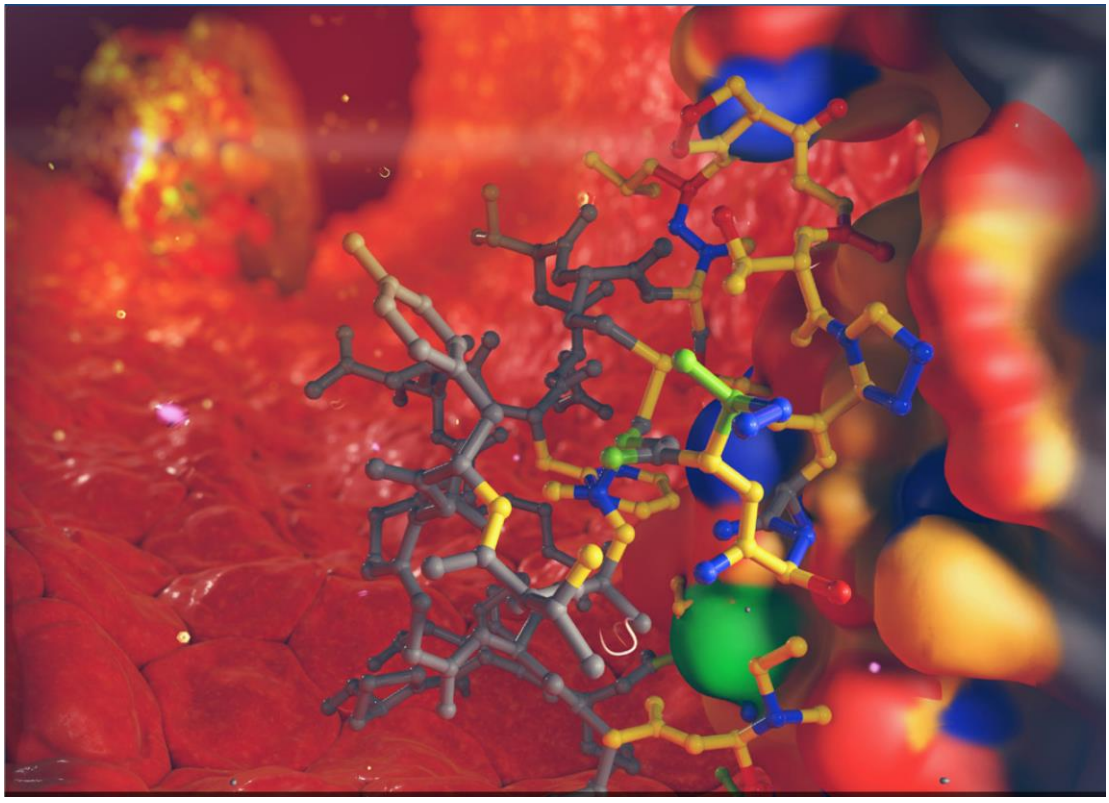
# Internal Medicine Grand Rounds

## University of Texas Southwestern Medical Center

## May 2, 2014

# New and Emerging Therapies for Hyperlipidemias

**Jay D. Horton, M.D.**



This is to acknowledge that Jay D. Horton, M.D. has disclosed financial interests or relationships with commercial concerns directly or indirectly related to this program. Dr. Horton will be discussing off-label uses in his presentation.

**Presenter**: Jay D. Horton, M.D.

**Rank**: Professor

**Division**: Digestive and Liver Diseases

**Purpose & Overview:**
To discuss and explain the mechanism of action and indications for use of new drugs available or soon to be available for the treatment of hyperlipidemia.

**Objectives:**
1. Understand the mechanism of action of Lomitapide and indications for use.
2. Understand the mechanism of action of Mipomersen and indications for use.
3. Understand the role of PCSK9 in cholesterol metabolism and the mechanism of action of molecules designed to block PCSK9 activity.

**Biosketch:**
Dr. Jay D. Horton is a Professor of Internal Medicine and Molecular Genetics, Chief of the Division of Digestive and Liver Diseases, and holds the Robert C. and Veronica Atkins Chair in Obesity and Diabetes Research at UT Southwestern Medical Center. He obtained his B.S. and M.D. degrees from the University of Iowa and completed his Internal Medicine residency, gastroenterology fellowship, and Howard Hughes post-doctoral fellowship at UT Southwestern. In clinical digestive diseases, Dr. Horton has an interest in conditions that lead to fatty liver disease and obesity. Currently a major focus of the laboratory is to determine how regulators of fat metabolism contribute to the development of fatty liver in various disease processes such as diabetes, obesity, and fatty acid oxidation defects. In recent work, Dr. Horton has delineated the function of PCSK9, a protein secreted into the blood that determines plasma cholesterol levels through its action on LDL receptors in liver.

## Facts Regarding Cardiovascular Disease

- Approximately **600,000 people** die of heart disease in the United States every year– that's **1 in every 4 deaths** (6)**.**
- Heart disease is still the leading cause of death for both men and women. (6).
- Every year about **720,000 Americans** have a heart attack (7).
- Coronary heart disease costs the United States **$108.9 billion** each year (8).
- Plasma LDL cholesterol concentrations represent the greatest risk factor for developing atherosclerosis.
- ~37 million U.S. adults with elevated LDL-C levels are not on an LDL-C lowering therapy.
- As many as 50% of patients stop taking statins within one year of initiating treatment. Poor statin adherence is associated with worse cardiovascular outcomes.
- ~ 5-20%, or 2-7 million adults in the U.S., ceased statin therapy because of muscle pain or weakness and can be considered statin intolerant.

In the 1970s, Joseph L. Goldstein and Michael S. Brown showed that familial hypercholesterolemia (FH) is caused by mutations in the LDL receptor gene, which leads to excessive accumulation of LDL-cholesterol (LDL-C) in the blood and atherosclerosis. LDL receptors in the liver remove the majority of LDL-C from the blood. They went on to win the Nobel Prize in 1985 for this work. Elevated LDL-C is now an established risk factor for cardiovascular disease and the CDC estimates that 71 million U.S. adults have elevated plasma LDL-C (hypercholesterolemia). Brown and Goldstein's work ultimately led to the development of HMG-CoA reductase inhibitors or "Statins", which lower plasma LDL-C levels by increasing the number of LDL receptors in liver and thereby reduce the risk for cardiovascular disease. However, there are sub-groups of individuals who either do not have a large enough response to stains (homozygous and heterozygous FH patients many of whom lack a functional gene for the LDLR receptor) or who do not tolerate statins largely owing to muscle pain. Thus, there is still an unmet need for additional therapeutic modalities to lower LDL-C. Understanding where therapeutic opportunities exist has largely been derived from human genetics as will be discussed throughout the presentation.

## Determinants of Plasma LDL Cholesterol Levels:

Plasma LDL-C concentrations are ultimately determined by the summation of the rates of VLDL production from liver and rates of LDL-C clearance from blood (**Fig. 1**). VLDL assembly in liver and chylomicron assembly in intestine are initiated in the endoplasmic reticulum (ER) by the translation of apolipoprotein B (apoB) (**Fig. 2**). The human *APOB* gene is located on chromosome 2 and encodes a ~20 kb mRNA that is translated into the full-length apoB-100 consisting of 4,536 amino acids in liver. A truncated form of apoB,

apoB-48, represents the N-terminal 48% of apoB-100 and is produced in the intestine by an mRNA editing mechanism and is used exclusively for chylomicron formation.

In humans, apoB-100 and apoB-48 are obligatory proteins for the assembly of hepatic VLDL and intestinal chylomicrons. Each VLDL is composed of one molecule of apoB, multiple copies of other apolipoproteins, together with varied amounts of triglycerides (TG) and cholesteryl esters. Among the different lipid and protein constituents of VLDL, the availability of apoB-100 and TGs are critical for the assembly and secretion of VLDL and chylomicrons.

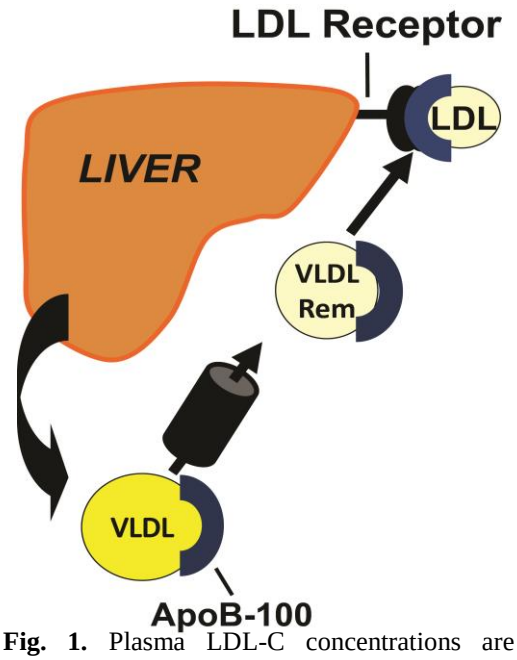



**Fig. 1.** Plasma LDL-C concentrations are determined by rates of hepatic VLDL production by LDL clearance.

While the details of the cellular and molecular mechanisms by which different lipid and protein components are brought together for VLDL assembly are not fully understood, it is known that an additional protein, microsomal triglyceride-transfer protein (MTTP), is absolutely required for VLDL and chylomicron production. MTTP is a heterodimer consisting of a 97-kDa lipid-binding and -transfer subunit and a 55-kDa protein disulfide isomerase. MTTP is expressed in the hepatocytes and enterocytes where it transfers lipids to apoB.

The obligatory roles of apoB and MTTP in VLDL and chylomicron assembly/secretion were first demonstrated through the identification of individuals who carried mutations in the genes encoding these proteins.

**Abetalipoproteinemia**

Familial abeta-lipoproteinemia is caused by mutations in *MTTP*. Abetalipoproteinemia was first reported in the medical literature by Bassen and Kornzweig in 1950 (9). It is an autosomal recessive disease of which there is only about 100 cases of reported. Approximately one-third of the reported cases result from consanguineous marriages. The clinical manifestations

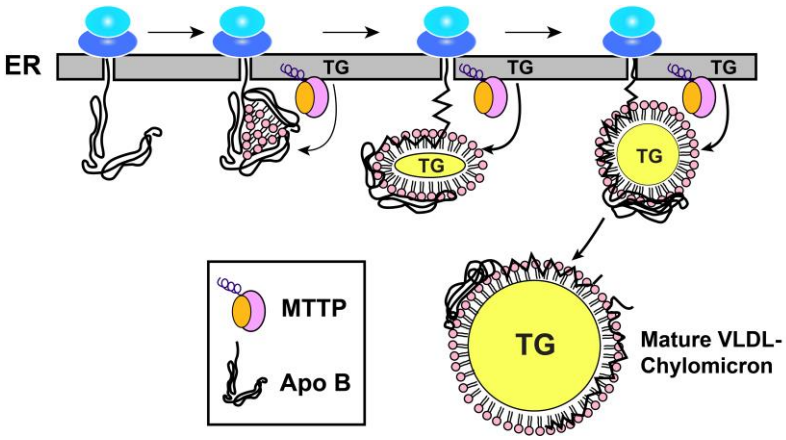



**Fig. 2.** Assembly of VLDL/chylomicrons. The process begins with the translation of ApoB, which acquires triglycerides through the action of microsomal triglyceride-transfer protein (MTTP).

of abetalipoproteinemia are those of lipid and fat-soluble vitamin malabsorption, and include acanthocytosis, pigmentary degeneration of the retina, ataxia, and hypocholesterolemia from having no apoB-containing lipoproteins (VLDL and LDL) (**Fig. 3**.). As a result, plasma cholesterol levels are very low (20–50 mg/dl) and TG levels are often <10 mg/dl. However, most heterozygotes (~80%) have normal lipid levels.

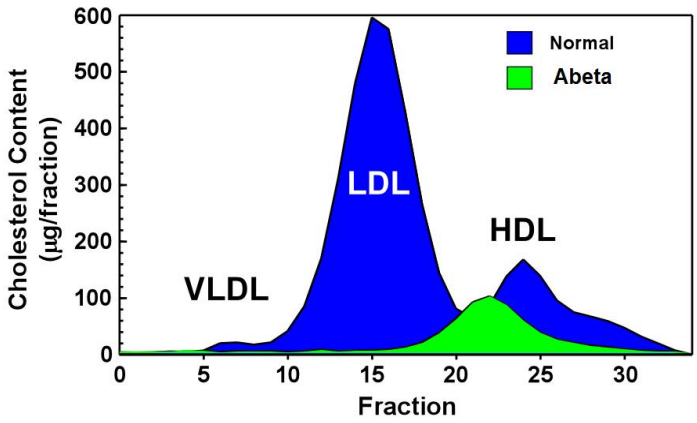



**Fig. 3**. Cholesterol content of VLDL, LDL, and HDL on plasma from a normal individual and an individual with abetalipoproteinemia.

**Steatorrhea**

Steatorrhea is always present in subjects with abetalipoproteinemia on a normo-lipidemic diet. Infants with abetalipoproteinemia develop diarrhea, vomiting, and abdominal swelling within months after birth. Later, the GI symptoms improve, in part because the patients self-institute a lipid-poor diet because of intolerance to fats. Endoscopic examination of the intestine reveals a yellow appearance, which reflects the accumulation of lipids in the mucosa. Histologically, biopsies taken from the duodenal region of fasting patients is of normal thickness with villi of normal height. The enterocytes are distended with a clarified cytoplasm owing to the presence of numerous vacuoles that predominate in the upper two-thirds of villi (**Fig. 4**).

**Hepatic Steatosis**

Liver biopsies from abetalipoproteinemia individuals consistently show hepatic steatosis. Of the 12 cases reported, 3 had moderate centro-lobular steatosis, which are not accompanied by elevated transaminases. Three other cases were associated with elevated transaminases. In 4 cases, an evolution to fibrosis was noted, with one of which progressed to cirrhosis and required liver transplantation (10).

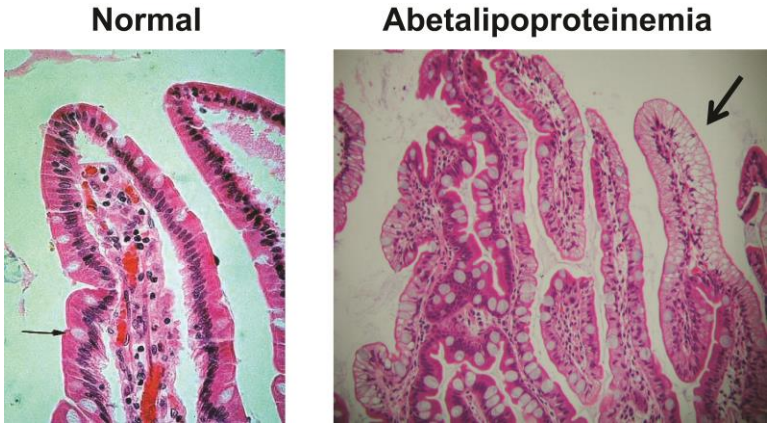



**Fig. 4**. Histology of intestinal biopsies from a normal individual and a patient with abetalipoproteinemia.

**Vitamin Deficiency**

Chronic malabsorption of fat and the lack of apoB-containing lipoproteins lead to fat-soluble vitamin deficiency in subjects with abetalipoproteinemia. The plasma transport and tissue distribution of these vitamins depend almost exclusively (vitamin E, beta-carotene) or in part (vitamins A, D, and K) on lipoproteins containing apoB. Plasma levels of vitamin E and beta-carotene are extremely low in abetalipoproteinemia. Because of alternate modes of transport, the levels of vitamins A, K, and D are

diminished, but not to the extent of vitamin E or beta-carotene. However, individuals with abetalipoproteinemia often develop alterations in night vision and decreased visual acuity follows.

**Neurological Signs**

The first neurological sign in abetalipoproteinemia is often the loss of deep tendon reflexes followed by alteration of proprioception (loss of position and vibratory senses and a positive Romberg sign), a cerebellar syndrome (dysmetria, ataxia, and wide-based spastic gait), and muscular weakness. These signs and symptoms are due to vitamin E deficiency.

**Hematological Signs**

Acanthocytes (erythrocytes with irregular cytoplasmic projections) are found in abetalipoproteinemia subjects and moderate to severe develop anemia can develop. The anemia is associated with hemolysis and shortening of the erythrocyte half-life.

## Familial Hypobetalipoproteinemia

Familial hypobetalipoproteinemia (FHBL) is defined by very low levels (<5th percentile) of total apoB and/or LDL-C in plasma. In most cases of FHBL, the genetic cause(s) are not known; however, the best-characterized cases are due to mutations in the *APOB* gene. Most mutations are missense and frame-shift mutations producing truncated proteins. In general, the signs and symptoms of individuals with FHBL are similar by usually significantly milder than those found in abetalipoproteinemia.

## Therapeutics Based on Lessons Learned from Human Genetics

**Lomitapide (Juxtapid): MTTP Inhibitor**

The discovery of the molecular basis of abetalipoproteinemia led directly to the concept that pharmacologic inhibition of MTTP could be a strategy to reduce LDL-C. Several compounds were developed but the only one that has been advanced is lomitapide. The development of this drug has largely been for the treatment of homozygous familial hypercholesterolemia (hoFH), an autosomal co-dominant disorder due to mutations in the LDL receptor gene. The disease is characterized by markedly elevated plasma LDL-C often greater than 300 mg/dl, tendon xanthomas and markedly premature and progressive atherosclerosis. The current mean age of death of patietns with hoFH is 33 years. Because of the impaired LDL receptor function, patients with hoFH have minimal responses to conventional drug therapies that work by upregulating the LDL receptor and most require LDL-C apheresis.

Lomitapide was approved by the FDA in December of 2012 as an orphan drug for the treatment of dyslipidemia in patients with hoFH. Proof of concept was first demonstrated in six patients with hoFH administered lomitapide at escalating doses of up to 1.0 mg/kg per day in four 4-week phases. Compared with baseline, maximal reductions in levels of LDL-C were 51% (11). The most-common lomitapide-related adverse events were gastrointestinal in nature and thought to be related to patients consuming a greater amount of fat than was recommended in the study protocol (<10% calories). All patients had increases in liver fat content, ranging from 10% to 40% at the highest dose of lomitapide, owing to the mechanism of action, which blocks VLDL and thus TG secretion from liver. Elevations in LFTs were observed in four of the six patients. After

drug cessation, liver fat and LFTs levels returned to baseline in all patients except one, whose hepatic fat accumulation persisted for 14 weeks following active treatment.

In phase III testing of lomitapide, 29 hoFH patients with mean baseline LDL-C level of 336 mg/dl, despite receiving aggressive lipid-lowering therapy received escalating doses of lomitapide, up to a maximum of 60 mg/day as tolerated (4). During the 26-week efficacy phase, LDL-C levels decreased by 50% from baseline (*P* <0.0001) in 23 patients (six patients withdrew from the study during this phase) with a median lomitapide dose of 40 mg per day (**Fig. 5**). During the 52-week safety phase, 23 patients continued lomitapide, with six stopping or decreasing the frequency of LDL apheresis. At the end of the study, LDL-C levels remained reduced by 38% from baseline (*P* <0.0001).

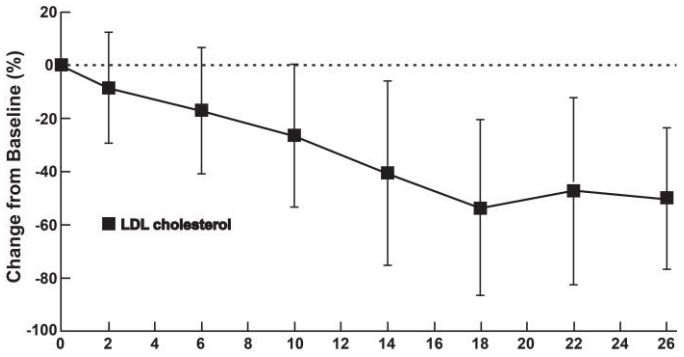



**Fig. 5.** Mean % changes in LDL-C from baseline to wk 26 (4).

Nearly all patients reported at least one mild-to-moderate adverse event, primarily GI in nature. Elevations in LFTs >3X normal occurred in 10 patients and resolved with dose reduction (**Fig. 6**). The mean hepatic fat content increased from 1.0% at baseline to 8.6% at week 26 (**Fig. 6**). These results were interpreted as having an acceptable risk/benefit ration that led to approval but the availability of lomitapide is limited to health-care providers and pharmacies that undergo certification regarding the potential for the drug to cause hepatotoxicity.

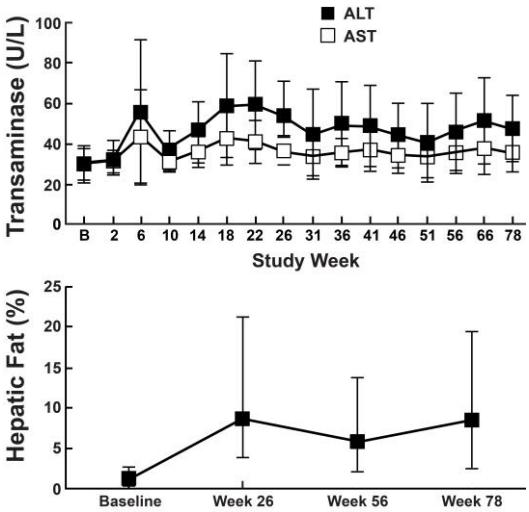



**Fig. 6.** Alanine transaminase (ALT) and aspartate transaminase (AST) levels and percentage of hepatic fat in the liver as measured by MRS.

**Mipomersen (Kynamro): ApoB Antisense**

Antisense technology uses short, single-stranded, chemically modified oligonucleotides that bind to the mRNA of a targeted protein, interfering with translation and protein synthesis. To date, only two drugs using antisense technology have been approved by the FDA. The first was fomivirsen (marketed as Vitravene) for the treatment for cytomegalovirus retinitis in 1998 and mipomersen (marketed as Kynamro) for the treatment of hoFH in 2013. Mipomersen is a second-generation 20-nucleotide antisense oligonucleotide that targets the coding region of apolipoprotein B-100 mRNA leading to reduced apoB protein and lower VLDL secretion from liver.

Mipomersen (200 mg) is administered by subcutaneous injection weekly. To date, four phase 3 clinical trials in which a total of 391 patients were given mipomersen or placebo have been published and all demonstrated substantial reductions in LDL-C and

triglycerides in patients already receiving maximal lipid-lowering therapy (3, 12-14). As with lomitapide, the mechanism of action of mipomersen causes hepatic steatosis and elevated LFTs.

The largest and most recent results from a phase 3 study comes from a planned interim analysis of an ongoing, open-label extension trial in patients with FH receiving mipomersen weekly as an add on to maximally tolerated lipid-lowering therapy (3). The mean changes in LDL-C from baseline to weeks 26, 52, 76, and 104 were −28, −27, −27, and −28%; respectively (**Fig. 7**). Reductions in total cholesterol, non-high-density lipoprotein-cholesterol, and lipoprotein(a) were comparable with decreases in LDL-C and apolipoprotein B levels.

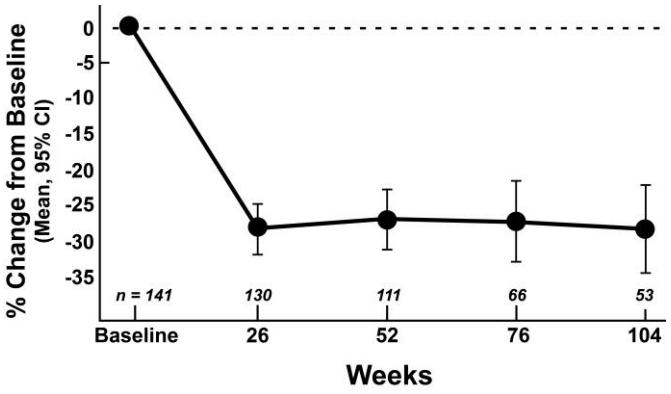



**Fig. 7.** LDL-C reductions with long-terrn mipomersen treatment (3).

Of the 141 patients treated, 77 (55%) discontinued treatment within the first 2 years of treatment; 61 (43%) due to treatment related AEs and 16 (11%) for reasons that were not AEs such as patient study fatigue, or starting LDL apheresis. The long-term safety profile of mipomersen was similar to that reported in the other randomized placebo-controlled Phase 3 trials. Adverse events included injection site reactions or flu-like symptoms in nearly 100% of the patients (**Fig. 8**). There was an increase in liver fat during the initial 6–12 months (**Fig. 9**). The median ALT level showed a similar trend to that of liver fat over time.

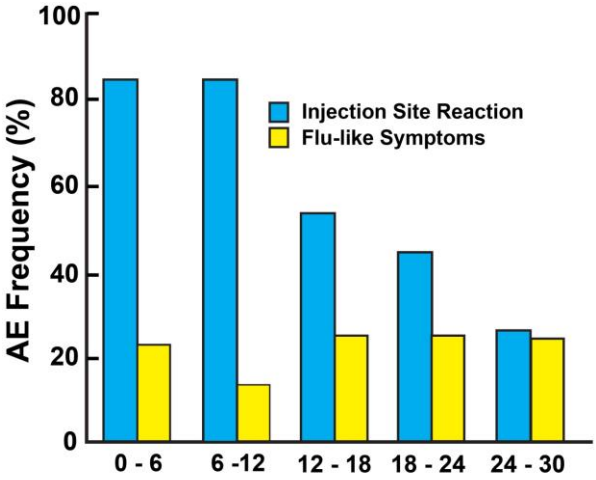



**Fig. 8**. Incidence of injection site reactions and flu-like symptom events occurring at least once during treatment (3).

Combined, patients receiving mipomersen in all phase 3 trials had higher rates of adverse events leading to study discontinuation than those receiving placebo (pooled data: 18.0% versus 2.3%). In the pooled data analysis, the most-common adverse events leading to discontinuation were injection-site reactions (5.0%), flu-like symptoms (2.7%), and abnormal LFTs (5.4%). Injection-site reactions were reportedly a painless erythema appearing within 24 hours of injection and affected the majority of patients receiving mipomersen (84.3%). Flu-like symptoms typically occurred within the first weeks of treatment and resolved within 1–2 days. Overall, 16% of mipomersen-treated patients had elevated LFTs >3X ULN and they returned to normal after discontinuation. No cases of severe hepatotoxicity as defined by Hy's law (aminotransferase elevation accompanied by

increased serum total bilirubin) occurred. Liver MRI was performed in two trials, and mipomersen caused a median 9.6% increase in hepatic fat, compared with a 0.02% increase in placebo-treated patients. As a result of the side-effects and like lomitapide, mipomersen is limited to health-care providers and pharmacies that undergo certification regarding the potential for the drug to cause hepatotoxicity.

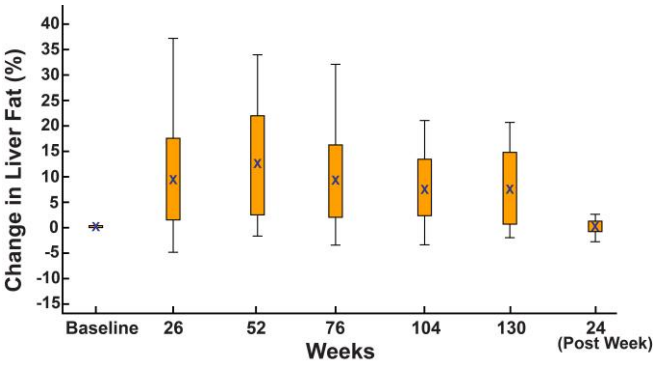



**Fig. 9.** Change from baseline in liver fat content (3).

## Proprotein convertase subtilisin-like kexin type 9 (PCSK9)

PCSK9 is a protease that destroys low density lipoprotein (LDL) receptors in liver and thereby controls the level of LDL-C in plasma. Mutations that increase PCSK9 activity cause hypercholesterolemia and coronary heart disease (CHD), whereas mutations that inactivate PCSK9 have the opposite effect, lowering LDL and reducing CHD. Although the mechanism of action of PCSK9 is not yet clear, the protease is an ideal therapeutic target to lower plasma levels of LDL-C and prevent CHD.

### Background

In 2003, four groups reported the characterization of a new member of the proprotein convertase gene family. First, Seidah and colleagues (15) characterized a transcript encoding a novel proprotein convertase previously shown to be up-regulated during apoptosis in neuronal cells. The link between PCSK9 and cholesterol metabolism rapidly followed with the discovery that selected mutations in the gene cause autosomal dominant hypercholesterolemia (16), and the observation that PCSK9 was regulated by SREBP-2, a transcription factor that regulates all enzymes of cholesterol biosynthesis and the LDL receptor (LDLR) (17, 18).

### Structural features of PCSK9

Prior to 2003, only two autosomal dominant forms of hypercholesterolemia were known: familial hypercholesterolemia (FH), caused by mutations in the genes encoding the LDL receptor (*LDLR*), and familial defective Apo-B100 (FDB), caused by

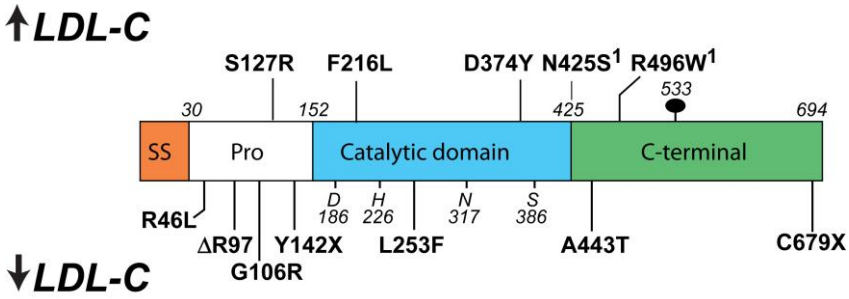



**Fig. 10**. A schematic of PCSK9 with the location of mutations associated with elevated (top) or reduced (bottom) plasma levels of LDL-C. The major domains of PCSK9 are delineated using different colors. The location of the aspartate (D), histidine (H) and serine (S) that comprise the catalytic triad and the site of binding of the single N-linked sugar (N533) are shown. Abbrev, used: SS, signal sequence; Pro, prodomain (5).

mutations in ApoB-100 (*APOB*) that disrupt binding of LDL to LDLR. Both disorders decrease LDLR-mediated endocytosis in the liver, the major route of clearance of circulating LDL-C. Initially, three missense mutations in *PCSK9* were identified in families with a clinical phenotype resembling FH: S127R, F216L, and D374Y (5) Subsequently, additional missense mutations were identified in hypercholesterolemic subjects (**Fig. 10)**. The only clinical findings reported in subjects with mutations in *PCSK9* are those related to lipoprotein metabolism suggesting that PCSK9 functions primarily in the cholesterol metabolic pathway.

Proprotein convertase (PC) enzymes are structurally related to the bacterial subtilisin-like serine protease kexin found in yeast. There are nine subtilisin-like serine proteinases in mammals designated PC1/3, PC2, furin, PC4, PC5/6, PACE4, PC7, S1P (site-1 protease) and PCSK9. Like other family members, the signal sequence (aa 1-30) in PCSK9 is followed by the prodomain (aa 31-152) and catalytic domain (**Fig. 10**) (5). The prodomain (~14 kDa) remains bound to the mature protein (63kDa) as it traverses the secretory pathway.

**Gain-of-function mutations in *PCSK9***

Most enzyme defects cause recessive disorders. The observation that *PCSK9* mutations cause dominant hypercholesterolemia suggested that the mutations confered a gain-of-function (16), either by increasing the normal activity of PCSK9 or by conferring a new activity to the protein. The first experimental evidence for a gain-of-function mechanism came from studies in which wild-type and mutant PCSK9 (S127R and F216L) were expressed at high levels in the livers of mice; hepatic LDLR protein levels fell dramatically in the mice receiving either the wild-type or mutant PCSK9 (**Fig. 11**) (5). No associated reductions in LDLR mRNA levels were observed. Thus, overexpression of PCSK9, whether mutant or wild-type, reduces LDLRs through a post-transcriptional mechanism.

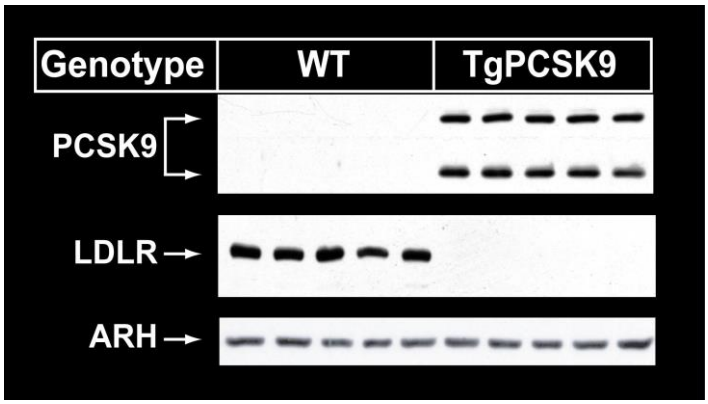



**Fig. 11.** Overexpression of PCSK9 reduces LDLR protein in livers of mice.

**Loss-of-function mutations in *PCSK9***

To test the hypothesis that loss-of-function mutations in *PCSK9* would cause hypocholesterolemia by increasing LDL clearance, Cohen *et al.* (19) sequenced the coding region of *PCSK9* in individuals with the lowest plasma levels of LDL-C ($<5^{th}$ %) in a population-based sample. One out of every 50 African-Americans in the population had a nonsense mutation in *PCSK9* (either Y142X or C679X) that lowered LDL-C levels by ~40% (**Fig. 12**) (19).

The loss-of-function mutations in *PCSK9* were sufficiently common to address a more general question regarding whether life-long reductions in plasma levels of LDL-C confer greater protection from CHD than cholesterol-lowering therapies instituted later in life**?** In a large biracial 15-year prospective study, nonsense mutations in PCSK9 that

reduced LDL-C levels by 8% decreased the frequency of CHD (defined as myocardial infarction, coronary death, or coronary revascularization) by 88% (20). The reductions in CHD associated with these mutations were significantly greater than those observed in more short-term clinical trials employing statins. These data suggest that earlier intervention might significantly magnify the clinical efficacy of cholesterol-lowering therapy by attenuating the development and progression of atherosclerosis.

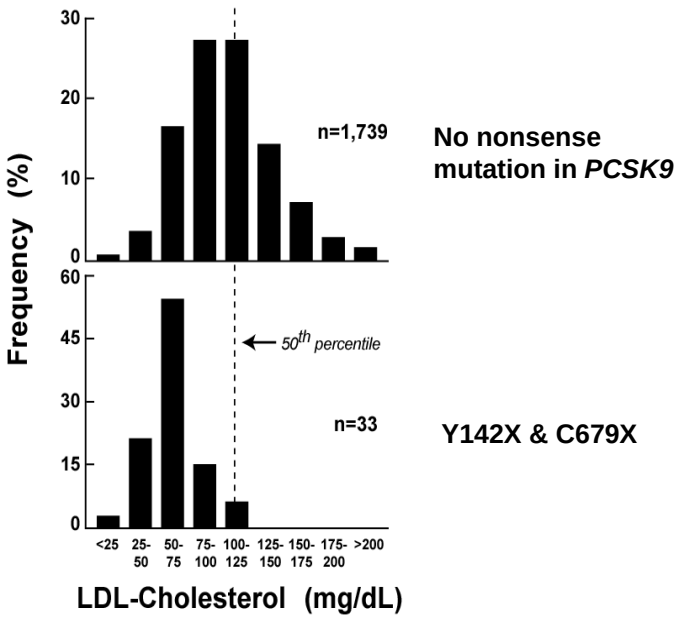



**Fig. 12.** Distribution of plasma LDL-C levels in African American subjects in the Dallas Heart Study without (top) and with (bottom) a nonsense mutation in *PCSK9*.

The mechanisms by which loss-of-function mutations in *PCSK9* reduce plasma cholesterol levels were investigated in mice in which *Pcsk9* was inactivated. These animals have increased hepatic LDLR protein levels, accelerated LDL clearance, and reduced plasma cholesterol levels (21). Thus, PCSK9 acts to tonically suppress LDLR levels, thereby limiting LDLR-mediated uptake of lipoproteins.

*PCSK9 directly binds the LDLR*

Studies in cultured cells initially showed that the PCSK9 can be co-immunoprecipitated with the LDLR after PCSK9 is added exogenously to the medium of cells, implying a physical association between the two proteins (22). Zhang *et al.* (23) showed that recombinant human PCSK9 interacted in a sequence-specific manner with the first epidermal growth factor-like repeat (EGF-A) in the EGF homology domain of the human LDLR. To determine the residues of PCSK9 that directly interacted with the LDLR, the crystal structure of PCSK9 in complex with the EGF-A domain of the LDLR

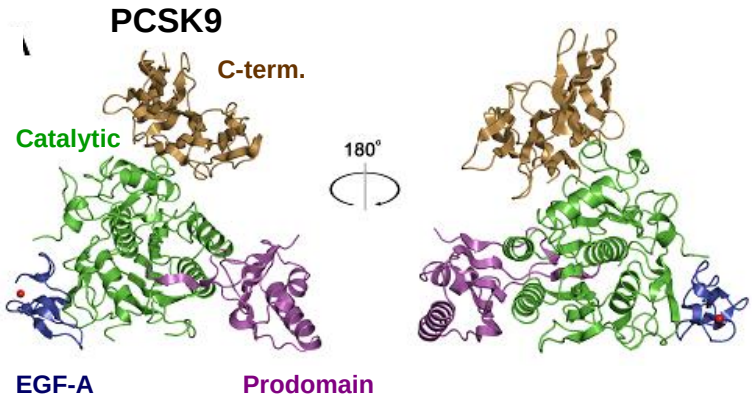



**Fig. 13.** The PCSK9:EGF-A complex. PCSK9, with the prodomain (magenta), the subtilisin-like catalytic domain (green), and the C-terminal domain (brown), and the EGF-A domain of LDLR (blue) are represented as a ribbon diagram (1).

was solved by Kwon *et al.* (1) (**Fig. 13**). EGF-A binds a surface of PCSK9 that is formed primarily by residues 367-381.

*Site of action of PCSK9 in cells*

The intracellular itineraries of PCSK9 and the LDLR are similar, but their paths diverge at the cell surface (**Fig. 14**). The LDLR remains associated with the cell membrane whereas PCSK9 is secreted into the medium. PCSK9 is also secreted *in vivo* by the liver and is present in human plasma (24). We developed an enzyme linked immunoabsorbent assay (ELISA) to measure circulating levels of PCSK9 and found the levels of plasma PCSK9 concentration range from ~50 to ~1000 ng/ml in subjects from DHS (24).

PCSK9 remains inactive while it migrates through the secretory pathway and acts on the LDLR only after it is secreted. The prodomain of PCSK9 remains tightly attached to the mature protein during its secretion, presumably inhibiting catalytic activity. In other proprotein convertases, the prosegment undergoes a secondary proteolytic processing event either in the Golgi or after secretion that relieves the inhibition and unmasks enzymatic activity. Evidence that PCSK9 functioned extracellularly came from parabiosis experiments were carried out in mice (22). The circulations of PCSK9 transgenic mice were connected to those of wild-type mice. Comparison of liver biopsies before and after parabiosis revealed a dramatic reduction in LDLR protein in livers of the recipient wild-type mice.

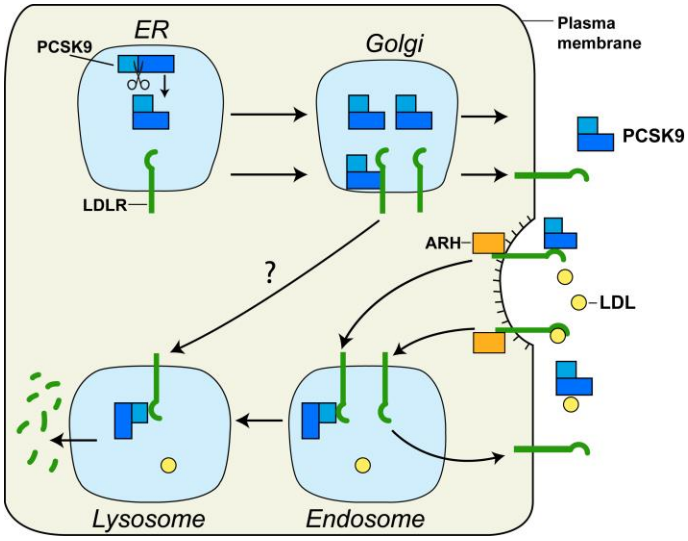



**Fig 14**. PCSK9 undergoes autocatalytic cleavage in the ER. The cleaved prodomain (light blue) associates with the catalytic fragment (dark blue) and acts as a chaperone permitting the mature protein to move from the ER into the secretory pathway. PCSK9 that is secreted binds to LDLRs on the cell surface. The LDLR/PCSK9 complex is internalized via the adapter protein ARH (orange). PCSK9 prevents the recycling of the LDLR from the endosome back to the cell surface and/or directs the LDLR to the lysosome (5).

The results of these studies suggest several possible mechanisms by which PCSK9 might promote LDLR degradation. PCSK9 could bind to the LDLR in a catalytically inactive state on the cell surface and then become active in the acidic environment of the endosome, resulting in LDLR degradation. Alternatively, by binding to the LDLR, PCSK9 might interfere with the normal recycling of the LDLR after internalization, redirecting the LDLR to lysosomes rather than back to the cell surface (**Fig. 14**). If the latter hypothesis is correct, the action of PCSK9 on LDLR might not involve catalytic activity.

*Is the catalytic activity of PCSK9 required for its function?*

Protein substrates are known for eight of the nine subtilisin-like serine proteinases. Cleavage of the substrates generally results in the production of mature bioactive proteins

as well as processing intermediates, or occasionally, the inactivation of the cleaved protein.

PCSK9 in which the catalytic histidine has been substituted to an alanine does not undergo autocatalytic cleavage and fails to exit the ER. LDLR levels do not change when a catalytically dead enzyme is expressed in liver (25). Thus, autocatalytic activity appears to be required for PCSK9 to leave the ER, but is it required for PCSK9-stimulated LDLR degradation? To address this question, the prodomain and the catalytic domain were expressed *in trans* in cells and the resultant recombinant protein complex was purified from the medium (2). The success of this approach permitted the introduction of a mutation in PCSK9 that abolishes catalytic activity but did not interfere with the secretion of the protein.

As shown in **Fig. 15**, the catalytically inactive protein could mediate the destruction of LDLRs when added to the medium of cultured HepG2 cells in a manner that was nearly identical to that of the wild-type protein. The infusion of catalytically inactive protein into mice also led to the reduction in hepatic LDLRs. These results support a model in which exogenous PCSK9 binds to the LDLR, which then either targets the LDLR to the lysosome for degradation or prevents the recycling of the receptor in a manner that is independent of inherent catalytic activity of the protein. These data also indicate that unlike other PCs, PCSK9 is unique as a subtilisin-like serine protease in that the protein carries out a biological function that is independent of its proteolytic activity.

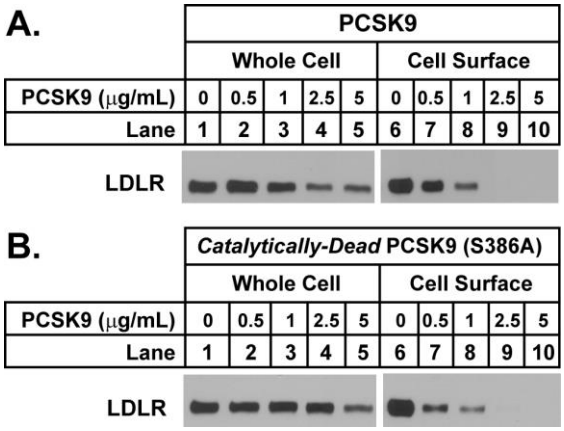



**Fig. 15.** Catalytically inactive PCSK9 degrades the LDLR when added to HepG2 cells. Cells were cultured with the indicated concentrations of: *A*, PCSK9; *B*, *Catalytically-Dead* PCSK9(S386A) for 4 h. Cell surface proteins and whole cell extracts were resolved by SDS-PAGE and immunoblot analysis was performed for LDLR (2).

**Therapeutic Approaches to Inhibit PCSK9 Activity**

Currently, two approaches are under development to block PCSK9 function. The first is using small interfering RNA (siRNA) to knockdown the mRNA for PCSK9 in liver and thus reduce plasma and functional PCSK9 levels. Small interfering RNA (siRNA) can direct sequence-specific degradation of messenger RNA, leading to suppression of synthesis of the corresponding proteins, as part of the natural biological process known as RNA interference (RNAi).

One siRNA compound, designated ALN-PCS, has been used in a Phase 1 randomized, single-blind, placebo-controlled, dose-escalation study in healthy adult volunteers with elevated LDL-C was recently reported (26). Of 32 participants, 24 were randomly allocated to receive a single dose of ALN-PCS (0.015 mg/kg [n=3], 0.045 mg/kg [n=3], 0.090 mg/kg [n=3], 0.150 mg/kg [n=3], 0.250 mg/kg [n=6], or 0.400 mg/kg [n=6]) and eight to placebo. In the group given 0.400 mg/kg of ALN-PCS, treatment resulted in a mean 70% reduction in circulating PCSK9 plasma protein ($p<0.0001$) and a mean 40% reduction in LDL-C from baseline relative to placebo ($p<0.0001$) (**Fig. 16**).

No drug-related serious adverse events occurred in any of the participants who received ALN-PCS. All treatment-emergent adverse events were mild to moderate in severity, with similar proportions affected in the ALN-PCS and placebo groups (79% *vs* 88%). The majority of events were a macular, erythematous rash that occurred with equal frequency in participants given ALN-PCS and those given placebo. Rashes were transient, occurring on the first day after treatment, and was asymptomatic without pain or pruritus.

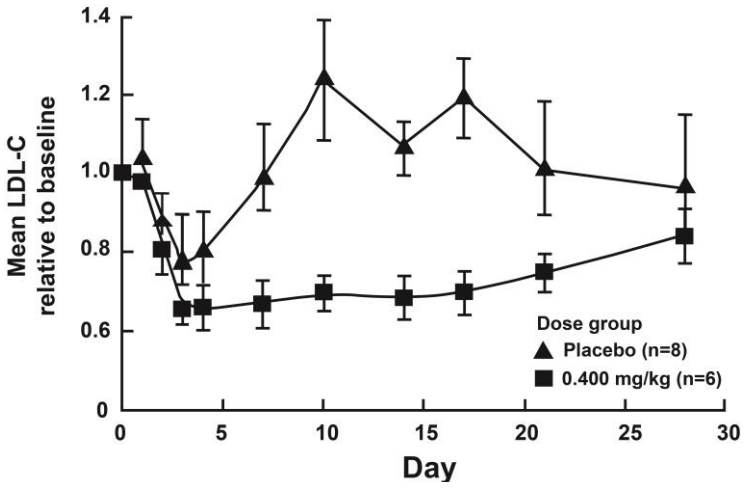



**Fig. 16.** Mean change in plasma LDL-C from baseline after a single injection of placebo or ALN-PCS.

The second approach to inhibit PCSK9 activity has been to develop antibodies that disrupt the interaction of PCSK9 and the LDLR. Multiple studies using two different antibodies (evolocumab and alirocumab) have been published and both antibodies are now in phase 3 studies. A third, bococizumab, is also in phase 3, but results have only been presented in abstract form. The reports from phase 1- 3 trials have all shown similar efficacy in various patient populations so only representative studies that have undergone peer-review will be presented.

The most recent study with the largest number of individuals treated for the longest period of time is the MENDEL-2 (27). This is a randomized, controlled, phase 3 trial using evolocumab in patients with hypercholesterolemia. Shown in **Fig. 17** are the changes in mean LDL-C as a function of time in patients given placebo or evolocumab 420 mg S.Q. every month. LDL-C levels decreased from baseline, on average, by 56.1% (–58.3%, –53.9%) versus 1.3% (–4.4%, 1.7%) for placebo.

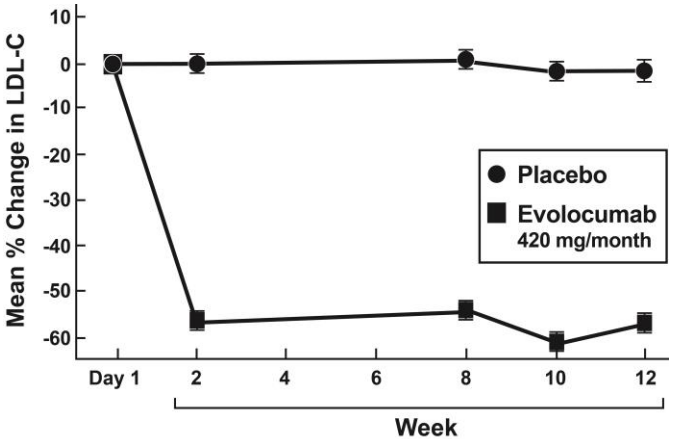



**Fig. 17.** Mean change in plasma LDL-C in patients given placebo (n=155) or evolocumab 420 mg/month (n=153).

Treatment-emergent AEs occurred in 134 (44%) of evolocumab-treated patients, 68 (44%) placebo-treated patients. No deaths or cardiovascular endpoints were reported. AEs led to study drug discontinuation in 6 (3.9%), and 7 (2.3%) patients in the placebo, and evolocumab groups, respectively. Rates of potential muscle-related AEs and laboratory abnormalities were comparable across treatment groups. Injection-site

reactions were reported in 5% of each group; none led to discontinuation of study drug. No neutralizing or binding antibodies were detected during the study.

An important question is whether PCSK9 inhibition will work additively with statins. This has been addressed in studies using evolocumab and alirocumab. Shown in **Fig. 18** are results from a phase 2, multicenter, double-blind, placebo-controlled trial involving 92 patients who had elevated LDL-C after treatment with 10 mg of atorvastatin for at least 7 weeks. Patients were assigned to receive 8 weeks of treatment with 80 mg of atorvastatin daily plus alirocumab 150 mg S.Q. once every 2 weeks, 10 mg of atorvastatin daily plus alirocumab, or 80 mg of atorvastatin daily plus placebo once every 2 weeks. The mean (± SE) % LDL-C reduction from baseline was 73% with 80 mg of atorvastatin plus alirocumab, as compared with 17.3±3.5 with 80 mg of atorvastatin plus placebo (P<0.001) and 66% with 10 mg of atorvastatin plus alirocumab. Thus, significant additional LDL-C lowering can be obtained by PCSK9 inhibition in patients on maximal doses of statins.

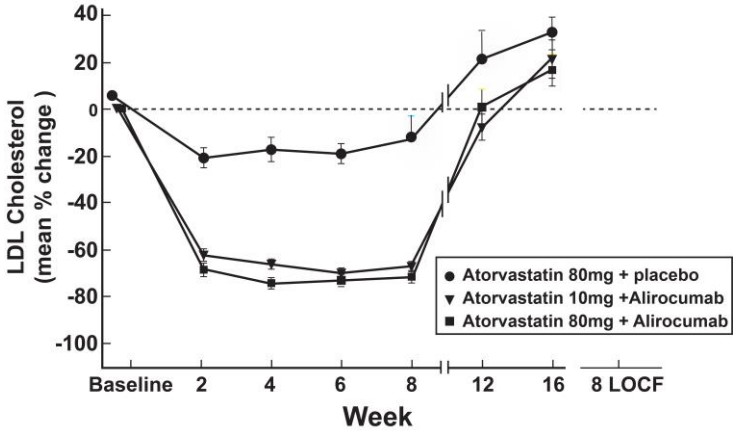



**Fig. 18.** Mean percent change from baseline in LDL-C, according to treatment group.

*Safety issues associated with PCSK9 inhibition*

Will pharmacological inhibition of PCSK9 be safe in humans? PCSK9 is expressed in the kidney and cerebellum of adult mice, in addition to the liver and small intestine (15). Although inactivation of PCSK9 in embryos of zebrafish results in disordered neuronal development and death, mice lacking PCSK9 develop normally and have no gross neurological defects. Humans heterozygous for loss-of-function mutations in PCSK9 appear to be healthy and have a normal life-span (20). Moreover, a compound heterozygote with two inactivating mutations in PCSK9 (Y142X and ΔR97) and consistently low plasma levels of LDL-C (14-34 mg/dL) was found to have no circulating PCSK9 (28). This African-American has grossly normal renal, hepatic, and neuronal function. Another individual homozygous for the C679X mutation was identified in Zimbabwe; she has a plasma LDL-C of 16 mg/dL. Careful clinical assessment of subjects with inactivating mutations in PCSK9 might reveal additional phenotypes, providing clues to substrates of PCSK9 other than the LDLR. Nevertheless, in March of 2014, the FDA said it is "aware of concerns raised with neurocognitive adverse events and other lipid-lowering therapies, including statins, and as part of our oversight of new drug development, we are carefully monitoring these events."

**Summary**

While the prevention and treatment of atherosclerotic vascular disease was revolutionized statins, there are still select populations that could benefit from additional or alternative LDL-C lowering. The lessons learned from human genetics have led to the development of three new therapeutic modalities to lower LDL-C. The first two, mipomersen and

lopidimide, both act by reducing VLDL secretion from liver (**Fig. 19**) and are FDA approved for use only in patients with hoFH. The third, PCSK9 inhibitors, work by increasing the number of LDLRs in liver thereby increasing LDL-C clearance from the blood (**Fig. 19**). It is likely PCSK9 inhibitors will have a broader application to include use in heterozygous FH and possibly statin-intolerant patients. However, no PCSK9 inhibitors are currently FDA approved but it is anticipated that some PCSK9 antibodies will be evaluated by the FDA in the next year or two.

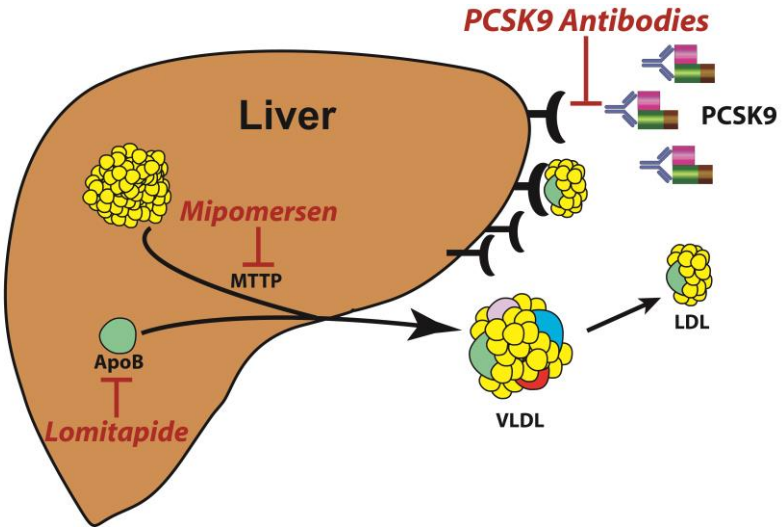



**Fig. 19.** Site and mechanism of action of the three new classes of drugs to lower plasma LDL-C.

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