

New targets and emerging therapies for reducing LDL cholesterol

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Purpose of review

Effective therapies for lowering LDL-cholesterol reduce the incidence of cardiovascular disease and provide associated decreases in morbidity and mortality. Progress in our understanding of metabolism and innovations in drug design have jointly identified promising new drug targets and alternative approaches to old targets. This review focuses on the mechanism, safety and efficacy of emerging LDL-cholesterol lowering therapies.

Recent findings

Decreasing apolipoprotein B expression or preventing the formation of a stable lipoprotein structure by inhibiting microsomal triglyceride transfer protein attenuates the secretion of atherogenic lipoproteins containing apolipoprotein B into the plasma. Increases in LDL receptor-mediated cholesterol clearance occur when hepatic cholesterol stores are reduced secondary to inhibition of squalene synthase or LDL receptor degradation is disrupted by reduced activity of proprotein convertase subtilisin kexin type 9. Each of these developing therapies demonstrably reduces LDL-cholesterol levels.

Summary

The emergence of modalities that act in series and in parallel with available agents may allow more effective LDL-cholesterol lowering in those patients intolerant of current therapy, and may permit decremental reductions in LDL-cholesterol for those unable to achieve aggressive LDL-cholesterol goals using existing agents.

Keywords

apolipoprotein B, LDL-cholesterol, microsomal triglyceride transfer protein, proprotein convertase subtilisin kexin type 9, squalene synthase

Abbreviations

apoB	apolipoprotein B
ASO	antisense oligonucleotides
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA
LDL-C	low-density lipoprotein cholesterol
mRNA	messenger ribonucleic acid
MTP	microsomal triglyceride transfer protein
PCSK9	proprotein convertase subtilisin kexin type 9
siRNA	small-interfering RNA
VLDL	very-low density lipoprotein cholesterol

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Introduction

Serum LDL levels are proportionate to the risk of atherosclerotic cardiovascular disease. Interventions that effectively lower LDL impart morbidity and mortality benefits referable to a decreased incidence of fatal myocardial infarction and other cardiovascular events. The definition of 'at risk' continues to expand, redefining the population of patients that stand to benefit from reductions in low-density lipoprotein cholesterol (LDL-C). The amount of LDL-C reduction providing maximum benefit is also a focus of ongoing clinical trials, and guidelines have routinely adopted more aggressive goals, and recent data support lowering these goals even further [1]. Moreover, the response to available LDL-C lowering therapies is highly variable, and the efficacy and tolerability of monotherapy and combination therapy will become even more limiting as goals become more aggressive. Collectively, the expanding target population, the progressively lower LDL-C goals, and the patient-specific variability in response and tolerability illustrate the need for additional therapies. The present article reviews four classes of LDL-C lowering therapies in development and the available data regarding their safety and efficacy (Table 1).

Inhibition of apolipoprotein B

Apolipoprotein B (apoB) is a large protein that forms the backbone of all lipoproteins other than high-density lipoprotein. From a single apoB gene, the intestine synthesizes a shorter form known as apoB-48 and the liver a longer form known as apoB-100 as a result of editing of the messenger ribonucleic acid (mRNA) in the intestine (but not the liver) at a specific codon to a nonsense (stop) codon. The apoB-100 protein provides key structural integrity to circulating very-low density lipoproteins (VLDL) and LDL, and also serves as the ligand for the LDL receptor (Fig. 1). Plasma apoB concentration is proportionate to the concentration of

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Table 1 Relative efficacy, advantages, disadvantages and clinical status of emerging LDL-C lowering therapies

Therapy	Efficacy as LDL-C lowering therapy	Potential advantages	Potential disadvantages	Clinical trials ^a
apoB inhibition ISIS-301012 (apoB ASO)	Up to 50% reductions in LDL-C	Additive to existing therapy, infrequent dosing	ASO therapies not conventional, need for SQ injection, injection site reactions	Phase II; orphan drug development for patients with homozygous familial hypercholesterolemia
MTP inhibition AEGR-733 (BMS-201038)	Up to 50% reductions in LDL-C	Additive to existing therapy, potential for weight loss	Variable increases in hepatic fat, potential GI side effects	Phase II; orphan drug development for patients with homozygous familial hypercholesterolemia
Imlitapide (AEGR-427) ^b	20% reductions in LDL-C	Limited available clinical data – advantages and disadvantages are theoretical and referable to class		Phase II
SLX-4090, Dirlotapide	No efficacy studies to date	Theoretically no hepatotoxicity, additive to ezetimibe, weight loss	May be less efficacious compared with hepatic MTP inhibition, risk of steatorrhea	Phase II
Squalene synthase inhibition TAK-475	Nearly 30% reduction in LDL-C	Decreased statin-associated myopathy, efficacy incremental to statins, devoid of pleiotropic statin effects	Efficacy inferior to statins, theoretically devoid of pleiotropic statin effects	Phase III
PCKS9 inhibition PCKS9 ASO or siRNA	No human studies to date, 38% reduction in LDL-C in animal studies	Theoretically additive to or synergistic with statins, human genetic data suggest CV benefit and safety	ASO/RNAi therapies not conventional, requires intact LDL receptor	Preclinical

ASO, antisense oligonucleotides; CV, cardiovascular; GI, gastrointestinal; LDL-C, low-density lipoprotein cholesterol; MTP, microsomal triglyceride transfer protein; RNAi, RNA interference; SQ, subcutaneous.

^aData from clinicaltrials.gov as of 31 July 2007.

^bLimited clinical trial data in public domain.

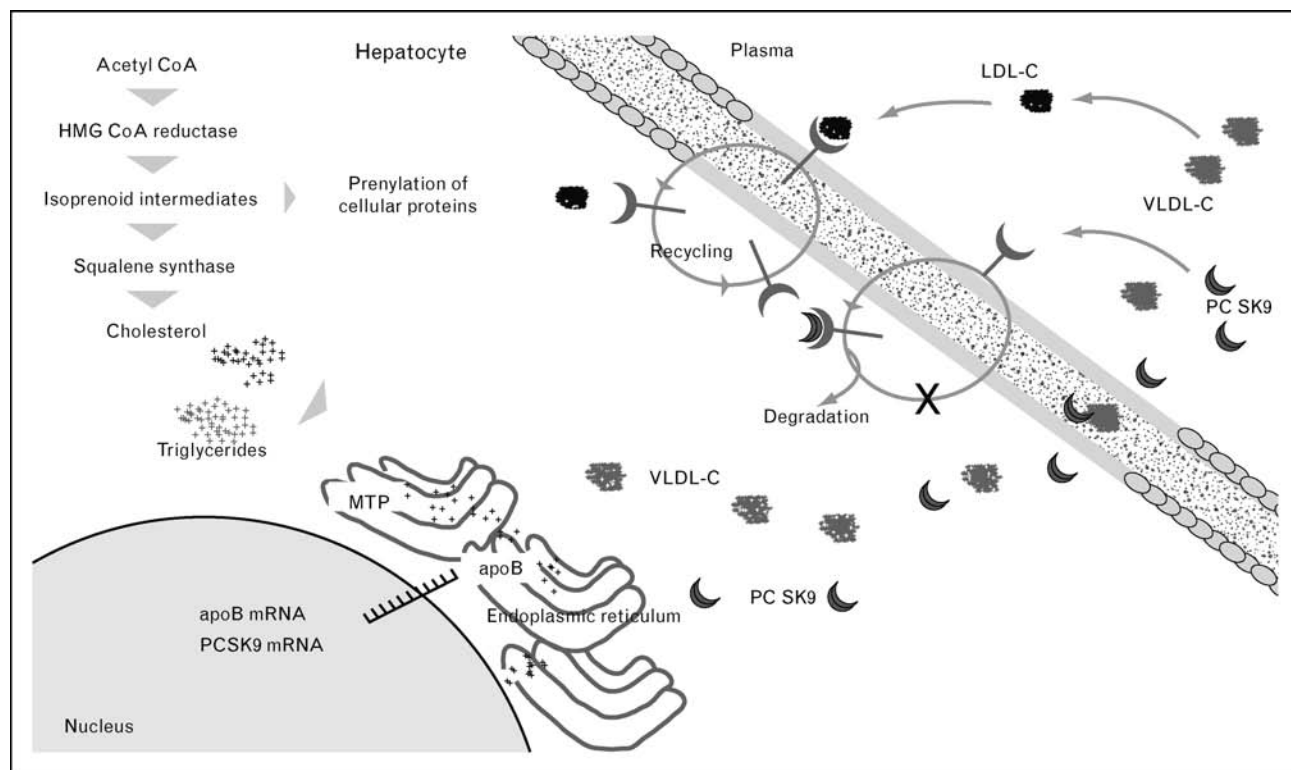
circulating atherogenic lipoproteins and a predictor of cardiovascular risk [2]. Mutations which result in decreased affinity of apoB for the LDL receptor result in familial defective apoB, an autosomal dominant hypercholesterolemia with impaired clearance of plasma LDL and increased risk of atherosclerotic cardiovascular disease [3]. Conversely, mutations in apoB that reduce translation or secretion, or enhance the catabolism of apoB are associated with reduced plasma LDL-C levels and reduced risk of coronary heart disease [4].

Inhibiting translation of apoB transcripts would theoretically decrease the concentration of circulating atherogenic lipoproteins by impairing the hepatic assembly of VLDL. 'Second-generation' antisense oligonucleotides (ASO) are chemically modified to have a greater affinity for target mRNAs than their first-generation predecessors and concentrate in the liver [5]. The efficacy of an ASO targeted to apoB (apoB-ASO) was initially demonstrated in mice [6,7]. Large time and dose-dependent reductions in hepatic apoB mRNA were achieved, along with large and statistically significant reductions in total cholesterol, LDL-C and apoB, independently of functional LDL receptors [6]. A humanized form of this ASO was produced by ISIS Pharmaceuticals (ISIS-301012, Carls-

bad, CA, USA), and validated in mice models expressing transgenic human apoB-100 [7].

In a phase I multiple-dose study in subjects with mild dyslipidemia, subcutaneous injection of ISIS-301012 reduced plasma apoB by up to 50% and LDL-C by up to 44% [8^{••}]. The effect was prolonged; apoB and LDL-C remained below baseline for up to 3 months after the last dose. In a subsequent phase II dose-ranging study, healthy patients with elevated LDL-C responded to seven weekly subcutaneous injections of ISIS-301012 with dose-dependent reductions in LDL-C of up to 70% [9]. When administered to patients on chronic statins but not at LDL-C goal, weekly administration of ISIS-301012 elicited up to 47% reduction in LDL-C [10]. A comparable effect was demonstrated with weekly 300-mg injections in three patients with homozygous familial hypercholesterolemia [11], suggesting a potentially effective treatment for this serious condition. Overall, the effects of ISIS-301012 are dose-dependent, show divergence from placebo as early as 48 h, and can persist for several weeks after the last exposure. Adverse effects with ISIS-301012 have included some local erythema at the injection site. The reported incidence of hepatic transaminase elevations in clinical trials has been relatively low to date, and

Figure 1 Targets of emerging LDL-lowering therapies



Squalene synthase inhibitors inhibit de-novo cholesterol synthesis downstream of the departure of isoprenoid intermediates. Subsequent depletion of intracellular cholesterol increases LDL receptor surface expression and LDL clearance from the blood stream. MTP inhibitors functionally inhibit apolipoprotein assembly in the endoplasmic reticulum, while ASO-apoB reduces apolipoprotein substrate – both effectively reducing the secretion of VLDL from the hepatocyte into the plasma, with subsequent reductions in circulating LDL. PCSK9 is secreted *in vivo*, and limits LDL receptor expression by marking the receptor for intracellular degradation. Quantitative disruption of PCSK9 limits LDL receptor degradation, increases LDL receptor expression, and increases plasma LDL clearance.

concordant with the lack of apparent triglyceride accumulation in animal models [6]. Ongoing clinical investigations aim to clarify the necessity of a ‘loading’ period prior to weekly injections, to assess the effect on hepatic lipid, and to evaluate an oral formulation. If the larger ongoing trials reveal a similar safety profile and efficacy as these earlier trials, apoB-ASO could benefit the population of patients unable to achieve LDL-C goals on existing therapies.

An alternative to ASOs could be the administration of small-interfering RNA (siRNA) molecules complementary to apoB mRNA. Indeed, siRNAs to apoB significantly reduced the expression of apoB mRNA and the plasma concentration of apoB-containing lipoproteins in nonhuman primates [12**]. The development of siRNA-based approaches to targeting apoB is less advanced than ASO-apoB, but a somewhat promising alternative approach.

Inhibition of the microsomal triglyceride transfer protein

Microsomal triglyceride transfer protein (MTP) is an endosomal protein concentrated in hepatocytes and intes-

tinal enterocytes. MTP mediates the transfer of cholesterol esters and triglycerides to nascent apoB – a step that stabilizes apoB secondary structure, diverts the lipoprotein from ubiquitination-degradation, and provides a scaffold for the subsequent addition of neutral lipids (Fig. 1). These steps are required for the secretion of apoB-containing lipoproteins from enterocytes and hepatocytes [13]. Complete absence of MTP results in abetalipoproteinemia, characterized by the absence of circulating apoB and markedly decreased LDL-C [14,15]. This discovery led to the concept that small-molecule MTP inhibitors would disrupt chylomicron (enterocyte) and VLDL (hepatocyte) assembly and secretion, with consequent reductions in circulating LDL [16].

Subjects with homozygous familial hypercholesterolemia have LDL-C levels exceeding 500 mg/dl, a lack of functional LDL receptors (limiting the efficacy of statin therapy), and a major risk of atherosclerotic disease in childhood and early adulthood [17]. An MTP inhibitor was shown to be effective in reducing cholesterol in the rabbit model of this disorder [18]. A clinical trial in six subjects with homozygous familial hypercholesterolemia

was performed with the MTP inhibitor BMS-201038 (Bristol-Myers Squibb, New York, NY, USA) using a dose-titration scheme over 4 months to a maximal dose of 1 mg/kg body weight [19^{••}]. It demonstrated large and statistically significant decreases in total cholesterol (–58%), LDL-C (–51%), and apoB (–55%) at the highest dose. Kinetic studies demonstrated a marked reduction in LDL apoB production. At the highest dose, liver transaminases and hepatic fat were increased in four of the six subjects, but returned to baseline in three of four subjects within 4 weeks off drug. High-dose MTP inhibition may thus be developed as an orphan drug approach for homozygous familial hypercholesterolemia patients, given their high level of cardiovascular risk.

Lower doses of MTP inhibition could potentially have a role in treating patients unable to reach LDL-C goal on present therapies, with a lower risk of hepatic side effects. BMS-201038 (developed as AEGR-733 by Aegerion Pharmaceuticals, Bridgewater, NJ, USA) was evaluated at doses significantly lower (5–10 mg) in subjects with LDL-C ranging from 130 mg/dl to 250 mg/dl, alone and in combination with ezetimibe [20]. In this population, MTP inhibition resulted in a significant dose-dependent decrease in LDL-C (–19% to –30%), which was additive to that achieved with ezetimibe (–20% ezetimibe alone, –35% to –46% combined) and coincident with decremental reductions in total cholesterol and apoB. Overall, there was no difference in the rate of discontinuations in the group treated with ezetimibe 10 mg alone compared with those patients treated with 10 mg ezetimibe plus 10 mg of AEGR-733. Up to 20% of those treated with up to 10 mg AEGR-733, however, did experience liver transaminase elevations. Another MTP inhibitor, CP-346086 (Pfizer Inc., Groton, CT, USA), was reported to effectively reduce total cholesterol by 47% and LDL-C by 72% in healthy human volunteers [21]. Implitapide is another MTP inhibitor with proven efficacy in animals [22] that is in clinical development. Inhibition of hepatic MTP clearly reduces LDL-C levels, but further work is necessary to establish the therapeutic window for efficacy in LDL-C reduction versus risk of hepatic fat accumulation [16].

In order to circumvent the hepatic effects of MTP inhibition, nonabsorbable, enterocyte-selective inhibitors of MTP have been developed. Animal studies revealed that SLx-4090 (Surface Logix, Brighton, MA, USA) was undetectable in the serum of mice after chronic administration with doses that reliably reduced LDL-C. Phase I studies with SLx-4090 recently demonstrated the absence of plasma drug after doses as high as 800 mg daily, while significant reductions in postprandial triglyceridemia (–50%) occurred with doses as low as 50 mg [23]. Dirlotapide (Pfizer Inc., Groton, CT, USA) is another enterocyte-selective MTP inhibitor, presently marketed

as a canine weight-loss therapy and providing significant (–51%) reductions in total cholesterol in preclinical animal studies [24,25]. Although intestinal-specific MTP inhibitors are likely to be effective in treating hyperchylomicronemia, their efficacy as LDL-C lowering agents is uncertain.

Inhibition of squalene synthase

Squalene synthase catalyzes the conversion of farnesyl pyrophosphate to squalene and represents the point at which cholesterol intermediates are strictly committed to cholesterol synthesis (Fig. 1). Like inhibitors of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, they decrease cellular cholesterol, the consequent secretion of atherogenic lipoproteins, and induce homeostatic increases in plasma LDL clearance [26]. Unlike the statins, squalene synthase inhibition occurs after the divergence of isoprenoid intermediates to nonsterol pathways (Fig. 1) and therefore does not limit the prenylation of membrane-directed proteins – a process that has been implicated in both the putatively favorable pleiotropic effects as well as the adverse myopathic effects of statins [27,28].

In animal models, including LDL receptor deficient mice and nonhuman primates, squalene synthase inhibition causes reductions in non-HDL cholesterol that are comparable to statins [29–31]. TAK-475 (Takeda Pharmaceuticals, Deerfield, IL, USA) has been developed for use in humans, and has progressed through early clinical trials. In a randomized, blinded study of healthy human volunteers with LDL-C levels between 130 and 210 ($n = 321$), TAK-475 significantly and dose-dependently reduced LDL-C at 8 weeks compared with placebo (maximum –27%), but was less efficacious than 10 mg of atorvastatin (–37%) [32]. A subsequent study indicated that TAK-475 provided decremental reductions in total cholesterol (–13%) and LDL-C (–20%) in healthy subjects ($n = 172$) on chronic atorvastatin but not at LDL-C goal [33]. Of the collective 277 patients randomized to TAK-475 in these studies, there was one incident of transient liver enzyme elevation. Ongoing phase III trials include the evaluation of TAK-475 as monotherapy, in combination with low and high-dose statins, and in those with familial hypercholesterolemia.

The absence of significant adverse events in these studies is encouraging, and the lack of apparent myopathy is a potential benefit of squalene synthase inhibitors. Indeed, animal and cell culture models have convincingly implicated the depletion of isoprenoid intermediates in statin-associated myopathy, and demonstrated a protective effect when isoprenoids are replenished directly or by TAK-475 given in tandem with high-dose statins [30,34,35^{••}]. By providing serial inhibition of the cholesterol biosynthetic pathway, squalene synthase inhibition

added to statin therapy may permit greater inhibition of de-novo cholesterol synthesis, and thus greater LDL-C reduction, than statin therapy alone.

Proprotein convertase subtilisin kexin type 9 inhibition

PCSK9 is a serine protease that is expressed in the human liver and negatively modulates LDL receptor expression [36]. PCSK9 transcription is induced by low intracellular cholesterol (like that associated with statin therapy), and undergoes autocatalytic cleavage in the endoplasmic reticulum. Once released into the plasma it binds to the LDL receptor in competitive fashion and diverts the receptor from the recycling pathway to the degradative pathway [37–40] (Fig. 1). Gain-of-function variants of PCSK9 that bind to the LDL receptor with greater affinity have been identified in humans with autosomal dominant hypercholesterolemia [41]. In contrast, loss-of-function mutations in PCSK9 have been shown to result in substantially reduced LDL-C levels [42]. There is a spectrum of PCSK9 sequence variations that have a range of effects on reducing LDL-C levels and also range considerably in frequency [43,44]. Importantly, these conditions are associated with substantial reductions in the incidence of atherosclerotic cardiovascular disease [45^{••}]. Accordingly, mice with a transgenic disruption of PCSK9 have increases in hepatic LDL receptor expression, increased LDL clearance rates, and a significantly greater response to statins [46].

Serine protease activity is not required for PCSK9-mediated decreases in LDL receptor surface expression [47], making the development of small molecule inhibitors challenging. One experimental approach has focused on quantitative reductions in PCSK9 expression with ASO administration to achieve LDL-C lowering effects. ISIS-394814 was administered subcutaneously for 6 weeks to mice fed a high-fat diet [48]. PCSK9 mRNA was reduced 90% in tandem with two-fold increases in LDL receptor expression and significant decreases in total cholesterol (–52%) and LDL-C (–38%). These effects were abolished in LDL receptor deficient mice, demonstrating that the effects are in fact mediated by the LDL receptor. Based on the success of PCSK9 inhibition in primate and nonprimate animal models, it seems likely that ASO-PCSK9 will enter clinical development. As with apoB, another approach to targeting PCSK9 could be with siRNA-based approaches [12^{••}].

Adverse effects of PCSK9 inhibition remain speculative, but are theoretically minor when inferred from descriptions of individuals with loss of function mutations. It is encouraging that the phenotypic variability in these individuals seems restricted to cholesterol metabolism, and devoid of gross maladaptive features. Indeed, a compound heterozygote with loss-of-function mutations

in both PCSK9 alleles has been reported to have a very low LDL-C but is in good health [49[•]]. Given reductions of up to 40% in baseline plasma cholesterol in heterozygotes with dysfunctional PCSK9, even submaximal inhibition of PCSK9 may provide effective LDL-C reduction. The upregulation of PCSK9 by statins may limit their efficacy, and the addition of a PCSK9 inhibitor to a statin could blunt this homeostatic response and result in additive or even synergistic reduction of LDL-C.

Conclusion

The expanding target population, the lowering of LDL-C goals and the difficulty achieving the LDL-C goals presently in place, collectively represent the unmet medical need for additional tolerable and effective LDL-C lowering therapies. The therapies described herein can decrease atherogenic lipoprotein secretion or increase LDL clearance by impinging upon new targets, potentially circumventing issues that limit the tolerability of available agents, and providing incremental benefits when efficacy is the primary barrier to achieving LDL-C goals (Table 1). The discovery of new targets coupled with the development and refinement of directed therapies represents a convergence of basic and clinical science that holds promise for the prevention of cardiovascular disease in a variety of at-risk populations.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 658).

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