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Chapter 2

Apolipoprotein B synthesis inhibition; results from clinical trials

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Abstract

**Purpose of review**
Mipomersen is a second generation antisense oligonucleotide developed to inhibit the synthesis of apolipoprotein B-100 in the liver. In this review we will summarize the results of recent preclinical and clinical studies addressing safety and low-density lipoprotein cholesterol (LDL-c) lowering efficacy of this promising new compound.

**Recent findings**
In phase III clinical trials, mipomersen has been shown to significantly reduce LDL-c in patients with homozygous and heterozygous familial hypercholesterolemia (FH) on maximally tolerated lipid-lowering therapy. Injections site reactions, flu-like symptoms and increases in liver transaminases were the main adverse events. A recent safety study, designed to investigate the effects of mipomersen on intrahepatic triglyceride content, failed to show evidence of clinically relevant hepatic steatosis after 13 weeks of treatment.

**Summary**
Mipomersen is a promising new agent to lower LDL-c in patients at increased risk of cardiovascular disease (CVD) and/or intolerant to statins. Whereas safety concerns have focused on hepatic fat accumulation, to date no evidence of clinically relevant increases of intrahepatic triglyceride content are reported. Ongoing and future studies are eagerly awaited to assess the impact of mipomersen on hepatic triglyceride content after prolonged exposure.
Introduction

Cardiovascular disease (CVD) is the most prevalent condition in western society and is associated with premature morbidity and mortality. The most important risk factor for CVD is low-density lipoprotein-cholesterol (LDL-c). As a consequence LDL-c lowering is the main focus of the current treatment guidelines in the management of CVD-risk\(^1\). Many clinical end-point trials have substantiated that LDL-c lowering is associated with a decreased CVD risk with more aggressive lowering being associated with the most significant CVD risk reduction\(^2\)-\(^4\). As a result, clinical practice guidelines continue to lower LDL-c goals for patients at high risk for CVD. Target goals for patients with existing CVD and additional risk factors are now set at <70 mg/dL (<1.8 mmol/L)\(^1\). A substantial proportion of patients remain, however, unable to achieve these target goals for LDL-c\(^5\)-\(^7\) despite the introduction of powerful statins and the use of combination lipid-lowering therapy. This includes both patients with familial hypercholesterolemia (FH), who have extremely high baseline LDL-c levels and are at increased risk of premature atherosclerotic disease\(^8\),\(^9\) but also patients who are intolerant to statins mostly due to severe myalgia or myositis\(^10\). Therefore, alternative therapeutic strategies, effectively lowering LDL-c as monotherapy or on top of statins, are required for patients at high risk of CVD who are not on target or intolerant to statins. Mipomersen, an apolipoprotein B (apoB) synthesis inhibitor, has been put forward as a promising new candidate to meet this emerging need\(^1\);\(^11\);\(^12\).

Apolipoprotein B

ApoB is a large protein that occurs in the plasma in two isoforms, apoB48 and apoB100. ApoB48 is exclusively expressed in the intestine and is present on chylomicrons and chylomicron remnants. ApoB100 is mainly expressed in the liver and is present on all atherogenic lipoprotein particles for example very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL and Lipoprotein a [Lp(a)]. ApoB100 is required for the formation of VLDL in the liver; binding of apoB to the microsomal transport protein (MTP) results in the incorporation of lipids into the apoB100 molecule and leads to the formation of triglyceride-rich VLDL particles\(^13\). In the bloodstream VLDL triglycerides are hydrolyzed by the enzyme lipoprotein lipase (LPL)\(^14\). The action of LPL results in the transformation of triglyceride-rich VLDL into IDL and eventually cholesterol-rich LDL. Following secretion by the liver, apoB remains tightly bound to its lipoprotein particle\(^15\). Therefore a single apoB molecule can be found on all atherogenic lipoprotein particles for example VLDL, IDL and LDL\(^16\). In clinical practice, apoB can be used...
as a marker to estimate the total number of atherogenic lipoprotein particles. Furthermore elevated apoB is a hallmark of several inherited disorders associated with atherosclerosis\textsuperscript{17,18}. In contrast, patients with extremely low levels of apoB (<5\textsuperscript{th}percentile) due to familial hypo-beta lipoproteinemia (FHBL), seem to be protected against CVD\textsuperscript{19,20}. On the basis of these observations apoB has been proposed to be an attractive target to reduce CVD risk. Mipomersen, an apoB synthesis inhibitor, is the first agent available for human use to directly target apoB.

Figure 1  Antisense mechanism of action

A) Sequence-specific recognition by Watson-Crick base pair interactions, B) RNase H is an endogenous nuclease that hydrolyzes the RNA strand of a DNA:RNA duplex. Reduction in target mRNA levels by ASOs that support the RNase H mechanism has proven to correlate directly with reduction in target protein levels.

Figure 2  Mipomersen is functionalized to support endogenous RNase H activity.
Antisense oligonucleotides (ASOs) are short, single-stranded, synthetic analogs of natural nucleic acids that inhibit production of a target protein through sequence-specific binding to the cognate messenger RNA (mRNA) (figure 1). Binding of an ASO to a complementary mRNA, by Watson-Crick hybridization, induces selective degradation of the mRNA and prohibits translation of the selected mRNA into protein. Degradation of the mRNA is largely mediated by endogenous RNase-H which recognizes the mRNA-ASO complex (figure 2)\(^{12}\). Mipomersen is a second-generation ASO designed to specifically inhibit synthesis of the human apolipoprotein B-100 by the liver. Second-generation antisense inhibitors are characterized by greater potency, reduced potential for non-specific side effects and longer half-life, compared to the first-generation ASO\(^{21}\). After subcutaneous administration, ASO’s distribute predominantly to the kidney and the liver\(^{12}\). The latter makes ASO’s suitable for the inhibition of apoB100 since apoB100 is selectively synthesized in the liver. Synthesis of apoB48 in the intestine is unaffected by ASO’s. Cytochrome P450 is not involved in mipomersen metabolism and potential drug-drug interactions are anticipated to be minimal\(^{22}\).

**Clinical experience**

After thorough evaluation of both pharmacokinetics and efficacy of antisense drugs in animal models, mipomersen has been investigated in clinical trials. In Phase I and II studies in multiple patient populations including primary hypercholesterolemia and FH, mipomersen has been shown to result in significant dose-dependent and prolonged reductions in LDL-c (24-70%) either when used as a single agent or in combination with other lipid-lowering medicine\(^{23-25}\). In addition to LDL-c, mipomersen lowered serum apoB, non-high density lipoprotein cholesterol (non-HDL-C), triglycerides and Lp(a). A dose of 200 mg mipomersen once weekly administered subcutaneously was selected for further evaluation in Phase III clinical trials. Reductions in LDL-c lasted up to 4 weeks after the last dose, corresponding to a mean terminal half-life of mipomersen of approximately 30 days. Consistent with the long half-life it is estimated that steady-state will not be achieved before 6 months of treatment. Pharmacokinetic studies showed no clinically relevant interactions of mipomersen with the disposition and clearance of simvastatin or ezetimibe, and vice versa, supporting the use of mipomersen in combination with oral lipid-lowering agents\(^{26}\).

Recently the results of the first phase III clinical trials have been presented. A double blind randomized placebo-controlled global multicentre trial was designed to determine safety and efficacy of mipomersen on top of lipid-lowering drugs...
in patients with homozygous FH. A total of 51 patients were randomized 2:1 to 200 mg mipomersen or placebo. After 26 weeks of treatment, a mean reduction in LDL-c was observed of 25% in the mipomersen-treated group versus 3% for placebo (p<0.001). In addition patients treated with mipomersen experienced a 27% reduction in apoB and a 21% reduction in total cholesterol. Statistically significant reductions were also observed in other atherogenic lipids, including triglycerides (18%) and Lp(a) (31%). Reduction in LDL-c in this study showed wide variability with changes ranging from 2% to -82%, with a majority of patients exhibiting an 18% or greater reduction in LDL-c. The treatment effect was independent of baseline LDL-c, age, race or sex. Although four patients carrying the same LDL-receptor gene mutations demonstrated smaller changes in LDL-c concentration compared to other participants, in general, there was no correlation between the LDL receptor mutation and response to therapy.

Preliminary data from a second phase III trial were consistent with the results of previous clinical trials. This double-blind randomized placebo controlled study was conducted in 124 patients with heterozygous FH and a history of coronary heart disease on maximally tolerated statin therapy. After 26 weeks of treatment the mean reduction from baseline for LDL-c was 28%. Average LDL-c at baseline for all patients was 150 mg/dL (3.9 mmol/L) compared to 104 mg/dL (2.7 mmol/L) at the end of the study for all patients treated with mipomersen. The LDL-c treatment goal for high risk patients of <100 mg/dL (2.5 mmol/L) was achieved by 45% of the mipomersen-treated patients.

Lp(a) is an LDL-like particle that is synthesized by the liver and consists of an apoB molecule covalently linked to a large glycoprotein, apolipoprotein(a). Since apoB is an essential component for the synthesis of Lp(a), the reductions in Lp(a) observed at higher doses of mipomersen, are most likely the result of reduced availability of apoB in the liver following apoB synthesis inhibition. Although the physiological function of Lp(a) remains unknown, Lp(a) has been identified as a risk factor for CVD. Unfortunately Lp(a) concentrations are unresponsive to statin therapy. Hence, mipomersen could represent a novel therapeutic approach targeting Lp(a). Nevertheless, since agents exclusively lowering Lp(a) are not available, it remains to be established if Lp(a) reductions may truly contribute to cardiovascular benefit.
Safety

Mipomersen is well tolerated. Common adverse events include injection site reactions (ISRs), flu-like symptoms and increases in alanine aminotransferase (ALT). These side effects are generally no reason for discontinuation of treatment. The most common adverse events are ISR following subcutaneous administration of mipomersen. More than 90% of the patients experience ISRs. ISRs are generally characterized by a painless transient erythema that occurs within 24 hours after the injection. ISRs do not worsen on repeated dosing. Histological analysis has shown activated polymorphonuclear leukocytes and macrophages. There was no evidence for necrosis, abscess formation, ulceration or giant cell reactions. In addition to the acute responses at injection sites, there have been two types of delayed responses for example hyperpigmentation and reappearance of erythema. Hyperpigmentation may be a common response to skin injury for people with high Fitzpatrick skin classifications. However, the pathophysiological concept for reappearance of the erythema is unknown. In clinical studies of other ASO's similar injection site responses have been observed. Whereas measurements for antibodies thus far have all been negative, careful monitoring will be needed to ensure that long-term administration of mipomersen does not lead to auto-immunity or treatment resistance.

Flu-like symptoms have been reported for some patients in the clinical studies. The flu-like symptoms appear shortly after mipomersen injection, resolve within 1-2 days and are generally limited to the first few doses of mipomersen. Flu like symptoms after mipomersen administration may be secondary to pro-inflammatory activation although, thus far, no significant changes in hsCRP during mipomersen therapy have been reported.

The most important long-term safety concerns regarding apoB synthesis inhibition have focused on potential intrahepatic triglyceride accumulation. Since apoB is essential for the transport of VLDL from the liver, lowering apoB concentration may result in reduced secretion of triglyceride-rich VLDL-particles from the liver. Inhibition of VLDL export from the liver may lead to accumulation of triglycerides in the liver. In fact this was the case with the inhibition of MTP, a protein acting in the same pathway as apoB. Pharmacological inhibition of MTP has been shown to result in significant increases in intrahepatic triglyceride content in both experimental animals and humans. Furthermore, the majority of patients with FHBL, the ‘natural variant’ of low apoB, are characterized by profound hepatic steatosis. By contrast, preclinical studies with apoB synthesis inhibition did not result in hepatic triglyceride accumulation. Interestingly, in these animal models,
intrahepatic triglyceride concentrations were even lower in the animals treated with apoB synthesis inhibition compared to placebo-treated animals. Results from microarray analysis of liver mRNA from these experimental models suggested a compensatory decrease in fatty acid synthesis combined with an increase in fatty acid oxidation during apoB synthesis inhibition. Such effects may in part explain the absence of intrahepatic triglyceride concentration following apoB synthesis inhibition in animal models.

In human, increases in ALT >3x the upper limit of normal have been observed in ~10% of patients, particularly following treatment with higher doses of mipomersen. ALT increases were accompanied by lesser aspartate aminotransferase increases but not by increases in total bilirubin, alkaline phosphatase, prothrombin time or by decreases in albumin. The laboratory findings were also not accompanied by symptoms or other clinical signs suggestive of impairment of hepatic function. After discontinuation of treatment, transaminases returned to normal in all patients.

The exact cause for transaminase elevations during mipomersen treatment is thus far unclear. Transaminase increases have been suggested to result from a direct pharmacologic effect of mipomersen, but increased transaminases may also point to the accumulation of fat in the liver. Indeed, hepatic steatosis was detected during follow-up of patients with elevated liver enzymes following mipomersen treatment. Unfortunately baseline values were absent in most of these patients and since hepatic steatosis is prevalent in the general population, a definite causal relationship between mipomersen administration and hepatic steatosis could not be established.

To evaluate the effects of mipomersen on intrahepatic triglyceride-content, a randomized, double-blind, placebo-controlled study was designed in 21 patients with heterozygous FH on conventional lipid lowering therapy. Patients received a weekly subcutaneous dose of 200 mg mipomersen or placebo for 13 weeks while continuing conventional lipid-lowering therapy. The primary endpoint was change in intrahepatic triglyceride-content as measured by localized proton magnetic resonance spectroscopy (¹H-MRS). One in 10 patients (10%) in the mipomersen-treated group developed mild hepatic steatosis (5.7%) which was reversible following mipomersen discontinuation. For the group, there was a trend towards an increase in intrahepatic triglyceride content. Whereas this study clearly shows the absence of a profound steatotic response of the liver following mipomersen treatment, it should be taken into account that this study does have some limitations. First the group size was modest and patients at increased risk for hepatic steatosis were excluded from the trial. Second, treatment period was relatively short given that tissue concentrations of mipomersen do not reach
steady state before 26 weeks of treatment. Since apoB concentration in the liver may determine the extent of hepatic triglyceride accumulation, the findings from this study do not exclude the possibility of mipomersen inducing hepatic steatosis following a longer treatment period.

Steatosis observed in non alcoholic fatty liver disease (NAFLD) usually follows a benign course although with the development of non-alcoholic steatohepatitis the risk of progression into fibrosis and cirrhosis increases\cite{40,41}. However, hepatic steatosis following apoB lowering may differ from NAFLD. In fact, although long-term follow-up data on FHBL patients are scarce, it is believed that FHBL rarely if even, results in progressive liver disease\cite{42}. Nevertheless, the increased hepatic triglyceride content in FHBL may increase the susceptibility to the effects of metabolic factors such as insulin resistance and abdominal fat and alcohol\cite{43}. Since hepatic steatosis is a potentially serious adverse event, future studies should focus on the effects of long term use of mipomersen on intrahepatic triglyceride content including patients with increased risk of hepatic steatosis.

**Future**

At present a randomized placebo controlled phase II study is being conducted, investigating safety and efficacy of mipomersen in 30 patients intolerant to statins and at high risk for CVD. Furthermore two phase III trials evaluating the administration of mipomersen 200 mg for 26 weeks are currently ongoing in respectively 58 patients with severe hypercholesterolemia and in 158 hypercholesterolemic patients at high risk for coronary heart disease. With proven LDL-c lowering efficacy, future trials will focus predominantly at safety and compliance of long-term administration of the compound.

**Conclusion**

Mipomersen is a first-in-class apoB synthesis inhibitor currently in phase III development as a new treatment strategy to lower LDL-c in patients at high risk of CVD not on target or intolerant to statins. Mipomersen administration, either alone or on top of statins, has been shown to result in significant reductions in LDL-c and all other atherogenic lipoprotein particles including Lp(a). Mipomersen is well tolerated. Safety concerns related to the drug centre around potential hepatic triglyceride accumulation. Whereas increases in liver enzymes have been observed to date, proof of significant hepatic steatosis has not become evident.
Future safety studies evaluating the effects of long-term use on intrahepatic triglyceride content are required prior to broader use of this compound.
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Reference List


