results. In early clinical trials, mipomersen rapidly reduced serum ApoB-100, LDL cholesterol, and total cholesterol levels.[37] In some volunteers, ApoB-100 and LDL cholesterol levels were reduced by 60% and 54%, respectively. In a randomized, double-blind, placebo-controlled, dose-escalation study (50–300 mg) that included 44 patients with heterozygous FH, Akdim et al[38] found that mipomersen treatment for 6 weeks yielded 21% and 34% reductions in LDL cholesterol from baseline in the 200-mg and 300-mg groups, respectively. In this study, patients continued to receive standard lipid-lowering treatment, mainly statins.[38] In a separate randomized, double-blind, placebo-controlled study in 51 patients with homozygous FH receiving maximum tolerated doses of lipid-lowering drugs, Raal and colleagues demonstrated that weekly mipomersen treatment at a dose of 200 mg subcutaneously for 26 weeks reduced LDL cholesterol by 25%.[39] The findings of these studies suggest that mipomersen may provide an effective and less burdensome adjunctive therapy for patients who might otherwise need to rely on LDL apheresis for lipid management.[40]

Caveats

In the first of these studies, 11% (4/36) of the patients receiving mipomersen experienced elevations in liver transaminase levels at least three times the upper limit of normal, with signs of steatosis. Most of these patients, however, were in the high-dose group (300 mg weekly).[38] Nevertheless, in the second study, alanine aminotransferase concentrations of more than three times the upper limit of normal occurred in 12% (4/34) of patients receiving mipomersen at a dose of 200 mg for 26 weeks, with one patient demonstrating evidence of steatosis.[39] Clearly, additional investigation is warranted to clarify the relationship between hepatic enzyme elevations and hepatic steatosis with mipomersen treatment at all clinical doses. Hepatic steatosis is frequent in ApoB-defective genetic forms of familial hypobetalipoproteinemia[41] and, thus, may be an inherent risk with ApoB-100 antisense therapy. Simple hepatic steatosis usually follows a benign course, however, and only rarely progresses to cirrhosis.[40] Nonetheless, increased hepatic fat may amplify the deleterious effects of central adiposity, insulin resistance, and potentially hepatotoxic agents including alcohol.[41]

Table 2 Overview of mipomersen clinical development trials

Phase	Title	N	Subjects	Purpose	Completion date
Phase 1	A Study to Evaluate the Effect of Mipomersen on Cardiac Repolarization Conducted in Healthy Subjects	60	Healthy	Assess the electrocardiogram (ECG) effects of mipomersen	May 2010
	A Study to Determine the Effects of Multiple Doses of Mipomersen (200 mg SC) on the Pharmacodynamics and Pharmacokinetics of Single-dose Warfarin	18	Healthy	Assess how blood clotting and thinning time is affected when a single dose of warfarin is given alone or with mipomersen	May 2010
	Study to Evaluate 3 Different Dosing Regimens of Mipomersen Administered Via Subcutaneous Injections to Healthy Volunteers	80	Healthy	Evaluate 3 different dosing regimens of mipomersen to determine how much of the drug is present in the circulation	March 2010
Phase 2	Open Label Extension of ISIS 301012 to Treat Familial Hypercholesterolemia	22	Familial hypercholesterolemia	Evaluate safety and efficacy	September 2011
	Measure Liver Fat Content After ISIS 301012 Administration	38	Varying degrees of risk for hepatic steatosis	Evaluate the effect that mipomersen has on liver triglyceride content	March 2010
	Safety and Efficacy Study of 301012 Administration in High Risk Statin Intolerant Subjects (ASSIST)	33	Intolerance to statins	Evaluate safety and efficacy	October 2010
	Study of ISIS 301012 (Mipomersen) in Heterozygous Familial Hypercholesterolemia Subjects on Lipid Lowering Therapy	44	Heterozygous familial hypercholesterolemia	Assess the safety and efficacy of varying doses of ISIS 301012 (mipomersen) as add-on therapy	
Phase 3	Study to Assess the Safety and Efficacy of ISIS 301012 (Mipomersen) in Homozygous Familial Hypercholesterolemia (RADICHOL 1)	51	Homozygous familial hypercholesterolemia	Evaluate safety and efficacy	April 2009
	Safety and Efficacy of Mipomersen in Patients With Severe Hypercholesterolemia on a Maximally Tolerated Lipid-Lowering Regimen and Who Are Not on Apheresis	58	Severe hypercholesterolemia	Evaluate safety and efficacy	October 2010
	Safety and Efficacy of ISIS 301012 (Mipomersen) As Add-on Therapy in High Risk Hypercholesterolemic Patients	158	High cholesterol and high risk for cardiovascular disease	Evaluate safety and efficacy	October 2010
	Efficacy and Safety Study of ISIS 301012 (Mipomersen) as Add-on in Familial Hypercholesterolemic Subjects With Coronary Artery Disease (RADICHOL II)	124	Heterozygous familial hypercholesterolemia and coronary artery disease	Evaluate safety and efficacy	May 2010
	An Open-label Extension Study to Assess the Long-term Safety and Efficacy of ISIS 301012 in Patients With Familial Hypercholesterolemia or Severe-Hypercholesterolemia	143	Familial hypercholesterolemia or severe hypercholesterolemia	Evaluate safety and efficacy of extended dosing (52 weeks)	April 2010

Data from U.S. National Institutes of Health. Search results for mipomersen. ClinicalTrials.gov. http://clinicaltrials.gov/ct2/results?term=MIPOMERSEN+. Accessed March 1, 2011.

In both randomized, placebo-controlled studies, injection-site reactions were the most common adverse events.[38,39] However, immune reactions may represent a potentially greater concern. Although antibodies to mipomersen have not been detected, careful long-term monitoring is prudent.[38]

Conclusions

Antisense therapy represents a promising and evolving approach to the management of numerous diseases in which a specific abnormality has been identified as a primary or major contributing etiologic factor. Although ASO therapy has been fraught with methodological challenges, many have been resolved, and its relative conceptual simplicity and specificity, versus conventional pharmacologic interventions, remain intriguing and attractive aspects from a therapeutic perspective. ASO therapy targeting ApoB synthesis appears to be a promising adjunctive treatment for refractory dyslipidemia in patients with FH who have not responded adequately or are intolerant to statins or conventional combination therapy. Clinical studies to date have established the feasibility and therapeutic benefits of antisense adjunctive therapy in the treatment of hypercholesterolemia. However, long-term studies are still required to establish safety in terms of the risk for steatohepatitis.

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