

Lipids

From proprotein convertase subtilisin/kexin type 9 to its inhibition: state-of-the-art and clinical implications

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Statins are recommended as first-line therapy for patients with hypercholesterolaemia. A sizable proportion of patients, however, does not reach therapeutic goals, is statin intolerant, or, despite optimal statin therapy, is at high risk of ischaemic events. Proprotein convertase subtilisin/kexin type 9 (PCSK9) plays a major role in lipid metabolism and several comorbidities. Monoclonal antibodies targeting PCSK9 are a new lipid-lowering approach with the potential to improve clinical outcomes in patients with dyslipidaemia. In this review, we discuss current experimental and clinical evidence of the role of PCSK9 and its inhibition on lipid metabolism and several pathologic conditions with a focus on clinical outcomes. A state-of-the-art analysis of current clinical evidence and future directions on PCSK9 and its inhibition is provided.

Keywords

Proprotein convertase subtilisin/kexin type 9 (PCSK9) • PCSK9 inhibitors • Clinical perspectives

Introduction

Coronary artery disease (CAD) caused by obstructive atherosclerosis remains the most frequent cause of morbidity and mortality worldwide.^{1,2} Lipid-lowering therapy represents the cornerstone of treatment of patients with atherosclerotic cardiovascular disease. For years statins have been regarded as a key intervention to lower lipids and improve clinical outcomes.³ However, despite statin therapy at maximally tolerated doses, many patients do not achieve their lipid goals and are burdened by a residual ischemic risk of developing cardiovascular events. Human monoclonal antibodies against proprotein convertase subtilisin/kexin type 9 (PCSK9) administered subcutaneously have been identified as an innovative lipid-lowering strategy. Several Phase III studies in different settings showed that the administration of PCSK9 antibodies combined with statins provided additional benefits in terms of reducing atherogenic lipid fractions in patients with hyperlipidaemia when compared with the sole statin therapy.^{4,5}

In this article, we address the role of PCSK9 in regulation of lipid metabolism and associated diseases, as well as its increasingly recognized effects beyond lipid metabolism, the clinical implications of a pharmacological inhibition of PCSK9, and its future therapeutic directions.

Regulation of low-density lipoprotein metabolism

Since the discovery of PCSK9 over a decade ago, when gain-of-function mutations of PCSK9 were linked to high values of low-density lipoprotein cholesterol (LDL-C) in patients with familial hypercholesterolaemia, the enzyme was found to interfere with various lipid metabolism pathways.^{6–8}

The main clearance route of circulating LDL-C from the blood occurs via hepatocyte endocytosis—a process mediated by binding of LDL-C to low-density lipoprotein receptors (LDL-R) on the

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hepatocyte cell membrane.⁹ The PCSK9 enzyme, a serine protease mainly expressed in the liver and the intestine, acts by promoting the degradation of LDL-R molecules expressed on the cell surface and reducing in turn the amount of LDL-R in hepatocytes (Figure 1). Low intracellular levels of cholesterol or gain-of-function mutations promote PCSK9 transcription and translation; the molecule's enzymatic activity permits its intracellular maturation, which is then followed by secretion. Circulating PCSK9 binds to the LDL-C and LDL-R complex on the cell surface and is subsequently cointernalized. This bond promotes the degradation of the receptor in the lysosome, rather than its recycling to the plasma membrane; PCSK9 can also bind the LDL-R intracellularly. Thus, since PCSK9 plays a major role in degradation of LDL-R, it has emerged as a key target to treat hypercholesterolaemia and coronary heart disease.

Inflammation

Several properties have been discovered for PCSK9 in addition to its known effects on lipid metabolism. Experimental data show that PCSK9 is markedly induced by diverse inflammatory stimuli, such as lipopolysaccharides, zymosan, and turpentine, resulting in a significant increase in LDL-C levels.^{10,11} Proprotein convertase subtilisin/kexin type 9 can directly act as trigger of inflammatory response. Proprotein convertase subtilisin/kexin type 9 administration was found indeed to enhance ox-LDL uptake and vascular cell adhesion

protein-1 expression in smooth muscle cells, which is a key event in the development of the inflammatory cascade that links cholesterol accumulation to the chronic inflammatory process of atherosclerosis¹² (Figure 1). On the other hand, PCSK9 inhibition is associated with reduced monocyte recruitment and attenuated ox-LDL-induced expression of pro-inflammatory chemokine synthesis and secretion. Additionally, reduced PCSK9 function is associated with increased pathogen lipid clearance via the LDL-R, a decreased inflammatory response, and improved outcome during septic shock.¹³

Atherosclerosis and lipoprotein (a)

The expression of plasma PCSK9, due to its systemic pro-inflammatory action and the effect on lipid metabolism, is directly related to severity of atherosclerotic phenotype.¹⁴ In atherosclerotic lesions, PCSK9 exerts a multifactorial action. By inhibiting LDL-R activity in macrophages, it impairs cholesterol efflux, eventually promoting lipid retention within macrophages and foam cell formation¹⁵ (Figure 1). Denis *et al.*¹⁶ demonstrated higher cholesterol accumulation and severe aortic lesions in transgenic PCSK9 mice compared with wild-type controls. In contrast, PCSK9-knockout mice accumulated four-fold less aortic cholesterol than the wild-type controls did. Proprotein convertase subtilisin/kexin type 9 has been found to promote the inflammatory response and the

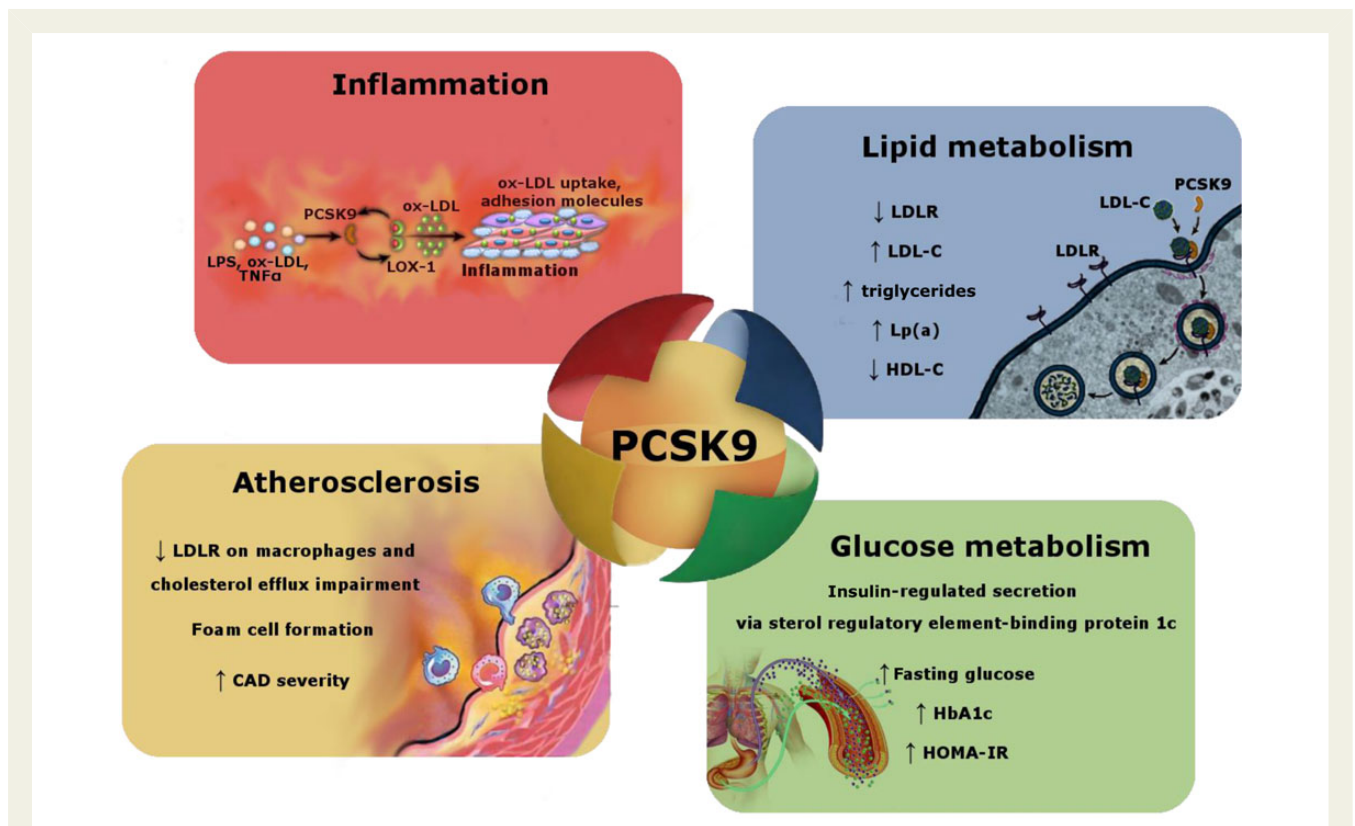


Figure 1 Multifactorial effect of proprotein convertase subtilisin/kexin type 9 on different pathways. CAD, coronary artery disease; LDLR, LDL receptor; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment insulin resistance; OxLDL, oxidized low-density lipoprotein; Lp(a), lipoprotein (a); LPS, lipopolysaccharide; PCSK9, proprotein convertase subtilisin/kexin type 9; TNF α , tumour necrosis factor alpha.

accumulation of oxLDL in the subendothelial space. Notably, in atherosclerotic lesions, PCSK9 is secreted by vascular smooth muscle cells, as well, suggesting also an intraplaque modulation of the atherosclerotic process.

A consistent and independent direct association with cardiovascular disease risk was observed for lipoprotein (a) [Lp(a)], which acts as a pro-inflammatory mediator that augments lesion formation in atherosclerotic plaques. Proprotein convertase subtilisin/kexin type 9 inhibition has been found associated with reduction of Lp(a) concentrations by ~20%.¹⁷ So far, no recommended medical treatment for high Lp(a) has been developed to target the increased residual risk of cardiovascular patients; thus, PCSK9 antibodies may find an application in this clinical scenario, as well.

Blood pressure

Some reports documented that PCSK9 promotes degradation of epithelial Na(+) channels, which regulate blood pressure by influencing epithelial sodium reabsorption in the collecting ducts;¹⁸ the hypothesis of its effect on blood pressure regulation is corroborated by an observational study reporting a correlation between blood pressure and PCSK9 level.¹⁹ Further studies are needed to evaluate the impact of PCSK9 antibodies in patients with different blood pressure levels and their impact on blood pressure itself.

Chronic kidney disease

Limited data are available with regard to the relationship between PCSK9 and chronic kidney disease (CKD). A recent study showed that circulating PCSK9 levels are negatively correlated with renal function.²⁰ After kidney transplantation or haemodialysis, PCSK9 is decreased and retains a positive correlation with LDL-C, suggesting that PCSK9 may remain a significant determinant of LDL-C in CKD subjects.²¹ These data suggest that the regulation of LDL-C by PCSK9 remains intact in CKD patients subject to haemodialysis.

Current proprotein convertase subtilisin/kexin type 9 antibody approval status and approved regimens

The Food and Drug Administration (FDA) approved Praluent (alirocumab at dose 75–150 mg every 2 weeks, 24 July 2015) and Repatha (evolocumab, at dose 140 mg every 2 weeks or 420 mg once a month, 27 August 2015) in addition to diet and maximally tolerated statin therapy for treatment of adults with heterozygous familial hypercholesterolaemia (HeFH) and homozygous familial hypercholesterolaemia (HoFH, Repatha only) or patients with clinical atherosclerotic cardiovascular disease such as heart attacks and strokes, who require additional lowering of LDL cholesterol.^{22,23} The European Medicines Agency approved Repatha (22 May 2015) and Praluent (24 July 2015) in patients with primary hypercholesterolaemia (heterozygous familial and non-familial) or mixed dyslipidaemia, who are unable to reach LDL-C goals with maximum tolerated statin therapy, either alone or in combination with a statin

or other lipid-lowering therapies.^{24,25} Additionally Repatha is indicated in patients with HoFH aged 12 years and older, in combination with other lipid-lowering therapies. Praluent was tested in 10 randomized placebo-controlled trials (RCTs) enrolling almost 5300 patients, and Repatha was evaluated in nine RCTs including ~5500 patients with HeFH and mixed dyslipidaemia; two studies specifically included participants with HoFH,^{26,27} leading to the drug approval for homozygous patients with higher LDL-C level and at greater risk for cardiovascular events. The decision to indicate the new drug for HoFH in these young patients when still no trial in this population was conducted is motivated by the lack of other treatment options. However, patients who are statin intolerant without established clinical atherosclerotic cardiovascular disease (CVD) are at the moment left out of the indications. A third PCSK9 inhibitor named bococizumab is currently evaluated in phase III randomized controlled trials by Pfizer and is expected to be approved in the near future.

Clinical settings

Proprotein convertase subtilisin/kexin type 9 inhibition and familial hypercholesterolaemia

Familial hypercholesterolaemia is a disorder characterized by genetic LDL receptor malfunction that significantly increases risk of CAD. Patients with HeFH usually have at least one normal LDL receptor allele; conversely in those with HoFH, both LDL receptor alleles are usually abnormal. Individuals affected by homozygous FH already develop significant atherosclerotic lesions in the first decade of life, which frequently leads to fatal myocardial infarction before reaching adulthood.²⁸ Owing to the reduced LDL-C liver clearance and lack of response to high-dose statin therapy, they reach total cholesterol levels of up to 19 mmol/L.²⁹ Heterozygous patients (prevalence ~1 : 200, one of the most frequent monogenetic diseases^{30,31}) experience first cardiovascular events in their third or fourth decade of life, which accounts not only from the increased LDL-C levels (however, lower than in homozygotes) but also from time to exposure to the high cholesterol levels. The clinical sequelae are tendon xanthomas, premature CAD and, in HoFH, valvular or supra-avalvular aortic stenosis. Despite the effectiveness of cholesterol-lowering therapies, most patients with FH cannot achieve plasma LDL-C targets recommended to prevent cardiovascular events. In TESLA Part B, 50 patients with homozygous hypercholesterolaemia were randomly assigned to receive monthly injections of evolocumab 420 mg or placebo.²⁶ Of the 49 patients who received their assigned treatment and completed the trial, 45 (94%) were homozygous for LDL receptor mutations—22 for the same mutations (true homozygotes) and 23 for different mutations (compound heterozygotes). Compared with placebo, evolocumab achieved an overall mean reduction in plasma LDL-C of 30.9% absolute reduction (2.4 mmol/L). Patients with defective mutations in one or both LDL receptor alleles responded better to treatment than did those with at least one null mutation. The homozygous patient with two null LDL receptor mutations and the patient with autosomal recessive hypercholesterolaemia did not respond to evolocumab. Remarkably, the response of LDL-C to active treatment

varied, even in people with the same LDL receptor mutation. In the RUTHERFORD-2 trial, the authors assessed the efficacy of evolocumab 140 mg administered subcutaneously every 2 weeks, or 420 mg every month, vs. placebo in lowering plasma LDL-C in 331 participants with heterozygous FH.³² The two drug regimens had similar efficacy, with ~60% reduction in plasma LDL-C from a baseline of 4 mmol/L, relative to placebo ($P < 0.0001$); >60% of patients attained an LDL-C concentration <1.8 mmol/L, a target rarely achievable with best available therapy.

At variance with the homozygous form of the disorder, the LDL-C responses did not depend on the genetic variant in the LDL receptor, which suggests that investigation of the molecular defect might be not necessary for prescription of PCSK9 monoclonal antibodies to patients with heterozygous FH. In the recent two randomized, double-blind studies (ODYSSEY FH I, $n = 486$; FH II, $n = 249$), patients were randomized 2:1 to alirocumab 75 mg or placebo every 2 weeks.³³ Alirocumab dose was increased at Week 12 to 150 mg Q2W if week LDL-C was ≥ 1.8 mmol/L (70 mg/dL). Primary endpoint (both studies) was percentage change in calculated LDL-C from baseline to week 24. Mean LDL-C levels decreased from 3.7 mmol/L at baseline to 1.8 mmol/L (-57.9% vs. placebo) at week 24 in patients randomized to alirocumab in FH I and from 3.5 to 1.8 mmol/L (-51.4% vs. placebo) in FH II. These reductions were maintained through Week 78 and suggest that also in patients with HeFH and inadequate LDL-C control at baseline despite maximally tolerated statin dose, alirocumab treatment resulted in a significant LDL-C lowering, greater achievement of LDL-C target levels, and was well tolerated. To evaluate the effect of LDL-C reduction in preventing early cardiovascular events and decreasing residual risk to population risk of the same age, the HAUSER-RCT study was initiated for children and adolescents with heterozygous familial hypercholesterolaemia—with results, however, not yet available.³⁴ Proprotein convertase subtilisin/kexin type 9 levels are associated with serum LDL-C levels and lipid metabolism even in neonates, with the correlation maintained through lifetime. However, the enzyme seems to have a multifactorial effect. The mutations that prevent secretion of PCSK9 are associated with a 30–40% reduction in LDL-C and remarkably with a 88% reduction in clinical events associated with CAD over a 15-year follow-up period.³⁵

Coronary artery disease

Healthy adults do not express a correlation between PCSK9 concentration, carotid intima media thickness, and cardiovascular event occurrence; however, the body mass index, insulin, LDL-C, and triglycerides are already independent predictors of PCSK9 levels.³⁶ Those correlations change with CAD progression (Table 1). Several reports showed that CAD patients have higher PCSK9 levels than the control group,³⁷ which in turn present a correlation of small density LDL-C with PCSK9 and becomes significant in patients with dyslipidaemia and stable CAD, but not in non-CAD group.³⁸ Higher PCSK9 concentrations were additionally associated with female gender, hypertension, statin treatment, C-reactive protein, HbA1c, and insulin level.³⁹

Proprotein convertase subtilisin/kexin type 9 levels are also correlated with the severity of CAD assessed by the use of a Gensini angiographic score system; among patients assigned to groups based

on angiographic score, those with the most severe coronary stenosis also had the highest serum PCSK9 levels.⁴⁰ By logistic regression analysis, PCSK9 levels were associated with an increased CAD risk.³⁷ In another independent recent analysis from the Emory Cardiology Biobank study, plasma PCSK9 levels were elevated in patients with CAD by angiography (385.0 ± 146.9 ng/mL) compared with controls (340.4 ± 125.2 ng/mL, $P < 0.001$). By multivariate analysis, plasma PCSK9 levels resulted an independent predictor of CAD. Of note, alirocumab was found to exhibit anti-atherogenic potential and dose-dependently decreased atherosclerotic lesion size and severity. These effects appear to be enhanced by adding atorvastatin. Similarly, monocyte recruitment was reduced and plaque composition improved its stability with increased content of smooth muscle cells and collagen, whereas macrophage and necrotic core content decreased.⁴¹

Impact of proprotein convertase subtilisin/kexin type 9 antibodies on cardiovascular outcomes

The first and most comprehensive evidence of cumulative effects on cardiovascular clinical outcomes with PCSK9 antibodies was observed in a large meta-analysis that included 24 trials with 10 159 patients and found reduced mortality in patients treated with PCSK9 antibodies.⁴² The odds reduction in mortality with PCSK9 antibodies was >50% (Figure 2). The finding, although preliminary, is encouraging and is further corroborated by a similar direction of reduction in the odds of cardiovascular mortality and myocardial infarction. Of note, no signal for heterogeneity was present across trials in the analysis of all-cause and cardiovascular mortality, and there was stability of the direction and magnitude of results in the sensitivity analyses. Moreover, the sensitivity analyses for type and dose of PCSK9 antibody, and the subgroup analyses stratified by placebo or ezetimibe as the control arm and by background statin therapy, all suggest that the overall effect is robust and justified.

Given the finding from cholesterol-lowering trials that each 1 mmol/L reduction of LDL-C translates to a 22% reduction in major cardiovascular events, the antibodies are expected to result in ~40% reduction in major cardiovascular events.³ Notably, since PCSK9 inhibition seems to have an additional, beyond LDL-C effect, its inhibition could result in even higher risk reductions. These favourable results are also confirmed in two publications of large-scale trials including subjects with hypercholesterolaemia. In the combined analysis from OSLER-1 and OSLER-2 studies, which evaluated long-term effects of evolocumab as an extension of the open-label, randomized controlled trials and included 4465 patients. Eligible patients had completed either of the trials without suffering any adverse events that required discontinuation of study drug. Participating patients were re-randomized to the PCSK9 inhibitor plus standard therapy vs. standard therapy alone without placebo control.⁴³ Throughout the 48- to 56-week trial period, no safety issues emerged during the follow-up period. Baseline LDL-C was 120 mg/dL before the initial randomization into the parent studies and was reduced by 61% to a mean of 48 mg/dL. Importantly, a composite cardiovascular event rate including death, myocardial infarction, unstable angina requiring hospitalization, coronary revascularization, stroke, transient ischaemic attack, and heart failure requiring

Table 1 Correlations of proprotein convertase subtilisin/kexin type 9 levels across different populations

Setting	Study	PCSK9 level
Neonates	Araki et al. ⁶³	The PCSK9 concentration in male newborns was significantly lower than that in females Circulating serum PCSK9 levels were positively correlated with total cholesterol and LDL-C No correlations between PCSK9 levels and birth weight, gestational age, or SGA Circulating PCSK9 levels and gestational age were independent predictors of the serum LDL-C levels
Children and adolescents	Baass et al. ⁴⁵	PCSK9 levels were significantly positively associated with fasting glucose, insulin, and HOMA-IR In multivariable analysis, a 10% higher fasting insulin was associated with a 1–2% higher PCSK9 in both sexes. There were also positive associations between PCSK9 and total cholesterol, LDL-C, and triglycerides, as well as with HDL-C and apolipoproteins A1 and B Sex modified the association between age and PCSK9 in youth. Differences between sexes and during pubertal development suggest an influence of sex hormones on plasma PCSK9 concentrations There were no sex differences in PCSK9 concentrations in young adults, whereas significant differences were observed in youth
Middle-aged men enrolled in the FATE study, free of vascular disease	Zhu et al. ³⁶	Multivariate linear regression analyses indicated that body mass index, insulin, low-density lipoprotein cholesterol, and triglycerides were independent predictors of PCSK9 Further modelling revealed no correlation between PCSK9 concentration and carotid intima media thickness, flow-mediated dilation, or reactive hyperaemic velocity time integral Analyses indicated no significant association between PCSK9 concentrations and cardiovascular event occurrences
Chinese population	Cui et al. ¹⁹	Serum PCSK9 levels were slightly higher in women than in men Compared with premenopausal women, postmenopausal women had significantly higher PCSK9 levels Serum PCSK9 levels were correlated with multiple metabolic variables including age, body mass index, total cholesterol, LDL cholesterol, triglycerides, fasting blood glucose, systolic blood pressure, and diastolic blood pressure After stepwise regression analysis, there was a significant positive association between serum PCSK9 levels and total cholesterol, triglycerides, and SP in men In women, there was a positive correlation between PCSK9 levels and total cholesterol, age, and DP
Indian Asian populations not taking statin therapy	Walton et al. ⁶⁴	PCSK9 levels were weakly correlated with male gender and number of diabetes years, and inversely with log ₁₀ of lipoprotein (a) concentration Gensini score was associated with age, established angina, duration of diabetes, low HDL-C, lipoprotein (a), creatinine, C-reactive protein, and PCSK9 concentrations PCSK9 concentrations are correlated with atheroma burden
Multi-ethnic population	Lakoski et al. ⁶⁵	PCSK9 levels were significantly higher in women than in men PCSK9 levels were significantly higher in postmenopausal women compared with premenopausal women, irrespective of oestrogen status Plasma levels of PCSK9 correlated with plasma levels of LDL-C and plasma levels of triglycerides, insulin, and glucose
JUPITER trial participants	Awan et al. ⁶⁶	At baseline, median PCSK9 concentrations were higher in women than in men During 1 year, there was no change in PCSK9 concentrations in the placebo arm, suggesting stability in time. In contrast, rosuvastatin increased PCSK9 by 35% in women and 28% in men Rosuvastatin increased plasma concentration of PCSK9 in proportion to the magnitude of LDL-C reduction; the LDL-C response to statin could not be inferred by PCSK9 concentrations
CAD and non-CAD patients	Li et al. ³⁷	The CAD group had higher PCSK9 levels than the control group when adjusting for the confounding factors PCSK9 levels were associated with the severity of CAD assessed by the Gensini score system Logistic regression analysis showed that PCSK9 levels were associated with an increased CAD risk Mediator analysis indicated that the effects of PCSK9 levels on severity of CAD were mediated by lipid and inflammation

Continued

Table 1 Continued

Setting	Study	PCSK9 level
Stable CAD and non-CAD patients	Zhang et al. ³⁸	The positive correlation of sdLDL-C with PCSK9 was only significant in patients with dyslipidaemia and stable CAD
Stable CAD	Werner et al. ³⁹	In a model adjusting for traditional risk factors including dyslipidaemia, PCSK9 was an independent predictor of high sdLDL-C in CAD group but not in non-CAD group Serum concentrations of PCSK9 predicted CV outcomes, the association was reduced after adjustment for fasting TG Higher PCSK9 concentrations were associated with female gender, hypertension, statin treatment, C-reactive protein, HbA1c, insulin, total cholesterol, and fasting triglycerides, but not with LDL-C or HDL-C
Ischaemic heart disease	Benn et al. ⁶⁷	46L allele carriers had a 12% (0.43 mmol/L) reduction in LDL-C and a 28% reduction in risk of ischaemic heart disease The observed 12% reduction in LDL-C theoretically predicted an only 5% reduction in risk of ischaemic heart disease
Cerebral ischaemic stroke	Han et al. ⁶⁸	Both rs1711503 and rs2479408 of PCSK9 genes were associated with cerebral ischaemic stroke

CAD, coronary artery disease; HDL-C, high density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PCSK9, proprotein convertase subtilisin kexin 9; SP, systolic pressure; DP, diastolic pressure; sdLDL-C, small, dense low density lipoprotein cholesterol.

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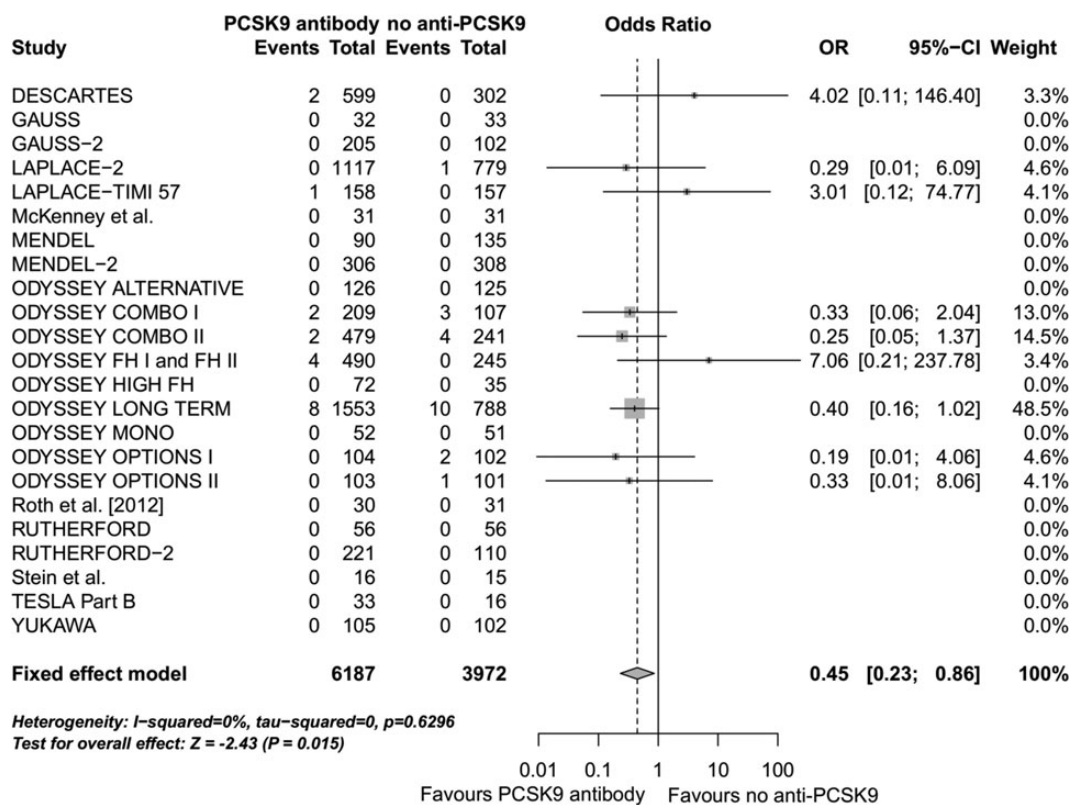


Figure 2 Mortality reduction with proprotein convertase subtilisin/kexin type 9 inhibitors—meta-analysis of randomized controlled trials. Adapted from Navarese et al.⁴²

hospitalization was halved in patients receiving evolocumab compared with the standard-therapy group.⁴⁴ These cardiovascular events occurred in a 1% of the evolocumab group vs. 2% of the standard-therapy group events.

Those results were in line with the evidence obtained from the randomized, double-blind, placebo-controlled trial, ODYSSEY LONG TERM trial, evaluating alirocumab administered for 78 weeks,⁵ which enrolled 2341 high-risk patients with HeFH, known

CAD, or CAD risk equivalent. Eligible patients had LDL-C levels above 70 mg/dL and were currently taking either high-dose statins or maximum tolerated dosages. In a *post hoc* analysis that assessed the pre-specified primary endpoint from the ongoing ODYSSEY Outcomes trial (a composite of death from coronary heart disease, nonfatal myocardial infarction, fatal or nonfatal ischaemic stroke, or unstable angina requiring hospitalization), a lower rate of adjudicated major adverse cardiovascular events was observed in the alirocumab (1.7%) compared with the placebo group (3.3%). In both trials, the cumulative incidence curves diverged progressively with time to exposure to the treatment. This evidence is especially encouraging, since to-date no single trial was yet powered to detect the effect of anti-PCSK9 on cardiovascular outcomes.

Proprotein convertase subtilisin/kexin type 9 and glucose homeostasis

Available human data concerning the effect of PCSK9 on glucose homeostasis are limited. A study by Baass *et al.*⁴⁵ interestingly showed that PCSK9 seems to have a negative impact on glucose metabolism even at early age. In children there was significant correlation between increased PCSK9 levels and fasting glucose, insulin, and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), to the extent that a 1–2% higher PCSK9 was associated with a 10% higher fasting insulin in both sexes (Figure 1). Additionally, the differences between sexes and during pubertal development suggest an influence of sex hormones on plasma PCSK9 concentrations. Another study in healthy and type 2 diabetic youth found that only hypercaloric high-fructose diet, neither high-fat or high-fat/high-protein diet, influenced plasma PCSK9 concentrations, which increased by 28% in healthy volunteers and by 34% in offsprings with diabetes.⁴⁶ On the other hand, physical exercise⁴⁷ as well as fasting⁴⁸ markedly decreased plasma PCSK9 concentrations. The enzyme level declined steadily during the fasting period, reaching a nadir at 36 h that was significantly ~58% lower than levels measured in the fed state.

Further data suggest PCSK9 to be secreted in insulin-dependent fashion.⁴⁹ Costet *et al.*⁵⁰ suggested that hepatic PCSK9 expression could be regulated by insulin via the sterol regulatory element-binding protein 1c, thereby providing a molecular connection between PCSK9 and insulin metabolism. Plasma PCSK9 levels were associated with the most detrimental lipoprotein-lipid profile including lower LDL particle size and higher apolipoprotein C-III levels and also higher HOMA-IR indices, additionally suggesting association with the insulin sensitivity index. Indeed, participants in the top PCSK9 levels tertile have ~40% lower insulin sensitivity indices compared with participants in the bottom tertile.⁵¹ This may hold further consequences on the lipid metabolism. On the other hand, carriage loss-of-function PCSK9 p.R46L was associated with insulin resistance, assessed indirectly through increased HOMA-IR, but only in those with the apolipoprotein E3/E2 genotype and in one report only;⁵² a study by Bonnefond *et al.*⁵³ failed to detect any significant association between p.R46L and markers of glucose homeostasis (fasting plasma glucose, HbA1c, fasting plasma insulin, HOMA-B, and HOMA-IR) and showed that p.R46L carriers did not have an increased incidence of type 2 diabetes over a 9-year follow-up, risk of type 2 diabetes in the case–control study, or in a total of

42 590 European participants in the DIAGRAM consortium. Consistent with those results, there was no increased risk of type 2 diabetes in subjects with PCSK9 loss-of-function variants in other cohorts.^{35,54}

Recently presented findings indicate safety of the PCSK9 antibodies administration in patients with metabolic disorders. A yearly treatment with evolocumab 420 mg monthly or placebo revealed in the interim, a *post hoc* analysis from DESCARTES that anti-PCSK9 agent was effective and well tolerated with no adverse signal for glycaemic control (including HbA1c, fasting plasma glucose, insulin, or HOMA measurements) in patients with pre-existing dysglycaemia or metabolic syndrome.⁵⁵ The incidence of new onset type 2 diabetes (HbA1c \geq 6.5% at any post-baseline visit) was similar in evolocumab and placebo groups (4.6 vs. 5.4%, respectively). Evolocumab reduced LDL-C by >50%, with similar reductions as in non-diabetic patients. A supportive results for evolocumab are derived from a pooled analysis of four Phase III studies: MENDEL-2, LAPLACE-2, RUTHERFORD-2, and GAUSS-2.⁵⁶ The LDL-C lowering potential with evolocumab was comparable in patients without type 2 diabetes (61%, two-weekly regimen and 62%, monthly regimen) and with type 2 diabetes (57 and 60%, respectively) with almost 90% of diabetic patients reached the <70 mg/dL LDL-C goal; the benefit was observed across all subgroups, regardless of insulin requirement, controlled HbA1c levels, renal function, and intensity of statin therapy.

The treatment with alirocumab was also proven to be as effective in patients with diabetes as in those without in a *post hoc* analysis of ODYSSEY LONG TERM, which included 2341 high cardiovascular risk patients, on maximally tolerated statin with or without other lipid-lowering therapy not reaching LDL-C target.⁵⁷ Over a third of patients had diabetes at baseline. Similarly to the OSLER patients, the magnitude of LDL-C reduction was comparable in patients with and without diabetes (59 and 63%, respectively), followed by changes in other lipid parameters, including Lp(a), triglycerides, and high-density lipoprotein cholesterol. No specific safety signals were observed in either group during 78 weeks of therapy. The frequency and profile of adverse events was similar in patients with and without diabetes (81.5 vs. 80.7%), when compared with the overall study population (81.0%); nasopharyngitis, upper respiratory infection, and urinary tract infection reported in \geq 5% of individuals in all subgroups, while injection-site reaction and myalgia were reported two-fold less frequently in patients with diabetes (3.4 vs. 7.2% and 3.1 vs. 6.7%, respectively) compared with non-diabetic group. Furthermore, a reduction in major cardiovascular events was also similar in patients with diabetes [hazard ratio (HR): 0.41; 95% confidence interval (95% CI): 0.18–0.96] and without diabetes (HR: 0.51; 95% CI: 0.23–1.13).

Ongoing clinical studies

The ODYSSEY Outcomes trial, which has just completed enrolment of 18 000 post-acute coronary syndrome subjects, examines the effects of alirocumab on the incidence of major cardiovascular events (composite primary endpoint of coronary heart disease death, non-fatal myocardial infarction, fatal and non-fatal ischaemic stroke, and unstable angina requiring hospitalization).⁵⁸ The results of observations derived from a minimum 36 000 patient-years of

follow-up are expected by 2017. In similar time frames, the results of the FOURIER trial will be available.⁵⁹ The trial has completed enrolment of 27 000 patients at high risk for a recurrent cardiovascular event and is evaluating 5-year evolocumab administration for the reduction of the major cardiovascular composite endpoint (cardiovascular death, myocardial infarction, hospitalization for unstable angina, stroke, or coronary revascularization). SPIRE-1 and SPIRE-2 are two other long-term outcome trials to test not yet FDA-approved PCSK9 monoclonal antibody bococizumab vs. placebo for the reduction of major cardiovascular events in high-risk patients at the background of lipid-lowering therapy.^{60,61} The results from that large database will provide further evidence of PCSK9 antibodies role in several subsets of patients at higher risk than those from previous trials. Potential broader use of PCSK9 inhibitors, such as in statin-intolerant patients, also raises questions as the criteria for statin-intolerance are still not clear, without generally accepted definition of this condition. Although parenteral application was at the beginning considered as a potential obstacle, this has not been confirmed so far in clinical trials. More recently, after the positive results of Phase II and Phase III trials, both patients and physicians became more open in discussing this treatment strategy.⁶² However, the presumed high cost of PCSK9 inhibitors might have an impact on their use. Nevertheless, similar to what happened with statins 10 years ago, it can be expected that the cost of treatment with PCSK9 inhibitors will decrease over time with a more extensive use and expanded labelling of the drug.

Summary

The use of proprotein convertase subtilisin/kexin type 9 (PCSK9) antibodies is an important novel therapeutic strategy for the management of patients with high LDL-C levels. Proprotein convertase subtilisin/kexin type 9 inhibition has the potential to improve clinical outcomes not only through the antidyslipidaemic effect but also anti-inflammatory action and potential effect on glucose metabolism. Future studies should address the clinical impact of PCSK9 inhibition beyond its lipid reduction. Statins have been the first line of treatment for patients with high cholesterol since the late 1980s, but this new class could offer an alternative to statin patients who experience unpleasant side effects or have insufficiently reduced LDL-C levels. Future trials are expected and to expand the indication and labelling of PCSK9 antibodies.

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