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PCSK9 inhibition to reduce cardiovascular disease risk: recent findings from the biology of PCSK9

Hagai Tavori, **Ilaria Giunzioni**, and **Sergio Fazio**

The Knight Cardiovascular Institute, Center for Preventive Cardiology, Oregon Health and Sciences University, Portland, Oregon, USA

Abstract

Purpose of review—Review novel insights into the biology of proprotein convertase subtilisin/kexin 9 (PCSK9) that may explain the extreme efficiency of PCSK9 inhibition and the unexpected metabolic effects resulting from PCSK9 monoclonal antibody therapy, and may identify additional patients as target of therapy.

Recent findings—For over 20 years, the practical knowledge of cholesterol metabolism has centered around cellular mechanisms, and around the idea that statin therapy is the essential step to control metabolic abnormalities for cardiovascular risk management. This view has been embraced by the recent AHA/ACC guidelines, but is being challenged by recent studies including nonstatin medications and by the development of a new class of cholesterol-lowering agents that seems destined to early US Food and Drug Administration approval. The discovery of PCSK9 – a circulating protein that regulates hepatic low-density lipoprotein (LDL) receptor and serum LDL cholesterol levels – has led to a race for its therapeutic inhibition. Recent findings on PCSK9 regulation and pleiotropic effects will help identify additional patient groups likely to benefit from the inhibitory therapy and unravel the full potential of PCSK9 inhibition therapy.

Summary—Injectable human monoclonal antibodies to block the interaction between PCSK9 and LDL receptor are demonstrating extraordinary efficacy (LDL reductions of up to 70%) and almost the absence of any side-effects. A more moderate effect is seen on other lipoprotein parameters, with the exception of lipoprotein(a) levels. We describe mechanisms that can explain the effect on lipoprotein(a), predict a potential effect on postprandial triglyderides, and suggest a new category of patients for anti-PCSK9 therapy.

Keywords

LDL-cholesterol; LDL-receptor; lipoprotein(a); PCSK9 inhibition; triglycerides

Correspondence to Sergio Fazio, MD, PhD, Oregon and Health and Science University, 3181 SW Sam Jackson Park Road, Mail Code HRC 504, Portland, OR 97239, USA. fazio@ohsu.edu.

Conflicts of interest

None.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in all countries [1]. Among the risk factors, hypercholesterolemia is directly linked to premature development of atherosclerosis, the pathology underlying coronary heart disease (CHD) and the most common form of stroke [1]. Familial hypercholesterolemia, commonly due to mutations in the low-density lipoprotein (LDL) receptor (LDLR), results in elevated LDL-cholesterol (LDL-c) levels and is known to cause premature atherosclerosis and aortic calcification in humans [2]. Statins and ezetimibe are currently the most common drugs used for reaching target LDL-c levels in patients with hypercholesterolemia, a strategy that improves CVD outcomes [3–5]. However, current lipid-lowering therapies have several limitations, including the fact that: approximately 50% of familial hypercholesterolemia patients still cannot reach desired LDL-c levels [6]; intolerance to statin is becoming increasingly common [7]; and increased incidence of new-onset diabetes reduces drug acceptance and compliance in some patient groups [8,9]. These unmet needs warrant the continuing search for new, potent, and safe cholesterol-lowering therapies.

Proprotein convertase subtilisin/kexin 9 (PCSK9) – a circulating serine protease – binds LDLR and leads to its intracellular degradation [10]. Mutations in the human PCSK9 gene can lead to hypercholesterolemia [11] or low cholesterol syndromes [12], depending on whether the mutation causes a gain or a loss of function, respectively. The impact of PCSK9 on LDLR degradation is being exploited with the development of PCSK9 inhibition therapies aimed at lowering serum LDL-c levels [13,14]. Clinical trials have shown that PCSK9 inhibition using either monoclonal antibodies (mAbs) to block serum PCSK9 or RNA interference (RNAi) to reduce PCSK9 production produces a large drop (>50%) in LDL-c levels [15,16▪,17]. The mAbs against PCSK9 also significantly reduce lipoprotein (a) [Lp(a)] by up to 30% in a dose-dependent manner [18▪,19▪]. The effect of PCSK9 inhibition on serum triglyceride levels is less clear, as most studies show a moderate reduction that does not always reach statistical significance; the effect on high-density lipoprotein cholesterol (HDL-c) is positive but modest [20,21]. All three mAbs [Evolocumab by Amgen (formally Applied Molecular Genetics, Thousand Oaks, CA, USA), Alirocumab by Sanofi (Paris, France)/Regeneron (Tarrytown, NY, USA), and Bococizumab by Pfizer (Groton, CT, USA)] are currently at phase III clinical trials, whereas the PCSK9 RNAi (ALN-PCSSC by Alnylam Pharmaceuticals) is still in phase I trials. In this review, we will summarize the most recent findings on PCSK9 inhibition, regulation, and function, focusing on the PCSK9–LDLR interaction, and on the effect of PCSK9 on atherogenic lipoproteins apart from LDL.

CROSS-TALK BETWEEN LOW-DENSITY LIPOPROTEIN RECEPTOR AND PCSK9: FROM PHYSIOLOGY TO INHIBITION THERAPY

The PCSK9 gene is 25-kb long and lies on the short arm of chromosome 1 in humans (chromosome 4 in mice), comprised 12 exons and 11 introns [22,23]. The proximal promoter of the PCSK9 gene contains a highly conserved hepatocyte nuclear-factor 1 (HNF1) binding site residing 28 base pairs upstream of a sterol regulatory element (SRE) motif that responds to changes in intracellular cholesterol levels [24]. In-vivo studies show

that the sterol-dependent regulation of PCSK9 is mediated predominantly by SRE-binding protein (SREBP) 2 [25]. Studies using primary mice hepatocytes or human hepatoma cell line (HepG2) showed that SREBP1c can also induce PCSK9 transcription [25,26]. Statins inhibit cholesterol synthesis, which in turn activates the SREBP pathway and up-regulates both LDLR and PCSK9 in hepatocytes [27]. Similarly, in patients taking statins, circulating PCSK9 levels are increased by up to 50% compared to controls [28–30]. Thus, in a state of cellular cholesterol deficiency, the cell increases production of LDLR to allow more internalization of cholesterol and at the same time also increases the synthesis of PCSK9, which degrades LDLR.

PCSK9 is synthesized as a precursor protein of 75 kDa that undergoes auto-catalytic cleavage to allow secretion of the mature product of 62 kDa [31,32]. It is important to mention that the catalytic activity of PCSK9 does not affect LDLR degradation [33]. The degradation of LDLR is mediated by the extracellular interaction between mature PCSK9 and the epidermal growth factor-like repeat A (EGF-A) of the LDLR, which leads to the internalization and degradation of both proteins [34]. In serum, mature PCSK9 can also undergo further cleavage by action of furin to generate a lower-molecular-weight form (55 kDa) that is less active than the mature form in causing LDLR degradation [35–37]. A detailed illustration of PCSK9 domains and their function is shown in Fig. 1. Of note, the mature form of PCSK9 was also shown to directly associate with the LDL compartment in the serum [38–41], although the clinical significance of this association remains to be determined.

Blocking antibodies to PCSK9 inhibit the extra-cellular interaction between PCSK9 and the LDLR [42,43]. The inhibition of PCSK9 leads to higher levels of hepatic LDLR, which causes more efficient clearance of LDL from the circulation and drastic lowering of LDL-c levels. As shown in the most recent phase II clinical trials [44–46] and reports from the ongoing phase III trials [47,48▪,49–51], PCSK9 inhibition via mAb injection once every 2 or 4 weeks results in a 50–65% drop in LDL-c, with small positive effects on HDL-c and triglyceride levels. Similar results were also shown in a phase I trial using small interfering RNA (siRNA) to inhibit PCSK9 production [16▪]. On the basis of the established correlation between LDL-c reduction and CVD events, recently reinforced by the outstanding results of the IMProved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE IT) trial [4], it is anticipated that PCSK9 inhibition therapy will significantly improve CVD outcomes, although trial results will not be available until 2018. Table 1 summarizes the ongoing phase III clinical trials and expected completion dates. What is striking is that PCSK9 inhibition therapy does not show any adverse side-effects – a property that will facilitate the approval process for this new class of medications. Moreover, a prospective analysis of a single-nucleotide polymorphism (SNP) in PCSK9 associated with low LDL-c levels demonstrated no association with cognitive performance, functional status, or nonvascular clinical events [52].

LDLR–ligand interactions can be classified in three major categories [1]: type I, or the canonical interaction with apolipoprotein (apo)B on LDL, when the ligand is degraded in the lysosome, whereas the LDLR recycles back to the cell surface to capture more LDL particles [53,54]; type II, or the interaction with apoE on remnant lipoproteins and possibly

HDL, when both receptor and ligand recycle to the surface [55]; and type III, or interaction with a noncanonical ligand such as PCSK9, when both receptor and ligand supposedly undergo degradation in the lysosome [10,56]. Type III receptor-mediated endocytosis suggests that not only does PCSK9 control surface LDLR levels but also that the LDLR acts as a key regulator of circulating PCSK9 levels [41,57]. Specifically, we were able to show that the deletion of one copy of the LDLR gene in mice results in a three-fold increase in circulating PCSK9 levels, whereas the complete loss of LDLR increases PCSK9 levels by 10-fold [41]. Similarly, transgenic expression of the inducible degrader of the LDLR, or IDOL [58], or of human PCSK9 [41] in mice reduced hepatic LDLR levels while increasing endogenous PCSK9 levels due to impaired clearance. In contrast, acute transgenic expression of the human LDLR in mouse liver causes an approximately 66% drop in serum PCSK9 levels [41]. This LDLR-mediated effect on PCSK9 levels also occurs in humans, where PCSK9 levels are lowest in normo-lipidemic patients, higher in hypercholesterolemic patients with heterozygous familial hypercholesterolemia, and highest in homozygous familial hypercholesterolemia (HoFH) patients [59].

There are over 1000 mutations identified in the LDLR gene [60], with a range of functional losses spanning the entire spectrum from slightly dysfunctional to completely nonfunctional (<2% of normal LDL uptake) [61]. Identification of the type of LDLR mutation (defective or negative) is of major interest in determining effectiveness of anti-PCSK9 therapies, as was shown in two recent clinical trials of patients with HoFH, when an average 30% drop in LDL-c levels was seen among patients with residual LDLR function, whereas there were no changes in LDL-c levels among the three homozygous familial hypercholesterolemia patients carrying two receptor-negative mutations [50,62]. These clinical studies suggest that an LDLR that is defective in LDL uptake can still bind (and be degraded by) PCSK9, but the complete absence of LDLR disallows the benefits of anti-PCSK9 therapy. By extrapolation, these results suggest the following scenarios in patients carrying hypothetical LDLR mutations with defective binding to PCSK9:

1. Hypercholesterolemia, caused by the presence of one mutant LDLR allele with impaired binding to both LDL and PCSK9: Here, the hypercholesterolemia caused by the reduced ability to bind and clear LDL from plasma is aggravated by the accumulation of PCSK9 in plasma and its increased degradation action on the normal LDLR allele product. It is likely that these patients will have a high ratio of PCSK9 to LDL-c, and may represent excellent candidates for PCSK9 inhibition therapy. Such a scenario was recently suggested to occur in patients carrying the rs688 polymorphism in the LDLR gene [63].
2. Hypocholesterolemia, caused by LDLR mutations with defective PCSK9 binding, but normal LDL binding: The effect of only one allele (heterozygous) with such a mutation is hard to predict, since the mutated receptor will be protected from degradation (and thus more efficiently clear LDL and lowering LDL-c levels) while causing a high circulating PCSK9 level that can degrade the normal LDLR allele product (and thus increasing LDL-c levels). A homozygous presentation, instead, is more likely to produce a low cholesterol syndrome, as both LDLR allele products will be virtually resistant to the action of PCSK9 and thus reproduce the phenotype

seen both in patients with absent PCSK9 in the circulation and in patients treated with anti-PCSK9 antibodies.

Recently, Somanathan *et al.* [64] have generated such an artificial LDLR mutation (L318D) and were able to show reduced serum cholesterol levels in LDLR/APOBEC double knockout mice, a model of human-like severe hypercholesterolemia.

ADDITIONAL EFFECTS OF PCSK9 INHIBITION ON APOLIPOPROTEINB-CONTAINING LIPOPROTEINS

Apart from the massive reduction in LDL-c achieved by PCSK9 mAbs, phase II clinical trials and reports of the ongoing phase III trials have shown variations in the levels of other classes of lipoproteins, such as Lp(a) and triglyceride-containing particles. Lp(a) is an established risk factor for cardiovascular disease [65,66], which consists of an LDL particle in which case the apoB moiety is covalently linked to apo(a) by a disulfide bond [67]. Apo(a) shares structural similarities with plasminogen and exerts prothrombotic and antifibrinolytic effects through competition for removal of the complex between plasminogen activator inhibitor and tissue-type plasminogen activator. Lp(a) is found and accumulates in the atherosclerotic plaque; influencing lesion size through mechanisms that involve accelerated lipid oxidation with induction of inflammatory changes and macrophage cell death, favoring both plaque progression and rupture [68]. Two recent pooled analyses of phase II trials with PCSK9 mAbs highlighted their effectiveness in reducing Lp(a) levels [18▪,19▪]. In the first analysis, administration of evolocumab for 12 weeks lowered Lp(a) levels in a dose-dependent manner [18▪]. The highest efficacy was obtained after injection of either 140 mg every 2 weeks, or 420 mg every 4 weeks, which reduced Lp(a) levels by 29.4 and 25.5%, respectively, compared to placebo [18▪]. Similarly, administration of 150 mg of alirocumab biweekly for 8 and 12 weeks reduced Lp(a) levels by approximately 30%, with the greatest reduction in individuals with higher starting Lp(a) concentration [19▪]. On the contrary, a recent phase I clinical trial of siRNA to inhibit PCSK9 production did not show any effect on Lp(a) levels [16▪]. Thus, although the mechanism by which anti-PCSK9 mAb reduces Lp(a) is unknown, it can be assumed that this effect is antibody-specific and thus linked to events occurring in the extracellular milieu. Alternatively, the reduction in Lp(a) levels may be mediated by reduced apoB synthesis, as was recently shown in clinical data using the apoB synthesis inhibitor mipomersen [69].

The association between plasma triglyceride levels and the risk of CVD has been extensively studied [70]. In this context, a panel of experts reviewed the most recent epidemiological studies related to fasting and nonfasting triglyceride levels and established their role as a risk factor for ischemic cardiovascular disease [71]. Interest in plasma triglycerides as a biomarker and target of therapy has been aroused after the identification of loss-of-function mutations in ApoCIII related to low plasma triglyceride levels and lower incidence of CHD [72,73]. The administration of evolocumab at 420 mg every 4 weeks in individuals with hypercholesterolemia, in addition to atorvastatin alone, or atorvastatin and ezetimibe, reduced triglyceride levels by only 11.5% after 52 weeks [48▪]. In the LDL-C Assessment with PCSK9 Monoclonal Antibody Inhibition Combined With Statin Therapy-2 (LAPLACE-2) trial, evolocumab administration, in combination with moderate to high-dose

statin, reduced triglyceride levels by 12–23% and 14–30% when administered every 2 and 4 weeks in hypercholesterolemic patients compared to placebo [47]. Triglyceride levels were also reduced by 20 and 12% after 12-week administration of 140 mg biweekly and 420 mg monthly in heterozygous familial hypercholesterolemia on stable lipid-lowering therapy, respectively [74]. However, other studies reported more modest reductions in triglyceride levels that did not reach statistical significance [20,21,51]. It is important to remember that measurements in all clinical trials are done under strict fasting conditions that highly affect the levels of both PCSK9 and triglycerides. Several in-vitro and in-vivo studies suggest a direct role for PCSK9 in the regulation of intestinal lipoprotein secretion and apoB48 production [75–78]. These studies should inform clinical investigations on the effect of PCSK9 mAbs specifically in the context of postprandial hypertriglyceridemia.

SUMMARY AND CONCLUSION

The discovery of PCSK9 as a circulating protein holding the power to regulate LDLR levels has changed our understanding of cholesterol metabolism, traditionally believed to be strictly under the control of cellular factors orchestrated by the SREBP transcriptional machinery [79,80]. Understanding the basic mechanism of PCSK9 action has led to a race for the therapeutic exploitation of this protein, whose inhibition produces safe and drastic lowering of LDL-c levels. A more refined knowledge of the complex interactions between PCSK9, LDL particle, and LDLR may help explain both the tremendous efficacy of anti-PCSK9 therapies and the unexpected effects on important predictors of CAD such as Lp(a) levels. Also, clarifying the inner secrets of PCSK9 biology will help identify additional patient groups likely to benefit from the inhibitory therapy, such as individuals with elevated PCSK9 levels and those prone to abnormal postprandial hypertriglyceridemia.

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KEY POINTS

- Inhibition of PCSK9 achieves low LDL-c goals in individuals with severe hypercholesterolemia.
- Reciprocity between PCSK9 and LDLR explains the extreme effect of inhibitory mAb on LDL-c levels.
- Anti-PCSK9 mAb therapy also reduces the levels of Lp(a), through mechanisms not yet identified.
- An effect on postprandial hypertriglyceridemia is suggested by novel data on PCSK9 regulation of intestinal lipoprotein assembly and secretion.

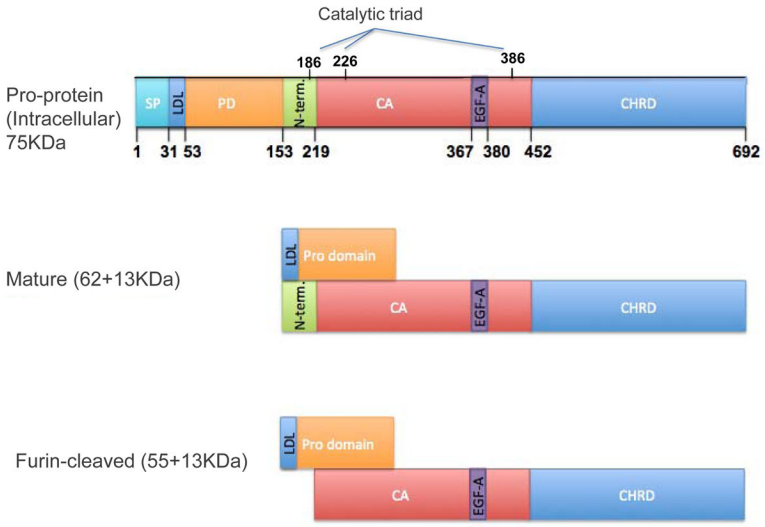

FIGURE 1.

Schematic representation of PCSK9 domains and molecular forms. (a) Full-length PCSK9 (692 amino acids and 75 kDa) has a signal peptide (SP), a pro-domain (PD) which contains the binding site of PCSK9 to LDL, a catalytic activity domain (CA) in the N-terminal section (N-term), an active site (catalytic triad Asp186, His 226 and Ser386) which is responsible for intracellular autocatalysis, and an EGF-A binding site (amino acids 367 to 380), responsible for the effects on LDLR. (b) Mature PCSK9 – upon autocatalytic cleavage, PCSK9 is secreted as a catalytically inactive protein and the cleaved PD remains noncovalently bound to the PCSK9 to carry it in the secretory pathway. This 62 (+13) kDa protein is the main active molecular form (mature), responsible for LDLR-mediated degradation. The mature PCSK9 can bind LDL particles via amino acid 31–53 in the PD. (c) Furin-cleaved PCSK9 – in serum, PCSK9 can be cleaved by furin to generate a lower molecular weight form of PCSK9 (55 + 13 kDa) that is less active in terms of LDLR degradation ability. CHRD, C-terminal histidine rich domain; EGF-A, epidermal growth factor-like repeat A; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; N-term, fragment cleaved by furin; PCSK9, proprotein convertase subtilisin/kexin 9.

Table 1

Ongoing phase 3 clinical trials using monoclonal antibodies against proprotein convertase subtilisin/kexin 9

Monoclonal antibody used	**Evolocumab by Amgen**	**Alirocumab by Sanofi/Regeneron**	**Bococizumab by Pfizer**
Phase 3 trial	FOURIER	ODYSSEY outcome	SPIRE I/II
Enrollment (*n*)	22 500	18 000	17 000/9000
Inclusion criteria	Age 40–85, History of CVD with high risk of recurrent event, LDL-c >70 mg/dl, triglycerides <400 mg/dl	Age >40, hospitalized of Acute Coronary Syndrome in the last 52 weeks before enrollment, LDL-c >70 mg/dl	I – Age >18, background of lipid-lowering treatment, at high risk of a cardiovascular event, LDL 70–100 mg/dl II – Age >18, background of lipid lowering treatment, at high risk of a cardiovascular event, LDL >100 mg/dl
Subcutaneous injection intervals	Once every 2 weeks		Once every 2 weeks
End date	02.2018	03.2018	06.2018/03.2018

FOURIER, Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk; LDL, low-density lipoprotein; ODYSSEY; SPIRE, The Evaluation Of PF-04950615 (RN316), In Reducing The Occurrence Of Major Cardiovascular Events In High Risk Subjects.