Low-density lipoproteins (LDLs) are a proven causal risk factor for the development of atherosclerotic cardiovascular disease. Plasma levels of LDL cholesterol (LDL-C) and its major protein, apolipoprotein B (apoB), are positively associated with incident cardiovascular events. Individuals with genetic conditions of extremely high LDL-C develop premature cardiovascular disease; conversely, those with genetically low LDL-C are protected from cardiovascular disease. Interventions that reduce LDL-C, including statins and bile acid sequestrants, are proven to reduce cardiovascular risk. Indeed, therapy to reduce LDL-C is a cornerstone of the treatment and primary prevention of atherosclerotic cardiovascular disease.

Despite the commercial availability of 3 major classes of LDL-lowering drugs (statins, bile acid sequestrants, and a cholesterol absorption inhibitor), a substantial unmet need has remained with regard to LDL-lowering treatment. Some patients are intolerant of statins because of myalgias. Others have genetic conditions that substantially elevate their LDL-C and make it challenging to achieve desirable LDL-C levels even with combination therapy. Perhaps the single best example of a genetic condition in which patients frequently fail to achieve acceptable LDL-C levels despite aggressive therapy is familial hypercholesterolemia (FH). This condition, classically caused by loss-of-function mutations in the LDL receptor, is associated with substantially elevated LDL-C and premature atherosclerotic cardiovascular disease. Patients with heterozygous FH typically have untreated LDL-C levels in the range of 200 to 400 mg/dL, and many cannot achieve desirable LDL-C levels on available combination therapy. Patients with 2 mutant LDL receptor alleles have homozygous FH (hoFH) with LDL-C levels usually >400 mg/dL and cannot achieve desirable LDL-C levels on available therapy. There are other causes of autosomal-dominant hypercholesterolemia resulting from mutations in the receptor-binding region of apoB (the ligand for the LDL receptor) and gain-of-function mutations in PCSK9 (a protein that targets the LDL receptor for degradation), and these patients can resemble patients with heterozygous FH in their clinical presentation and difficulty in achieving adequate LDL-C control.

LDL-C levels are regulated by the rate of LDL production and the rate of LDL catabolism (Figure 1A). The liver synthesizes and secretes very-low-density lipoproteins (VLDLs), which are lipolyzed in the plasma compartment and eventually converted to LDL. Thus, the hepatic production of VLDL is a major determinant of plasma levels of LDL-C. LDL is catabolized largely by the liver via the LDL receptor, and this process plays a key role in determining plasma LDL-C levels. Importantly, the 3 major classes of LDL-lowering drugs (statins, bile acid sequestrants, and cholesterol absorption inhibitor) all work by reducing cellular cholesterol in the hepatocyte and thus resulting in upregulation of the LDL receptor in the liver. This explains why patients with LDL receptor defects, particularly those with mutations in both alleles, respond less well to these therapies (Figure 1A). Indeed, in controlled trials of patients with hoFH, high doses of potent statins reduced LDL-C by only 0% to 25%, bile acid sequestrants by 0% to 10%, and ezetimibe by 0% to 10%. The variability in response is likely due to variation in the amount of residual functional LDL receptor activity, with those patients with no receptor activity (receptor negative) having essentially no response to drugs that work through LDL receptor upregulation.

Therefore, there has long been interest in developing therapies that work by reducing VLDL production and thus reducing LDL-C levels in a manner that is independent of the LDL receptor. Within the past year, 2 new first-in-class drugs have been approved by the US Food and Drug Administration that reduce LDL-C by inhibiting VLDL production through different mechanisms (Figure 1B). Here, we review the concepts and data behind both of these drugs.

Lomitapide and Microsomal Triglyceride Transfer Protein Inhibition

Microsomal Triglyceride Transfer Protein (MTP) is an endoplasmic reticulum–localized protein that transfers neutral lipids, especially triglycerides, onto newly synthesized apoB.
as a critical step in the assembly of chylomicrons in the intestinal enterocyte and VLDL in the hepatocyte. The physiological importance of MTP in humans was demonstrated by the discovery that loss-of-function mutations in the gene encoding MTP (MTTP) are the proximate cause of the rare genetic condition abetalipoproteinemia. Abetalipoproteinemia is characterized by marked hypocholesterolemia with the absence of apoB-containing lipoproteins in plasma. Patients with this condition were shown to lack MTP protein in the intestine and subsequently to have loss-of-function mutations in both alleles of the MTTP gene. In the absence of functional MTP, lipidation of apoB in the endoplasmic reticulum does not occur, and it is targeted for proteasomal degradation, leading to failure of secretion of both chylomicrons by the intestine and VLDL by the liver. Because VLDL serves as the metabolic precursor to LDL, LDL-C is also absent in the plasma.

The physiological consequences of the failure to secrete apoB-containing lipoproteins include impaired absorption of dietary fat and fat-soluble vitamins and reduced ability to transport vitamin E from the liver to the periphery (a function of the VLDL-LDL pathway). Patients have impaired oral fat tolerance with gastrointestinal symptoms on eating a high-fat meal. In addition, as a result of reduced export of lipid, hepatic fat is generally increased. The most important clinical manifestation of the disease is progressive retinal and spinocerebellar degeneration caused by neurological tissue deficiency of vitamin E. Because abetalipoproteinemia is very rare, there are no systematic studies of its relationship to cardiovascular disease; a few case reports have reported that on autopsy patients had no atherosclerotic vascular disease.

Lomitapide: An Inhibitor of MTP Activity
The discovery of the MTTP mutations in abetalipoproteinemia spurred interest in the concept that pharmacological inhibition of MTP could be a strategy to reduce LDL-C. One of the first MTP inhibitors to be discovered and to enter into clinical development was BMS-201038, now known as lomitapide. Lomitapide is a small molecule that inhibits lipid transfer by directly binding to MTP in the liver and intestines. In in vitro experiments using unilamellar vesicles, lomitapide inhibited rat, hamster, and human MTP with an inhibitory concentration of 50% (IC_{50}) of 5 to 7 nmol/L. In vitro studies demonstrated a reduction in apoB secretion by hepatocytes on treatment with lomitapide. Lomitapide was shown to substantially reduce plasma cholesterol in several hypercholesterolemic animal models, including Watanabe heritable hyperlipidemic rabbits, an animal model of hoFH. In a Zucker rat model of hyperlipidemia, administration of lomitapide reduced plasma cholesterol and triglycerides, improved insulin sensitivity, and reduced atherogenesis. Another MTP inhibitor, implimatide, reduced plasma cholesterol and inhibited progression of atherosclerosis in hypercholesterolemic apoE-deficient mice.

MTP Inhibition With Lomitapide: Early Clinical Studies and Pharmacology
Lomitapide was evaluated in a phase 1 placebo-controlled, ascending-multiple-dose study designed to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of ascending doses of 10, 25, 50, and 100 mg lomitapide or placebo administered once daily for 14 days in 36 modestly hypercholesterolemic subjects (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/2038858_juxtapid_toc.cfm). All subjects in the 10-, 25-, and 50-mg dose cohorts received 14 days of dosing as planned; dosing in the 100-mg dose group was stopped after day 8 as a result of gastrointestinal symptoms. Results of the study showed that the decreases from baseline in LDL-C were dose dependent, with maximal decreases noted between days 8 and 11; reductions in LDL-C were ≥30% with 10 mg, 55% with 25 mg, 70% with 50 mg, and 85% with 100 mg.

Consistent dose-response results were observed in the phase 2 studies in which the response of hypercholesterolemic subjects to lomitapide was evaluated. The LDL-C reduction achieved with the 5-mg dose was generally 14% to 19%, with the 10-mg dose was 30% to 37%, and with the 25 mg dose was ≥65%. An additive reduction in LDL-C was observed...
when lomitapide was coadministered with other lipid-lowering drugs. For example, lomitapide 10 mg was administered alone or in combination with ezetimibe 10 mg to moderately hypercholesterolemic subjects in a placebo-controlled trial. Lomitapide 10 mg alone reduced LDL-C by 30% and in combination with ezetimibe by a significantly greater 46%. In another study, lomitapide 10 mg reduced LDL-C by 37% and in combination with atorvastatin reduced LDL-C by 50% (www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203858Orig1s000MedR.pdf).

Lomitapide is administered orally and is readily absorbed, with a high first-pass effect in the liver. It has a terminal half-life of ≈29 hours, consistent with the time it takes to reach pharmacokinetic steady-state (6 days), and its pharmacokinetics appears approximately dose proportional between 10 and 50 mg after multiple-dose oral administration. A plateau in the LDL-C–lowering effect is observed after 14 days of daily dosing.

Lomitapide is extensively metabolized by CYP3A4 and is a direct inhibitor of CYP3A4 (www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203858Orig1s000MedR.pdf). Specifically, coadministration of the CYP3A4 inhibitor ketoconazole with lomitapide substantially increased plasma concentrations of lomitapide. Conversely, coadministration of lomitapide 60 mg with simvastatin 40 mg increased exposure to simvastatin acid ≈1.7-fold compared with simvastatin alone. Pharmacokinetic interaction studies with other lipid-lowering drugs showed minimal interactions. In addition, lomitapide increases warfarin plasma concentration by ≈30%, so vigilance should be exercised in patients taking warfarin.

Lomitapide in hoFH

Given its mechanism of action in reducing VLDL secretion, it was reasonable to speculate that lomitapide would be effective in reducing LDL-C levels in hoFH. Indeed, a study in Watanabe heritable hyperlipidemic rabbits, which are homozygous for an LDL receptor mutation, have severe hypercholesterolemia, and thus are a natural animal model for hoFH, supported this concept. Treatment of Watanabe heritable hyperlipidemic rabbits with lomitapide markedly reduced plasma levels of LDL-C. A subsequent study confirmed that this effect was due to reduced VLDL secretion. Additional studies in LDL receptor–deficient mice confirmed that MTP inhibition was effective in reducing LDL-C in the absence of the LDL receptor.

On the basis of these preclinical and earlier clinical experience, a phase 2 proof-of-concept study of lomitapide in 6 patients with hoFH was performed. A dose-escalation regimen was used for the first time in this study, with the concept that it would allow gastrointestinal tolerability and hepatic safety to be established at each dose level before moving to the next dose level. Dosing was based on weight and started at 0.03 mg/kg daily, with half-log increases every 4 weeks to a maximum dose of 1 mg/kg. All 6 subjects completed dose titration to the maximal 1-mg/kg dose, which resulted in a substantial decrease in LDL-C of 51%. Plasma apoB was reduced by 56% and triglycerides by 65%. ApoB kinetic studies confirmed that the reductions in LDL-C and apoB levels were due to a significant decrease in apoB production.

Based on the results of this study, a phase 3 study of lomitapide in hoFH was performed. Twenty-nine patients were enrolled in a single-arm, open-label trial in which lomitapide was dose titrated from a starting dose of 5 mg daily up to a maximum of 60 mg daily on top of standard of care, including LDL apheresis. The primary efficacy end point was at 26 weeks, before which concomitant lipid-lowering therapy remained unchanged; during a subsequent 52-week safety phase, changes in lipid-lowering therapy were permitted. Twenty-three subjects completed the 26-week efficacy phase, and the median dose of lomitapide at the end of the efficacy phase was 40 mg/d. An intention-to-treat analysis of all 29 subjects showed an LDL-C reduction of 40% (Table), which was statistically significant compared with baseline. Figure 2A shows a waterfall plot of the percent change in LDL-C of all 29 subjects at week 26. In the intention-to-treat analysis, apoB was decreased by 39% and lipoprotein(a) by 13% at 26 weeks. An analysis of the 23 subjects who completed the efficacy phase showed a mean LDL-C reduction of 50% and apoB reduction of 49% at 26 weeks. Although all subjects were genotyped, the study was not powered to determine the effect of genotype on response to lomitapide. All 23 subjects who completed the efficacy phase subsequently completed the safety phase, and LDL-C remained substantially reduced at the end of the 78-week trial despite changes in concomitant treatment that were allowed after week 26. Indeed, 3 subjects discontinued and 3 subjects reduced the frequency of LDL apheresis during the safety phase of the trial. Overall, 16 subjects achieved LDL-C levels <100 mg/dL at some point during the trial. An ongoing long-term extension study including up to 4.5 years of follow-up indicated stable reduction in LDL-C and apoB.

A decrease in high-density lipoprotein cholesterol (HDLC) levels had been previously observed during short-term treatment with lomitapide at both high and low doses. In the long-term phase 3 hoFH trial, HDLC levels were significantly reduced by 12% at week 26 but returned to baseline levels by week 78. The mechanism by which MTP inhibition transiently reduces HDLC levels could be related to reduced dietary fat absorption, which is known to reduce HDLC levels; the basis for the rebound with longer treatment is uncertain.

Clinical Adverse Effects of Lomitapide

Gastrointestinal Tolerability

Lomitapide treatment is associated with mechanism-related gastrointestinal adverse events, including nausea, flatulence, and diarrhea. The basis of these symptoms is likely related to a mechanism-based increase in intracellular triglyceride in the enterocyte rather than reduced absorption of fat from the gut lumen, given that steatorrhea is not a feature. These gastrointestinal effects can be minimized with adherence to a low-fat diet, dosing in the fasted state, and a gradual dose-escalation regimen. Furthermore, most substantial gastrointestinal-related effects occur relatively early in treatment. In the long-term phase 3 trial, 3 of 29 subjects discontinued within the first 12 weeks because of gastrointestinal symptoms, but the frequency and intensity of gastrointestinal events decreased substantially after 12 weeks, and there were
Table. Effects of Lomitapide and Mipomersen on Atherogenic Lipoprotein Parameters in hoFH (Phase 3 Studies)

<table>
<thead>
<tr>
<th></th>
<th>Lomitapide* (n=29)</th>
<th>Mipomersen† (n=34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C</td>
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<td></td>
</tr>
<tr>
<td>Baseline, mg/dL</td>
<td>336</td>
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<tr>
<td>End point, mg/dL</td>
<td>190</td>
<td>324</td>
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<td>Mean change, %</td>
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<tr>
<td>Non–HDL-C</td>
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<td></td>
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<tr>
<td>Baseline, mg/dL</td>
<td>386</td>
<td>463</td>
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<tr>
<td>Mean change, %</td>
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<td>Total cholesterol</td>
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<tr>
<td>Baseline, mg/dL</td>
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<td>502</td>
</tr>
<tr>
<td>Mean change, %</td>
<td>−36</td>
<td>−21</td>
</tr>
<tr>
<td>apoB</td>
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<td></td>
</tr>
<tr>
<td>Baseline, mg/dL</td>
<td>260</td>
<td>280</td>
</tr>
<tr>
<td>Mean change, %</td>
<td>−39</td>
<td>−27</td>
</tr>
<tr>
<td>Lp(a)‡</td>
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<td></td>
</tr>
<tr>
<td>Baseline, mg/dL</td>
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<td>60</td>
</tr>
<tr>
<td>Change, %</td>
<td>−13</td>
<td>−32</td>
</tr>
</tbody>
</table>

apoB indicates apolipoprotein B; HDL-C, high-density lipoprotein cholesterol; hoFH, homozygous familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol; and Lp(a), lipoprotein(a).

*Based on values at 26 weeks in all 29 subjects in an intention-to-treat analysis with the last observation carried forward. Change from baseline was statistically significant for all parameters.

†Based on values obtained at the visit closest to 14 days after the last dose of study treatment. Change from baseline was statistically significant for all parameters.

‡Values are the median.

no additional discontinuations as a result of gastrointestinal adverse events through 78 weeks.

Fat-Soluble Vitamins

Because MTP is involved in the absorption of fat-soluble vitamins, this issue has been studied extensively in the lomitapide clinical development program. Lomitapide treatment had no significant effect on plasma levels of vitamins A and D and on international normalized ratio (in patients not on warfarin) as a bioassay of vitamin K status. Lomitapide was associated with reductions in plasma levels of vitamin E, which is transported mostly by LDL. However, in the context of daily vitamin E supplementation (400 IU/d) during the phase 3 hoFH study, the ratio of vitamin E to total lipids remained within the normal range, consistent with a decrease proportional to the decrease in LDL-C. Additionally, patients with hoFH have high vitamin E levels at baseline and, even after lomitapide treatment, had vitamin E levels that were within or above the normal range.

Liver Effects

Treatment with lomitapide is associated in some patients with increases in serum transaminase levels that are generally transient and reversible. In the phase 3 study, 10 of the 29 subjects had at least 1 alanine aminotransferase level ≥3 times the upper limit of normal (ULN). Transaminase elevations were not accompanied by concomitant increases in serum bilirubin or alkaline phosphatase or with associated symptoms. None of the subjects in any of the lomitapide clinical studies had an alanine aminotransferase or aspartate aminotransferase elevation to >3 times the ULN with a corresponding total bilirubin value that was >2 times the ULN. In subjects with alanine aminotransferase or aspartate aminotransferase elevations >5 times the ULN, dose reduction or interruption led to a rapid decrease in the transaminase levels. In subjects with elevations that were <5 times the ULN, continuation of lomitapide at the same dose was generally associated with a decrease in transaminase levels to baseline.

Consistent with the mechanism of action of lomitapide, increases in hepatic triglyceride content were observed with lomitapide treatment. The inhibition of VLDL triglyceride secretion presumably results in some triglyceride accumulation in the hepatocyte. In the phase 3 study in patients with hoFH treated with lomitapide, liver fat content was measured by magnetic resonance imaging in all subjects at baseline and at 6, 12, and 18 months. Hepatic fat increased during the dose-titration phase from an average of 1% at baseline to an average of 8.6% ± 6% at 6 months, at which time the mean dose was 40 mg/d. Hepatic fat remained stable at 12 and 18 months and was 8.3% ± 8% at the end of the study. Changes in hepatic fat content were highly variable from patient to patient. The greatest elevations in transaminases and liver fat in patients with hoFH treated with lomitapide were observed in patients with high alcohol consumption. In vitro, alcohol has been shown to inhibit MTP activity. Both alanine aminotransferase and aspartate aminotransferase were significantly correlated with maximum hepatic fat (P=0.0006 and P=0.0018, respectively) with r² values of 0.433 and 0.377, respectively. The changes in hepatic fat content were negatively correlated with the change in LDL-C levels. In the phase 2 hoFH study, a follow-up magnetic resonance image was obtained 4 weeks after discontinuation of lomitapide, and the hepatic fat was found to be rapidly reversible. Markers of insulin action such as fasting glucose and insulin levels and hemoglobin A1c remained unchanged with lomitapide treatment.

The clinical implications of increased hepatic fat with lomitapide treatment are discussed below.

Mipomersen and ApoB Inhibition

ApoB and Hypobetalipoproteinemia

ApoB is the key structural protein in chylomicrons and VLDL and provides the framework for the packaging and distribution of both dietary and endogenously produced cholesterol and triglycerides by lipoproteins. The human APOB gene encodes a single RNA transcript from which 2 isoforms, apoB-48 and apoB-100, are translated as a result of mRNA editing. In humans, apoB-48 is produced predominantly by enterocytes, whereas apoB-100 is produced predominantly by hepatocytes. Accordingly, apoB-48 is an essential structural component of chylomicrons, and apoB-100 is an essential structural component of VLDL and LDL.

The importance of apoB to lipoprotein metabolism was established by studies of the inherited condition familial hypobetalipoproteinemia (FHBL). FHBL is an autosomal-dominant condition characterized by very low levels of apoB and
The APOB gene was the first gene to be identified as causally linked to FHBL. Unequivocal evidence was initially provided on detection of an aberrant apoB protein in the plasma of affected individuals from a 3-generation kindred. Subsequent DNA sequencing of the respective gene revealed a deletion in the coding region that introduced an upstream stop codon to produce a truncated species of apoB. Since this discovery, about 60 unique mutations in the APOB gene have been identified that cause synthesis of truncated protein products. Truncation of apoB decreases lipidation and secretion of the apoB-100 protein from hepatocytes and in some cases can also affect the rate of clearance from circulation; both factors contribute to the reduced plasma levels of apoB and LDL-C. Most patients with FHBL have mutations in 1 allele of the APOB gene; individuals who have mutations in both APOB alleles can be similar in clinical presentation to those with abetalipoproteinemia. Notably, truncating mutations in APOB can compensate for the LDL-raising effects of mutations in the LDLR and APOB genes that cause autosomal-dominant hypercholesterolemia, leading to normal plasma LDL-C levels.

The majority of individuals with heterozygous FHBL are clinically asymptomatic. Accumulation of hepatic fat can occur secondary to the reduced capacity of hepatocytes to export triglycerides via VLDL secretion. The presence of hepatic steatosis in this context, however, is variable across mutations and even among individuals carrying the same APOB mutation. Notably, hepatic steatosis that results from this form of FHBL is not associated with insulin resistance and evidence of advanced liver disease is rare. Determination of the effects of FHBL on risk of coronary heart disease has been limited by the availability of data and the absence of systematic analyses of affected individuals and families. However, a prospective case-controlled study involving a relatively large FHBL cohort demonstrated a decrease in arterial wall stiffness in the presence of nonlipid risk factors, suggesting that lifelong low apoB levels resulting from APOB mutations may be protective against coronary heart disease. Furthermore, common APOB variants with modest effects on lowering LDL-C levels repeatedly demonstrate a positive impact on cardiovascular health to reduce the risk of coronary heart disease.

Mipomersen: An Inhibitor of ApoB Synthesis

The discovery of truncating apoB mutations leading to low LDL-C supported the concept of inhibiting apoB synthesis as a strategy to reduce LDL-C levels. Mipomersen is a synthetic single-strand antisense oligonucleotide analog designed to bind sequence-specifically to the mRNA that encodes apoB-100. Hybridization of mipomersen to the target apoB mRNA via Watson-Crick base pair interactions creates a substrate for RNase H1, a ubiquitous intracellular ribonuclease that cleaves the RNA strand of the heteroduplex. RNase H1 activity leads to a decrease in target apoB-100 mRNA to reduce the production of apoB by the liver. Mipomersen is 20 nucleotides in length, with each internucleotide linkage chemically modified for stability and reduced immunogenicity. In clinical trials, mipomersen has demonstrated a significant reduction in LDL-C levels compared to placebo, as well as other LDL-C components and triglycerides. It is currently approved by the FDA for the treatment of homozygous familial hypercholesterolemia (HoFH) in combination with other lipid lowering therapies.
as a phosphorothioate diester and with 2′-methoxyethyl sugar residues incorporated into the first and last 5 positions. The phosphorothioate modification is a first-generation chemistry that attenuates the rate of hydrolysis or degradation by nucleases and increases binding to plasma proteins to facilitate drug distribution and absorption by tissues. The 2′-methoxyethyl modification is a second-generation chemistry that imparts additional drug stability and increases the affinity for the target mRNA for greater potency, whereas the central 2′-deoxy sugar residues support RNase H1 activity.

The effects of antisense inhibition of apolipoprotein B (apoB) synthesis on lipid metabolism and cardiovascular pathologies have been studied extensively across a number of species and models of hyperlipidemia and atherosclerosis. In all species tested, including mouse, hamster, rabbit, and monkey, the species-specific antisense apoB inhibitors reduced hepatic apoB-100 mRNA and protein, as well as serum levels of apoB, LDL-C, and total cholesterol, in a dose- and time-dependent manner. Significant reductions in serum triglycerides were also noted in certain species, for example, rabbit and hamster. Compensatory changes in liver lipid metabolism occurred with an antisense reduction of apoB in both mouse and monkey animal models, including those based on dietary challenge. Other key observations were the absence of steatosis in the intestine, with minimal effect on chylomicron production and dietary fat absorption, and abrogation of arterial atherosclerosis in LDL receptor–deficient mice by treatment with an antisense apoB inhibitor.

After subcutaneous injection, mipomersen is readily absorbed and distributed to tissues. Preclinical studies show highest drug concentrations in the liver and kidney. The drug is metabolized by nucleases, with parent drug and metabolites excreted predominantly in urine. The plasma terminal elimination half-life ranges from 1 to 2 months, which allows relatively infrequent dosing. The clinical dosing regimen for mipomersen is weekly.

### ApoB Inhibition With Mipomersen: Early Clinical Studies and Pharmacology

Mipomersen was evaluated in a randomized, double-blind, placebo-controlled multiple-ascending-dose study that involved 36 healthy subjects with mild hypercholesterolemia. Subjects were randomized at a ratio of 4:1 (active drug to placebo) and treated for 4 weeks at doses ranging from 50 to 400 mg. A dose-dependent and prolonged reduction in plasma apoB and LDL-C was demonstrated, with a maximum mean percent change from baseline of 50% and 35%, respectively, in the 200-mg dose group. Pharmacological effects were prolonged in the high-dose groups, with the majority of subjects remaining below baseline for 3 months after the last dose. The prolonged pharmacological response was attributed to the extended terminal plasma elimination half-life of mipomersen.

Three randomized, double-blind, placebo-controlled dose-ranging phase 2 studies evaluated mipomersen in subjects with mild to moderate hypercholesterolemia on therapeutically safe doses of statin therapy and as an add-on therapy in subjects with heterozygous familial hypercholesterolemia. Mipomersen produced statistically significant reductions in LDL-C and apoB levels as a single agent and as an add-on to statins and other lipid-lowering agents in all phase 2 study populations tested. The extent and duration of the pharmacological response correlated directly with dose and concentration as measured by drug plasma trough concentrations. After phase 2, the dose of 200 mg weekly was chosen for the phase 3 program.

No drug-drug interactions have been identified in healthy volunteer studies that evaluated the effects of mipomersen on the pharmacokinetic properties of simvastatin, ezetimibe, and warfarin. In line with the clinical results, cytochrome P450 isozyme activity and P-glycoprotein remain unchanged in the presence of mipomersen in vitro. In addition, no interactions have been identified in association with mipomersen treatment in phase 3 evaluations involving patients on maximum tolerated lipid-lowering therapies.

### Mipomersen in hoFH

Given its mechanism of action in reducing VLDL production, mipomersen was predicted to be effective in hoFH. Indeed, preclinical studies of mipomersen in LDL receptor–deficient mice indicated a substantial reduction in plasma cholesterol. A phase 2 open-label, dose-ranging study of mipomersen conducted in subjects with hoFH on maximally tolerated lipid-lowering therapies suggested the potential for clinical benefit. As a result, a 6-month placebo-controlled phase 3 trial in hoFH was performed. A total of 51 hoFH patients were randomized in a 2:1 ratio, with 34 allocated to mipomersen 200 mg weekly and 17 allocated to placebo. Patients were on maximally tolerated drug therapy, but those on LDL apheresis were excluded from the trial. Twenty-eight subjects randomized to mipomersen completed the 6-month trial. For all subjects, LDL-C reduction was defined as the visit closest to 14 days after the last dose of study treatment. Mipomersen produced a mean 25% reduction in LDL-C from baseline (Table). This response to treatment was statistically significant compared with placebo (−3%; P=0.0003). In addition to LDL-C, concordant reductions in serum apoB levels and all other apoB-containing atherogenic lipoproteins, including lipoprotein(a), occurred in subjects treated with mipomersen (Table). ApoB was decreased by 27% and lipoprotein(a) by 32% in the mipomersen-treated subjects. Figure 2B shows a waterfall plot of the percent change in LDL-C of all subjects. Response to mipomersen varied substantially and was independent of baseline LDL-C levels, age, race, or sex. Although the basis of FH was genetically characterized for 44 of 51 patients, the study was not powered to determine the effect of genotype on response to treatment owing to the large number of different variants. A total of 3 pediatric hoFH patients <18 years of age were included in the mipomersen arm of the trial and had a response to mipomersen treatment similar to that of the adults. In addition to reductions in atherogenic lipoproteins, a significant increase in LDL-C of 15% was observed with mipomersen treatment compared with placebo (4%; P=0.03).

In addition to the phase 3 trial in hoFH, 3 other phase 3 trials of mipomersen have been completed. As with the hoFH trial, all phase 3 studies were designed with a randomization ratio of 2:1 active to placebo and 26 weeks of therapy.
200-mg SC weekly dosing with no dose adjustments allowed. Patients were on a stable low-fat diet and maximum tolerated lipid-lowering therapy, excluding LDL apheresis. Efficacy analyses were performed on all patients with a valid baseline LDL-C measurement who received at least 1 injection of study drug and had at least 1 postbaseline LDL-C assessment. Across these phase 3 studies, average reductions from baseline ranged from −28% to −36% for LDL-C, −26% to −36% for apoB, −25% to −34% for non–HDL-C, and −21% to −33% for lipoprotein(a). Triglyceride levels were also significantly reduced by treatment with mipomersen compared with placebo, which could be due in part to the reduction in apolipoprotein C-III.63 Substantial reductions in total LDL particle numbers and size have also been reported in conjunction with mipomersen treatment.64

An open-label extension study is ongoing from the phase 3 studies for up to 4 years of treatment. An interim analysis has found that reductions in LDL-C and apoB levels are maintained for up to 2 years of treatment (www.isispharm.com/Site_Gfx/pdf/2012-03-28%20_Ph3_Extension_Study_ISA_Santos.pdf).

Clinical Adverse Effects of Mipomersen

Injection-Site Reactions
Mild to moderate erythema or pain at the injection site was the most frequently reported adverse event in patients who received subcutaneous doses of mipomersen in the 6-month placebo-controlled phase 3 studies (84% mipomersen, 33% placebo). The incidence of severe injection-site reactions was limited in the controlled studies (0.4%–3%), as was treatment discontinuation as a result of this type of event (5%). Notably, the incidence of injection-site reactions decreased incrementally at 6-month intervals with continued treatment during the extension study, in which the overall injection-site reaction event rate was 1 per 10 injections. Injection-site reaction events are generally self-limited and resolve spontaneously within 2 to 5 days.

Flu-Like Symptoms
Some patients experienced flu-like symptoms, with an incidence of 30% (versus 16% for placebo) in the phase 3 studies. Flu-like symptoms were typically characterized by some combination of fatigue, chills, and aches. As with injection-site reactions, the incidence of severe flu-like symptoms events was limited in the controlled studies (0.8%), as were respective dose discontinuations resulting from flu-like symptoms (3%). The incidence of flu-like symptoms events remained relatively constant at 6-month intervals with continued treatment during the extension study, in which the overall event rate was 1 per 50 injections or about once per year.

Inflammatory Signals
High-sensitivity C-reactive protein is a biomarker of inflammation and cardiovascular risk.65,66 Transient increases in high-sensitivity C-reactive protein levels have been observed in some patients treated with mipomersen within 1 to 2 days after the initial subcutaneous injection of 200 mg mipomersen. The mechanism of this effect remains uncertain. These increases are modest and are not associated with changes in other markers of inflammation. A predefined assessment of long-term changes in high-sensitivity C-reactive protein levels in each of the 4 placebo-controlled phase 3 studies found no significant change between baseline and end-of-treatment high-sensitivity C-reactive protein levels in patients treated with mipomersen. Furthermore, there was no evidence of an effect of mipomersen on erythrocyte sedimentation rate or IgG levels over time.

Hepatic Effects
Elevations in serum liver transaminases occur in some patients treated with mipomersen. In the controlled phase 3 studies, 8% of mipomersen-treated patients had alanine aminotransferase levels ≥3 times the ULN on 2 consecutive measurements at least 7 days apart. These elevations were not associated with clinically significant changes in other measures of liver function such as total bilirubin, alkaline phosphatase, prothrombin, coagulation factors, and albumin. In addition, they were largely reversible on continued treatment.

Increases in liver fat content occur in some patients as a result of mipomersen treatment. Analysis of the pooled data from phase 3 studies in the subset of subjects in whom hepatic fat was acquired at baseline and at the end of treatment revealed a median 10% increase in percent fat fraction in the mipomersen-treated subjects.60,61 The change in liver fat content partially correlated inversely with the percent change in apoB level at the end of treatment (r=−0.6). This effect was reversible; liver fat content returned to baseline in the posttreatment follow-up period. Periodic liver assessments in the extension study have indicated stabilization in fat content with continued treatment beyond 1 year. This observation may reflect compensatory changes in hepatic lipid metabolism as discovered in preclinical animal model studies on antisense inhibition of apoB.65,51 Other markers of metabolic status such as fasting glucose levels and hemoglobin A1c remained unchanged.

In a limited number of cases, liver biopsies were procured from mipomersen-treated patients for cause by study investigators. All biopsies confirmed hepatic steatosis and found minimal signs of inflammation and minimal to no liver fibrosis. These results are consistent with a simple steatosis and are similar to findings in FHBL. The implications of increased hepatic fat with mipomersen treatment are discussed below.

Benefit–Risk in the Use of Lomitapide and Mipomersen
Lomitapide and mipomersen are both approved by the US Food and Drug Administration for the treatment of patients with hoFH. As noted above, hoFH is characterized by very early cardiovascular morbidity and mortality.67,68 and other options for the treatment of hoFH are limited, with poor response to classes of LDL-lowering drugs that require LDL receptor upregulation for their efficacy. LDL apheresis is considered standard of care but is not widely available, and because of its transient nature, LDL apheresis requires repeated weekly or biweekly procedures. Indeed, a recent report suggested that even on maximal available therapy, the average life expectancy of patients with hoFH is 33 years.6 Although 2 new classes of LDL-lowering drugs, PCSK9 inhibitors and cholesteroy lester transfer protein inhibitors,
are in late-stage clinical development, it remains uncertain whether they will have any greater efficacy in hoFH than statins. PCSK9 inhibitors work by increasing LDL receptor protein, and it has been suggested that LDL reduction with cholesteryl ester transfer protein inhibition may also be LDL receptor dependent. A recent study in 8 hoFH subjects with a PCSK9 inhibitor showed a mean LDL-C reduction of ~15% with no response in Z receptor-negative patients.

Both lomitapide and mipomersen are approved as an adjunct to lipid-lowering medications and diet to reduce LDL-C, apoB, total cholesterol, and non–HDL-C in patients with hoFH. On the basis of the extensive evidence for the causal and graded relationship of LDL-C concentrations with coronary heart disease risk, the magnitude of reduction in LDL-C in patients with hoFH brought about by treatment with lomitapide or mipomersen would be expected to substantially reduce the risk of atherosclerotic cardiovascular disease and premature mortality in hoFH patients.

The approval of lomitapide and mipomersen for hoFH has sparked substantial discussion of the diagnosis of hoFH. There are no universally accepted criteria for the diagnosis, and more than a dozen criteria have been proposed over the years. Although classic hoFH may be easily recognizable because of its presentation in childhood with skin xanthomas and total cholesterol >500 mg/dL, it is becoming increasingly recognized that certain patients with severe hypercholesterolemia who may not meet the classic description of hoFH have mutations in both LDL receptor alleles or have 2 mutations in >1 gene regulating LDL receptor function. Sequencing of the LDL receptor, and potentially other genes such as apoB and PCSK9, is necessary for a definitive molecular diagnosis. However, some sequencing approaches may miss certain causal mutations that contribute to the phenotype of severe hypercholesterolemia. Furthermore, it currently is not standard clinical practice to perform sequencing for molecular diagnosis of severe hypercholesterolemia, nor is it typically reimbursable. Given these realities, the US Food and Drug Administration approvals for lomitapide and mipomersen did not mandate molecular diagnosis but rather accepted a clinical diagnosis consistent with hoFH.

Both lomitapide and mipomersen are required by the US Food and Drug Administration to have risk evaluation and mitigation strategy programs to ensure appropriate use and to maximize the benefit-to-risk ratio of these drugs. Clinicians who wish to prescribe either of these drugs must undergo a certification process, after which they make a clinical assessment of whether a patient is a candidate for either drug. For both drugs, registries of patients prescribed the drugs are being created to permit the collection of long-term safety and efficacy data.

Increased Hepatic Fat and Long-Term Hepatic Safety Are the Major Issues for Both Drugs

Both lomitapide and mipomersen result in mechanism-based variable increases in hepatic fat, accompanied by variable increases in hepatic transaminases. As noted above, the transaminase elevations are reversible on dose reduction/discontinuation or even transient with continued treatment, and the hepatic fat increases occur early and are stable over time. The long-term clinical consequences of increased hepatic fat as a result of these pharmacological interventions are unknown. Hepatic steatosis in other settings has been associated with the development of other pathologies, ranging from nonalcoholic steatohepatitis and cirrhosis to insulin resistance. The complete MTP deficiency state, abetalipoproteinemia, is variably associated with some degree of hepatosteatosis; however, liver fibrosis is rare and has generally been observed in the setting of medium-chain triglyceride supplementation, which has been implicated as a possible culprit contributing to progressive liver disease. Patients with hypobetalipoproteinemia resulting from apoB mutations generally have increased hepatic fat but do not appear to progress to fibrotic liver disease.

Hepatic steatosis is associated with insulin resistance; however, this association is not necessarily causal in that many factors predispose to both hepatic steatosis and insulin resistance. In subjects with hypobetalipoproteinemia, a condition that results in increased hepatic fat similar to that observed with MTP inhibition, steatosis is not associated with insulin resistance. In addition, steatotic livers isolated from rats fed a high-fat diet show a response to insulin similar to that of control livers. Finally no increases in fasting insulin or glucose levels have been observed with lomitapide or mipomersen. Formal insulin clamp studies in subjects before and after lomitapide and mipomersen treatment are necessary to definitively resolve this issue.

Currently, it is not possible to anticipate the long-term consequences of the hepatic fat increases seen in patients with hoFH treated with lomitapide and mipomersen. Patients need to be followed up with care. Of note, a patient with hyperchylomicronemia and fatty liver at baseline was treated with lomitapide with prevention of pancreatitis but was reported to develop steatohepatitis and fibrosis after 13 years of treatment.

Summary

Of the 3 previous classes of approved LDL-lowering drugs, bile acid sequestrants have been used to lower cholesterol for many decades, statins were first approved in 1987, and ezetimibe was approved in 2002. In a remarkable turn of events, 2 new first-in-class LDL-lowering drugs were approved within a month of each other: lomitapide in December 2012 and mipomersen in January 2013. Both drugs/classes lower LDL-C by reducing hepatic VLDL production, making them independent of the LDL receptor and thus effective in hoFH. However, as a result, they both cause a mechanism-based increase in hepatic fat. It is primarily for this reason that they were approved only for the most serious form of inherited hypercholesterolemia, namely hoFH. Both drugs reduce LDL-C in hoFH patients to a degree that would be expected to yield clinical benefit with regard to cardiovascular disease. Although the 2 drugs have not been directly compared head to head, in patients with hoFH, lomitapide at doses ≥20 mg daily appears to have somewhat greater mean efficacy in LDL-C reduction than mipomersen at the fixed dose of 200 mg SC every week. Lomitapide can cause gastrointestinal side effects such as nausea and diarrhea, which are often manageable through dose titration and maintenance of a low-fat diet. Mipomersen can cause injection-site reactions (which diminish over time) and occasional flu-like symptoms. Lomitapide is a CYP3A4 inhibitor and has potential...
for drug-drug interactions with CYP3A4 inhibitors and drugs metabolized by CYP3A4, whereas mipomersen has no known drug-drug interactions. Both drugs increase hepatic fat and can variably increase hepatic transaminases. Although the increase in hepatic fat is often modest and appears to stabilize after 6 months, the long-term implications of this increase are unknown at this time. For patients with hoFH, who are at risk for markedly accelerated atherosclerosis and premature cardiovascular death, treatment with lomitapide or mipomersen has the potential to reduce the risk of atherosclerotic cardiovascular disease and premature mortality.

Disclosures
Dr Rader has previously been a consultant to Isis Pharmaceuticals, is currently a consultant for and has stock options in Aegerion Pharmaceuticals, and is an inventor on a patent related to lomitapide that is owned by the University of Pennsylvania. Dr Kastelein pharmaceuticals, and is an inventor on a patent related to lomitapide.

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46. Rader and Kastelein New Drugs for Lowering LDL in hoFH 1031


Lomitapide and Mipomersen: Two First-in-Class Drugs for Reducing Low-Density Lipoprotein Cholesterol in Patients With Homozygous Familial Hypercholesterolemia
Daniel J. Rader and John J.P. Kastelein

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