

REVIEW

Lipid-lowering interventions targeting proprotein convertase subtilisin/kexin type 9 (PCSK9): an emerging chapter in lipid-lowering therapy

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Abstract

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease that is mainly expressed in the liver but can also be found in the intestine and kidneys. PCSK9 promotes the degradation of low density lipoprotein receptors (LDLR) by reducing their recycling and targeting the receptors for lysosomal destruction, thereby decreasing the rate of removal of LDL-cholesterol from the circulation. Thus, interventions targeting PCSK9 by reducing its expression may lead to significant reductions of LDL-cholesterol and possibly decrease cardiovascular risk. The present review aims to present and discuss the current clinical and scientific data pertaining to lipid-lowering interventions targeting PCSK9.

Keywords: AT04A vaccine, low density lipoprotein cholesterol, LDL-C, low density lipoprotein receptors, LDLR, proprotein convertase subtilisin/kexin type 9, PCSK9, small interfering RNA, siRNA.

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Introduction

The role of the low-density lipoprotein cholesterol (LDL-C) in the pathophysiology of atherosclerosis is well known. Statins continue to represent the standard of care. However, there are still cases in which patients fail to achieve the desired goals. It has been shown that only 20–26% of high-risk patients treated with statins as monotherapy for >90 days have reductions of LDL-C <70 mg/dL, and 67–77% had LDL-C <100 mg/dL [1]. There are cases in which patients are intolerant to statins, particularly at high dose, mostly owing to myalgia and weakness. Furthermore, an increased risk for diabetes mellitus, as well as some reports of statin-induced cognitive impairment, has made the Food and Drug Administration (FDA) to mandate additional safety labeling warnings. There are some other non-statin LDL-C-lowering drugs; however, those have produced limited only LDL-C reductions of up to 20%. For example, fenofibrate has been shown to decrease LDL-C by up to 20%, although, in cases of hypertriglyceridemia, it causes an increase in LDL-C levels. Extended-release niacin has been shown to decrease LDL-C by up to 17%. Colesevelam, a bile acid sequestrant, and ezetimibe, which selectively inhibits

the absorption of cholesterol in the small intestine, have been shown to cause a decrease in LDL-C levels by up to 18%. Thus, extensive research is being conducted to identify new LDL-C lowering drugs with a favorable side effect profile, which (used alone or in combination with statin therapy) would be able to produce LDL-C reductions of greater magnitude and decrease cardiovascular risk [2].

PCSK9 Inhibitors

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease, which is expressed mainly in the liver but can also be found in the intestine and kidneys. The human PCSK9 gene is located in the human chromosome 1p32.3 and encodes a 692-amino acid inactive glycoprotein, which undergoes an intramolecular self-catalytic cleavage in the endoplasmic reticulum [3]. This molecule binds to the LDL receptors (LDLR) and targets the receptors for lysosomal degradation, thereby reducing their recycling and reducing the removal rate of circulating LDL-C [4–8]. PCSK9 binds to the LDLR at the cell surface with the catalytic domain of PCSK9 binding to the epidermal growth factor repeat A of the LDLR. The LDLR–PCSK9

complex is then internalized via clathrin-mediated endocytosis. Due to an additional electrostatic interaction at acidic pH between the C-terminal domain of PCSK9 and the ligand-binding domain of the LDLR, PCSK9 remains bound to the LDLR in the sorting endosome. Consequently, the LDLR cannot adopt a closed conformation and is thus degraded instead of being recycled. The mechanism responsible for this failure appears to involve the ectodomain cleavage of the extended LDLR by a cysteine cathepsin in the sorting endosome. The cleaved LDLR ectodomain remains confined in the vesicular part of the sorting endosome for eventual degradation via the endosomal/lysosomal system [4]. On the other hand, interacting partners of PCSK9 have also been found in plasma that may influence its versatility, concentration, and function and modulate its action. Plasma lipoproteins act as important extracellular partners for PCSK9, and PCSK9–LDL complexes have been found both in mouse and human plasma. Besides LDL, PCSK9 may also have other interacting partners in plasma that can modulate its activity. For example, PCSK9 expression in liver cells may be increased by resistin, which is a small protein secreted by murine adipocytes and human macrophages. The levels of resistin are elevated in obesity and may contribute to insulin resistance and inflammation in patients with metabolic syndrome. Furthermore, resistin levels are linked to atherosclerotic cardiovascular diseases in humans [9].

Genetic studies have shown that gain-of-function mutations of PCSK9 in humans are associated with hypercholesterolemia and increased risk for coronary artery disease [10,11]. On the other hand, loss-of-function mutations of PCSK9 result in low LDL-C and significantly reduced cardiovascular risk [12]. Ironically, it has been shown that statins upregulate PCSK9 in a dose-dependent manner, which may lead to attenuation of their lipid-lowering effect [13]. Thus, inhibition of PCSK9 would be an attractive target to maximize the LDL-C-lowering effect of statins [2]. Moreover, aside from plain lipid-lowering, PCSK9 inhibition may also exert additional beneficial effects. More specifically, there are data supporting the hypothesis that reduced PCSK9 function increases LDLR-mediated pathogen lipid clearance and thus reduces the inflammatory response and improves outcomes in sepsis in both mice and humans [14]. Several therapeutic strategies have been developed to decrease levels of circulating PCSK9. The drugs in development include some drugs that block PCSK9 self-cleavage to an active form, that increase its cleavage by furin, and that impede binding with the LDL receptor [15]. However, up to date, monoclonal antibodies have emerged as the new class of lipid-lowering agents causing a remarkable reduction in the levels of LDL-C and other apoB-containing lipoproteins, such as lipoprotein (a) [16,17].

Evolocumab

Evolocumab is a human monoclonal immunoglobulin G2 antibody directed against PCSK9, which was approved by the FDA in August 2015. It was approved as an add-on treatment

to diet and maximally tolerated dose of statins for patients with heterozygous familial hypercholesterolemia (HeFH), homozygous familial hypercholesterolemia (HoFH), or clinical atherosclerotic cardiovascular disease, who require additional lowering of LDL-C [18].

In the Open-Label Study of Long-Term Evaluation against LDL Cholesterol 1 (OSLER-1) and 2 (OSLER-2), a randomized, open-label design was applied in a total of 4465 patients with different degrees of cardiovascular risk. The results of these two studies were analyzed and reported together. The results revealed that after 12 weeks evolocumab, compared to standard therapy, reduced the LDL-C level by 61%, and this effect remained consistent over time. Evolocumab increased the levels of high density lipoprotein (HDL) cholesterol and apolipoprotein A1 by 7.0 and 4.2%, respectively ($p < 0.001$ for both comparisons) [19].

The adverse events of the drug were non-specific, such as injection site reactions, arthralgias, headache, limb pain and fatigue, although some neurocognitive adverse events were found to be more frequent in the evolocumab group compared to the standard-therapy group. The rate of cardiovascular events at 1 year was reduced from 2.18% in the standard-therapy group to 0.95% in the evolocumab group [19].

In a study, which was conducted to determine the effects of evolocumab on progression of coronary atherosclerosis in statin-treated patients, it was shown that addition of evolocumab, compared with placebo, resulted in a greater decrease in percent atheroma volume (PAV) after 76 weeks of therapy [20].

The Further cardiovascular Outcomes Research with PCSK9 Inhibition in subjects with Elevated Risk (FOURIER) trial was a randomized, double-blind, placebo-controlled trial that enrolled 27,564 patients aged 40–85 years with stable atherosclerotic cardiovascular disease and additional risk factors. The primary endpoint of the FOURIER trial was the composite of cardiovascular death, myocardial infarction, stroke, coronary revascularisation, or hospital admission for unstable angina and the key secondary endpoint was the composite of cardiovascular death, myocardial infarction, or stroke [21].

In this trial, the primary outcome was reduced by 15% (95% confidence interval [CI]: 8–21), and the key secondary endpoint was reduced by 20% (95% CI: 12–27) over an average follow-up period of 2.2 years. Beyond 12 months, the reduction in the key secondary outcome with evolocumab was 25%, compared to 16% during the first 12 months. The efficacy results were consistent across subgroups, including men and women and quartiles of baseline LDL-C. The effect appeared to increase over time. There were no significant differences between the evolocumab and the placebo group in the overall incidence of adverse events, serious adverse events, or adverse events that are considered to be related to the study agent and leading to its discontinuation. Furthermore, there were no significant

between-groups differences in the incidence of muscle-related adverse events, cataract, neurocognitive adverse events, allergic reactions, new-onset diabetes, and hemorrhagic stroke (5.0 vs 4.8%, 1.7 vs 1.8%, 1.6 vs 1.5%, 3.1 vs 2.9%, 8.1 vs 7.7%, and 0.21 vs 0.18%, in the evolocumab group vs the placebo group, respectively). In addition, there were no significant between-groups differences in the incidence of aminotransferase levels >3 times the upper limit of normal range or creatine kinase levels >5 times the upper limit of normal range (1.8 vs 1.8% and 0.7 vs 0.7%, in the evolocumab group vs the placebo group, respectively). Only the injection site reactions, although rare, were more frequent in the evolocumab group, as compared with the placebo group (2.1 vs 1.6%, respectively) [21].

In a prespecified secondary analysis of the FOURIER trial, which dealt with the effects and potential safety concerns of very low LDL-C concentrations, it was shown that there was a monotonic relationship between achieved LDL-C and major cardiovascular outcomes down to LDL-C concentrations of less than 0.2 mmol/L (7.7 mg/dL). Furthermore, there were no safety concerns with very low LDL-C concentrations over a median of 2.2 years. These data support further LDL-C lowering in patients with cardiovascular disease to well below current recommendations [22].

In another prespecified analysis of the FOURIER trial, which investigated the efficacy and safety of evolocumab by diabetes status and the effect of evolocumab on glycaemia and risk of developing diabetes, it was shown that evolocumab significantly reduced cardiovascular risk in patients with and without diabetes (hazard ratio [HR] of 0.83 and 0.87, respectively). Furthermore, evolocumab did not increase the risk of development of new-onset diabetes and did not worsen glycaemia [23].

Alirocumab

The 'Long-Term Safety and Tolerability of Alirocumab in High Cardiovascular Risk Patients with Hypercholesterolemia Not Adequately Controlled with Their Lipid Modifying Therapy (ODYSSEY LONG TERM)' was a double-blind, randomized, controlled trial of alirocumab (150 mg subcutaneously every 2 weeks) compared with placebo for 78 weeks in 2341 patients at high risk for cardiovascular events, who were already being treated with the maximum tolerated doses of statins [24].

In this trial, alirocumab was administered in patients receiving maximally tolerated dose of statin, alone or in combination with other lipid-lowering agents. Alirocumab, as compared with placebo, caused an additional 61.9% reduction in LDL-C levels. At week 24, there were 79.3% of alirocumab-treated patients and 8.0% of the patients in the placebo group who achieved an LDL-C level <70 mg/dL ($p<0.001$). This LDL-C-lowering effect of alirocumab was consistent from week 4 to week 78 of the trial and was similar in patients with or without HeFH. Moreover, as compared with placebo, alirocumab reduced levels of apolipoprotein B by 54%, reduced levels of

total cholesterol by 37.5%, reduced levels of non-HDL-C by 52.3%, reduced levels of lipoprotein (a) by 25.6% and reduced levels of fasting triglycerides by 17.3%. On the other hand, as compared with placebo, alirocumab, raised levels of HDL-C by 4.6% and raised levels of apolipoprotein A1 by 2.9% [24,25].

In a *post hoc* analysis, alirocumab, as compared with placebo, reduced the rate of major adverse cardiovascular events (death from coronary heart disease, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, or unstable angina requiring hospitalization) by 48% (1.7 vs 3.3%; 95% CI: 0.31–0.90; nominal $p=0.02$) [24,25].

In addition, alirocumab was shown to be effective in patients with intolerance to statins. In a study, which included patients with statin intolerance at moderate to high cardiovascular risk, treatment with alirocumab caused a mean LDL-C reduction of 45.0%, whereas treatment with ezetimibe decreased mean LDL-C by 14.6% (mean difference 30.4%, $p<0.0001$). Skeletal muscle-related adverse events were less frequent in the alirocumab group, as compared to a group of patients who were rechallenged with atorvastatin (hazard ratio 0.61, $p=0.042$) [25,26].

Owing to the significant LDL-C reduction achieved with alirocumab, the need for lipoprotein apheresis in certain patients with familial hypercholesterolemia may be reduced or even obviated. In one study, treatment with alirocumab led to discontinuation of lipoprotein apheresis in 63.4% of patients with HeFH, who were previously undergoing regular apheresis. Furthermore, the frequency of apheresis was at least halved in 92.7% of patients [25,27].

The results of a large, ongoing outcome trial (ODYSSEY Outcomes: Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab) [28] are awaited with great interest and will provide important data regarding the potential benefits of alirocumab in the reduction of cardiovascular risk. This trial has enrolled 18,600 patients and will compare the effect of alirocumab versus placebo on the rate of cardiovascular events (coronary heart disease (CHD) death, nonfatal myocardial infarction (MI), fatal and nonfatal ischemic stroke, unstable angina requiring hospitalization) in patients who had suffered an acute coronary syndrome (ACS) event 4–52 weeks prior to randomization and are being treated with modern, standard of care, evidence-based medical therapy, as well as dietary management of dyslipidemia [25,28]. The results of this trial are expected to be available in late 2017 or in the first quarter of 2018.

Small interfering RNA

Small interfering RNA (siRNA) is a class of double-stranded RNA molecules, 20–25 base pairs in length, similar to microRNA (miRNA), and operating within the RNA interference (RNAi) pathway. siRNA interferes with the expression of specific genes with complementary nucleotide sequences by causing degradation of mRNA post-transcription, thus preventing translation [29].

The siRNA molecule has been used recently to decrease PCSK9 levels. The siRNA molecules follow the natural pathway of RNA interference (RNAi) by binding intracellularly to the RNA-induced silencing complex (RISC), thus enabling it to cleave messenger RNA (mRNA) molecules specifically encoding PCSK9 [30].

Inclisiran (ALN-PCSSc) is a long-acting, synthetic siRNA that is delivered subcutaneously, directed against PCSK9 that is conjugated to triantennary *N*-acetylgalactosamine carbohydrates. These carbohydrates bind to abundant liver-expressed asialoglycoprotein receptors, leading to inclisiran uptake specifically into the hepatocytes [30,31].

The ORION-1 trial is a Phase 2 dose finding trial. The study evaluated the efficacy of different inclisiran dosing regimens among patients who had elevated LDL-C levels despite receiving the maximum tolerated dose of a statin and who were considered to be at high risk for atherosclerotic cardiovascular disease. The trial also evaluated the safety and efficacy of inclisiran in lowering LDL cholesterol levels [32].

In ORION-1, 501 individuals were randomly assigned to one of eight different arms: two placebo arms and six intervention arms of increasing doses of inclisiran and varied dosing interval. Levels of PCSK9, cholesterol, and lipoproteins in the blood were then measured at 180 days. At the time of enrollment in the trial, all individuals were receiving maximum tolerated statin therapy and were at high cardiovascular risk [32].

At 180 days, a single dose of inclisiran 500 mg decreased the blood PCSK9 levels by 59.3%, LDL-C levels by 41.9%, and total cholesterol levels by 26.6% ($p < 0.001$). This dose also decreased the triglyceride levels by 12.2% and increased the HDL cholesterol levels by 6.9%. Inclisiran was well tolerated with similar numbers and proportions of adverse events reported in the inclisiran and placebo arms, the injection site reactions being the most common occurring in 4% of individuals [32].

Larger outcome studies with inclisiran have been planned for the near future to confirm its LDL-C-lowering effects and assess its benefits in reducing cardiovascular risk.

The AT04A vaccine against PCSK9

Transgenic mice were generated using a genomic 27-kilobase DNA construct isolated from the APOE*3-Leiden proband to study the effect of the APOE*3-Leiden mutation in vivo. Three strains that showed human APOE and APOC1 expression were generated. All strains had significantly elevated levels of total plasma cholesterol and triglycerides on a regular diet [33]. Because of its humanized lipoprotein metabolism, the double-transgenic mice represent a valuable model for the preclinical evaluation of interventions on atherosclerosis development. The diet-induced development of atherosclerosis in these mice has a proinflammatory plaque phenotype, and

exhibits responsiveness to all lipid-modulating interventions used [34].

The control and AT04A vaccine-treated mice were fed western-type diet for 18 weeks. The AT04A vaccine induced high and persistent antibody levels against PCSK9, leading to a significant decrease of plasma total cholesterol ($-53%$, $p < 0.001$) and LDL-C compared with controls. Furthermore, there was a significant reduction in the levels of plasma inflammatory markers, such as serum amyloid A (SAA), macrophage inflammatory protein-1 β (MIP-1 β /CCL4), macrophage-derived chemokine (MDC/CCL22), cytokine stem cell factor (SCF), and vascular endothelial growth factor A (VEGF-A) in AT04A-treated mice. As a result, treatment with the AT04A vaccine led to a decrease in atherosclerotic lesion area ($-64%$, $p = 0.004$) and aortic inflammation, as well as in more lesion-free aortic segments ($+119%$, $p = 0.026$), compared to the control [34].

Although the AT04A vaccine is in its initial stages of development, notwithstanding it appears as a very promising lipid-lowering intervention targeting PCSK9. AT04A is currently being tested in a Phase I clinical trial.

Conclusions and future directions

From the above review of the clinical and scientific data, it becomes apparent that interventions targeting PCSK9 by reducing its expression may lead to significant reductions of LDL-cholesterol and possibly decrease cardiovascular risk.

However, despite their cardiovascular benefits, the cost effectiveness of the treatment with PCSK9 inhibitors remains in question. In a recent study, which analyzed the cost effectiveness of PCSK9 inhibitor therapy, it was concluded that, assuming 2015 prices, the PCSK9 inhibitor use in patients with HeFH or atherosclerotic cardiovascular disease did not meet generally acceptable incremental cost-effectiveness thresholds and was estimated to increase the US health care costs substantially [35]. In another very recent study, again it was shown that, at current prices, the addition of PCSK9 inhibitors to statin therapy was estimated to provide an additional quality-adjusted life year for \$337,729, and that significant discounts were necessary to meet the conventional cost-effectiveness standards [36]. On the other hand, the UK's National Institute for Health and Care Excellence (NICE) has recently approved the limited use of PCSK9 inhibitors in some high-risk patient groups, although the NICE results were based on undisclosed price discounts negotiated with the pharmaceutical companies [37]. Thus, a significant reduction in the price of PCSK9 inhibitors may be necessary, particularly in the United States, in order to maximize cost effectiveness.

Other potential future strategies targeting PCSK9 in development include small molecule inhibitors that disrupt the processing of PCSK9, as well as the use of adnectins, which block the binding of PCSK9 to the LDL receptor [38].

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