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REVIEW

Potential of PCSK9 as a new target for the management of LDL cholesterol

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Abstract: A large proportion of patients at high risk for cardiovascular disease continue to suffer from cardiovascular events despite current therapies. The need for additional therapies to lower the residual risk has led to research on new pharmacological approaches. The discovery of proteins regulating the activity of the low-density lipoprotein receptor has been a major breakthrough in the development of new cholesterol-lowering drugs. This review describes inhibition of proprotein convertase subtilisin/kexin type 9 (PCSK9) as a promising treatment for familial hypercholesterolemia, especially the relatively good short-term safety of PCSK9 inhibitors. In particular, we focus on its additive effect with statins and its advantage as a monotherapy in statin-intolerant patients. The additional low-density lipoprotein cholesterol lowering obtained with PCSK9 inhibition will be able to reduce the additional risk, but its effect on cardiovascular events has to be evaluated in future studies.

Keywords: proprotein convertase subtilisin/kexin type 9, PCSK9, additional or replacement therapy to statins, statin intolerance, residual cardiovascular risk

Introduction

In 2003, Abifadel et al discovered in two French families a new locus associated with an autosomal dominant form of familial hypercholesterolemia (FH3) and described two gain-of-function mutations in the 12-exon gene *PCSK9* (1p34-32), which encodes for proprotein convertase subtilisin/kexin type-9 (PCSK9).[1]

Initially identified as neuronal apoptosis-regulated convertase-1, expressed in cerebellar neurons that undergo apoptosis,[2] PCSK9 is a serine protease that plays a crucial role in cholesterol homeostasis, promoting degradation of the low-density lipoprotein (LDL) receptor. It is synthesized by the liver as a 692-amino acid soluble zymogen, made up of a signal peptide, a prodomain, and a catalytic triad, followed by a C-terminal domain.[3] By autocatalytic intramolecular processing in the endoplasmic reticulum, the prodomain undergoes cleavage at position 152 (Asp_{151}/Gln_{152}), allowing maturation and secretion of PCSK9.[2]

Role of PCSK9

PCSK9 binds to the extracellular domain of the LDL receptor, ie, in the first epidermal growth factor-like repeat homology domain,[4] and is internalized along with the receptor in the cells.[5] The binding site for the LDL receptor is on the surface of the catalytic domain containing Asp374.[6] The C-terminal domain is not required for LDL receptor binding, but is necessary for internalization of the complex.[7] Interestingly, PCSK9 may also bind to LDL receptor molecules intracellularly and regulate LDL receptor expression

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on the cell surface.[8] Annexin A2, a protein involved in diverse cellular processes, binds to the C-terminal in the same way, but inhibits degradation of the receptor, demonstrating that this protein exerts an inhibitory function on PCSK9.[9]

Particularly important are the amino acid residues of the first epidermal growth factor-like repeat homology domain that coordinates the binding of calcium ions (Asp_{295}, Glu_{296}, Asp_{310}, Tyr_{315}, and His_{306}), which are responsible for the specificity of the interaction with PCSK9.[10] Some studies have demonstrated that PCSK9 locks the LDL receptor in an extended form, disrupting normal recycling of the LDL receptor by means of the cell surface.[11] The PCSK9/LDL receptor complex moves to the endosomes by clathrin-mediated endocytosis, where the lower pH increases the strength of this binding against LDL/LDL receptor binding. This results in release of LDL cholesterol and direction of the PCSK9/LDL receptor complex to the endosome[12] (Figure 1). The ability of PCSK9 to promote cellular degradation of the receptor is not dependent on its catalytic activity, as demonstrated by discovery of a mutation that prevents autocatalytic processing and is associated with low LDL cholesterol levels.[13] Subsequent studies investigated the molecular mechanism by which PCSK9 influences metabolism of LDL cholesterol.[14] Beyond this activity, PCSK9 seems to be involved in degradation of the very low-density lipoprotein (VLDL) receptor and apolipoprotein E receptor 2,[15] suggesting a role in modulation of cellular functions. Recent intriguing studies have identified a direct association with plasma triglycerides, suggesting a potential effect of PCSK9 on triglyceride-rich lipoproteins.[16,17] Confirming this observation, subjects carrying a PCSK9 gain-of-function mutation showed a three-fold elevation in apolipoprotein (apo)B100 production rates compared with normal subjects.[18] Chan et al found that PCSK9 and apoC-III are inversely associated with the catabolic rate of triglyceride-rich lipoprotein-apoB48, suggesting a role of PCSK9 in the post-prandial coordination of the catabolism of this lipoprotein.[19] Studies in hepatocytes found that PCSK9 binds to and reduces the intracellular degradation of apoB100 independently of LDL receptor levels and positively modulating the output of apoB.[20] Contrasting data show that PCSK9 levels do not correlate with VLDL secretion or clearance in obese patients.[21]

Even if the liver is the main organ that regulates plasma PCSK9 levels, other tissues contribute to the production of this convertase[2,22–24] (Figure 2). Enterocytes express the LDL receptor on their basolateral surface and PCSK9 can possibly activate their degradation, modulating metabolism of cholesterol and chylomicrons.[25] Some recent studies suggest a possible role of PCSK9 expressed in the kidney in modulating absorption of sodium by degradation of the

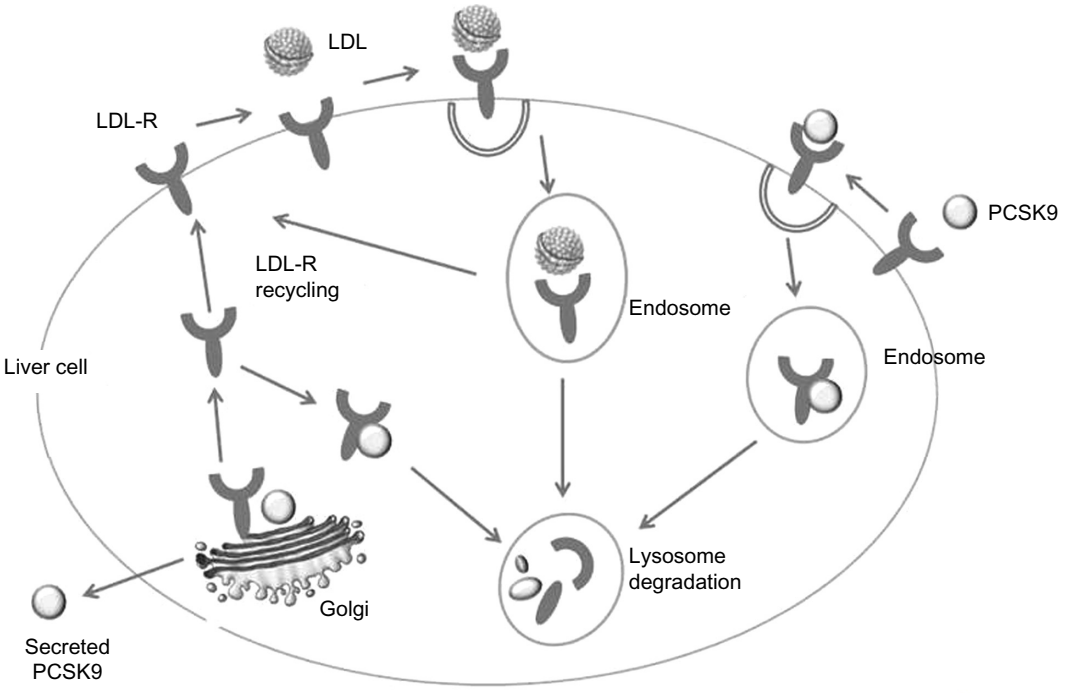

Figure 1 PCSK9 and LDL-R degradation.
Notes: PCSK9 regulates LDL-R expression. Circulating PCSK9 protein binds to the LDL-R. Once internalized into the liver cell, the PCSK9 protein directs the LDL-R to the lysosome for degradation. Intrinsically, PCSK9, secreted from the Golgi apparatus, binds the LDL-R before it reaches the cell surface and lead it to lysosomal degradation.
Abbreviations: LDL-R, low-density lipoprotein cholesterol receptor; PCSK9, proprotein convertase subtilisin/kexin type 9.

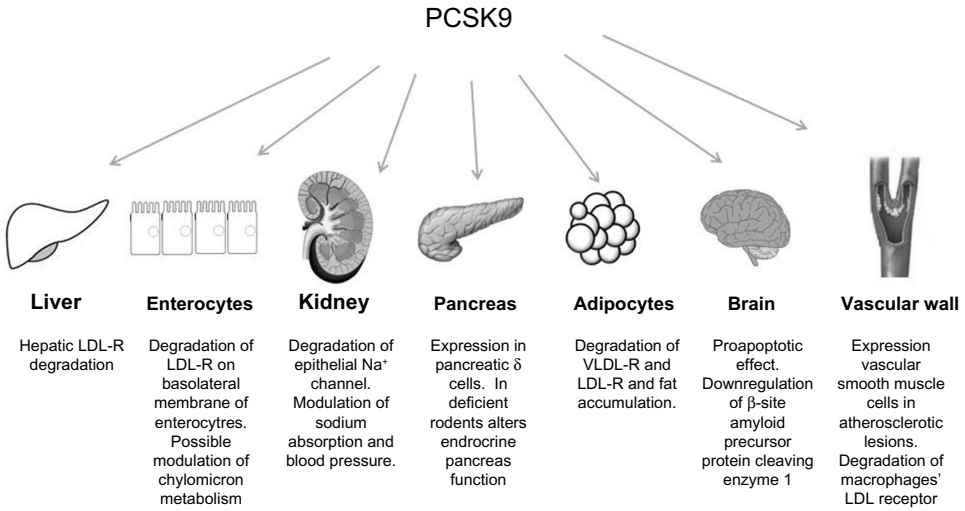

Figure 2 Role of PCSK9.
Abbreviations: LDL-R, low-density lipoprotein cholesterol receptor; VLDL-R, very low-density lipoprotein cholesterol receptor; PCSK9, proprotein convertase subtilisin/kexin type 9.

epithelial sodium channel and consequently playing a part in regulating blood pressure.[26] PCSK9 was found in human δ pancreatic cells, but while in rodents its deficiency alters endocrine pancreatic function, such as insulin secretion,[24] no effects have been demonstrated as yet in humans.[27] Although adipocytes do not express PCSK9, they are rich in LDL and VLDL receptors, which play a fundamental role in hydrolysis of triglyceride-rich lipoproteins and are important for fat storage in these cells.[28] Lakoski et al[17] reported a positive correlation between plasma PCKS9 and body mass index.

In a recent animal study, the authors observed a proapoptotic effect in cerebellar neurons of PCSK9 (mediated by apoE receptor 2 degradation) that was independent of N-methyl-D-aspartate receptor function.[29] Some studies suggested involvement of this convertase in the development of Alzheimer's disease,[30,31] in that it is able to generate amyloid β-peptide.[32] Controversial findings came from other research.[33] Interesting from a cardiovascular (CV) point of view is the discovery by an Italian group of the presence of PCSK9 in human carotid atherosclerosis lesions, in particular in vascular smooth muscle cells.[22] The proliferation of vascular smooth muscle cells express PCSK9, reducing macrophages LDL receptor levels. Other authors have observed an inverse correlation between carotid artery intima media thickness and expression of PCSK9, independently of LDL cholesterol.[34,35] PCSK9 does not modulate the LDL receptor in all body tissues, but the existence of a tissue-specific cofactor has been suggested, which could explain the preferences for the liver for carrying out its activity.[36]

Regulation of PCSK9

Circulating PCSK9 follows a circadian rhythm in parallel with that of the biosynthesis of cholesterol, and seems to be regulated by hormones and nutritional status.[37,38] In physiological conditions, plasma PCSK9 levels increase post-prandially, with a diurnal rhythm that mirrors hepatic cholesterol synthesis.[39] The plasma concentration declines during the fasting period, reaching a minimum at 36 hours (28% lower than in the fed state).[38] Some authors found a correlation between PCSK9 levels and fasting serum glucose and insulin levels, suggesting a role of PCSK9 in the development of diabetes.[17] This finding was not confirmed by other studies demonstrating that the degree of glucose intolerance is not associated with plasma PCSK9 levels; rather, the prevalence of diabetes modifies the relationship between plasma PCSK9, non-HDL (high-density lipoprotein) cholesterol, and apoB levels.[40] Postmenopausal women have significantly higher plasma levels of PCSK9, which are unaffected by estrogen replacement therapy.[17]

Expression of PCSK9 is mainly controlled by cholesterol levels and transcription factor sterol-responsive element-binding protein (SREBP)-2,[41,42] which coordinates numerous genes involved in cholesterol homeostasis, such as 3-hydroxy-3-methylglutaryl-coenzyme A reductase and LDL receptor expression.[41,43] Mice that lack SREBP-2 have reduced PCSK9 mRNA levels.[43]

Circulating levels of PCSK9 can be explained by plasma LDL cholesterol concentrations,[17,44] but LDL cholesterol levels do not fully reflect the activity of the protein for the following reasons: PCSK9 carries out its function in the intracellular compartment, and the antibodies used to dose

this protein do not show selectivity for the active convertase, but bind furin-cleaved PCSK9.

Plasma PCSK9 is associated with LDL-apoB catabolism,[45] and increased LDL catabolism effectively lowers plasma LDL cholesterol.[46] PCSK9 and the LDL receptor are both regulated by SREBP, expression of which is upregulated in conditions of cellular cholesterol deficiency,[37] and they are removed together in the lysosome. Using a validated mouse model, it has been demonstrated that the LDL receptor and PCSK9 are reciprocally regulated in a homeostatic pathway, where loss of the LDL receptor (eg, in patients with FH) leads to accumulation of PCSK9,[47] and in contrast, low levels of functional PCSK9 are followed by an increase in surface LDL receptor expression, which removes further plasma PCSK9.[48]

Statins increase PCSK9 levels and reduce those of LDL cholesterol.[44,49] Other lipid-lowering treatments also affect plasma PCSK9 concentrations. Davignon et al showed that they are increased by increasing the statin dose and further increased when ezetimibe is added.[50] A possible explanation lies in the reduction of intestinal cholesterol absorption and thus reduction of hepatic cholesterol that feedback regulates SREBP-2.[51] Later studies reported different results showing that ezetimibe alone or combined with simvastatin is not associated with an increase in PCSK9.[52] Fibrates significantly modified PCSK9 concentrations, but indirectly by modulating cholesterol levels.[53] Bile acid binding resins were found to increase gene expression of PCSK9 in human liver biopsies[54] and lipoprotein apheresis reduces PCSK9 levels by 50%, trapping LDL-bound and apoB-free PCSK9 in the apheretic column.[48]

Inactivation of PCSK9 seems to be mediated in hepatocytes by two proprotein convertases, ie, furin and PC5/6A,[55] which cleave mature PCSK9, releasing an inactive truncated protein. Lack of these two convertases leads to elevated plasma LDL cholesterol levels.

Genetic variants related to PCSK9

Since the discovery in 2003 of two rare missense mutations (Ser127Arg and Phe216Leu), some other genetic variants of PCSK9, having a negative or positive effect on plasma concentrations of LDL cholesterol and CV disease have been described in the scientific literature. A gain-of-function mutation, Glu670Gly, has been associated with increased LDL cholesterol and coronary atherosclerosis, but not in those of Caucasian or African descent.[56–59] In the Italian population, the same variant was associated with increased intima media thickness.[60] The Asp374Tyr variant has been found in Utah, Norway, and England.[61,62] Asn425Ser and Arg496Trp have been observed in patients with FH and concomitant mutations in the LDL receptor.[63] In the Dallas Heart Study, two nonsense mutations (Tyr142X and Cys679X) were identified, which led to a loss-of-function of PCSK9 and were associated with VLDL cholesterol.[56] The Ala443Thr variant in African Americans and the Arg46Leu variant in European Americans were associated with hypocholesterolemia.[59] In the ARIC study, three single nucleotide polymorphisms that cause loss-of-function mutation (R46L, Y142X, and C679X) were associated with decreased LDL cholesterol and a lower risk of coronary events[64] (Figure 3).

CV disease and lipids

CV disease is a leading cause of morbidity and mortality worldwide.[65] In most cases, CV disease is caused by an atherosclerotic process strongly related to cholesterol levels, as many epidemiological studies have demonstrated.[66] Increased LDL cholesterol is closely associated with the pathophysiology of CV disease, and accordingly, is a major risk factor for coronary artery disease (CAD).[66] Therefore, investigation of the mechanisms and clinical management of LDL cholesterol in patients at high CV risk is of fundamental importance. Several scientific reports have focused attention on raised LDL cholesterol, considering this as the main lipid target for prevention of CV disease. These reports have included identification of LDL receptor mutations as the cause of FH[67] and the LDL oxidation hypothesis, which has focused attention on LDL cholesterol.[68]

In contrast, HDL cholesterol is widely known as the "good cholesterol", because a number of studies have found a negative correlation between HDL cholesterol levels and

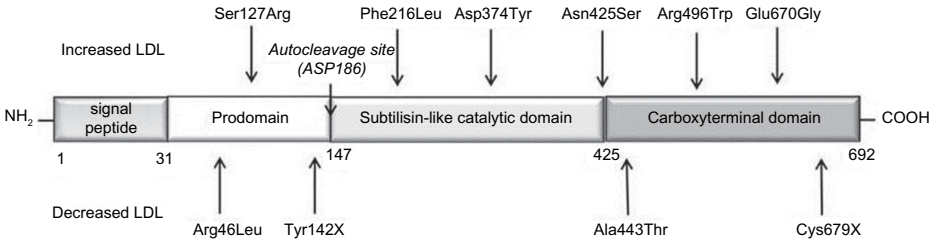

Figure 3 PCSK9 protein and its variants.
Abbreviations: LDL, low-density lipoprotein cholesterol; PCSK9, proprotein convertase subtilisin/kexin type 9.

the risk of CV events.[69,70] HDL cholesterol has antiatherogenic properties, for instance, cholesterol is transported from peripheral tissues such as the cells in the arterial walls to the liver by HDL particles, where it is used for the composition of lipoproteins and synthesis of bile acids, steroid hormones, fat, and soluble vitamins,[70] whereas low HDL cholesterol has often been observed as a component of metabolic syndrome, together with other determinants such as hyperglycemia, hypertriglyceridemia, high blood pressure, and high waist circumference. Each of these CV risk factors can promote the process of atherosclerosis.[68]

Indeed, Glomset et al[71] have introduced the concept of reverse cholesterol transport and speculated that HDL promotes this process, becoming a protective factor against CAD. An additional CV risk factor is high triglyceride levels. Several meta-analyses have shown that raised fasting and non-fasting triglyceride concentrations are associated with increased risk of CAD, even after adjustment for HDL cholesterol concentrations.[72]

Varbo et al[73] reported that high triglyceride concentrations are strongly associated with low HDL cholesterol, and this has resulted in more detailed research on HDL in the past 15 years, with less focus on triglycerides. In the Emerging Risk Factors Collaboration,[74,75] that included 302,430 individuals from 68 long-term, prospective studies, the authors reported that high triglyceride levels were associated with an increased risk of CAD and stroke (adjusted for age and sex).[76] A final emergent independent CV risk factor is lipoprotein (a), ie, Lp(a), an interesting particle that combines the atherogenic properties of LDL cholesterol with the thrombogenic properties of inactivation of plasminogen. The phenotypic diversity of familial hyperlipoproteinemia (a) and FH as a population at high risk, and their frequent presence together with other CV risk factors, indicates that critical revision of the current diagnostic and therapeutic recommendations established for isolated familial hyperlipoprotein (a) and FH is needed.

Recently, Di Angelantonio et al[76] discussed the importance of Lp(a) in stratification of people with intermediate and high CV risk, as advised by the European Atherosclerosis Society Consensus Panel, even if this recommendation was not supported by the American Heart Association Task Force.[77] Konishi et al[78] have published evidence that Lp(a) ≥30 mg/dL could be associated with a poor prognosis after percutaneous coronary intervention, even in patients who have achieved target lipid levels. Perhaps in this case we have to think of Lp(a) as being a residual risk factor in secondary prevention patients who achieve target lipid levels. Therefore, it could be concluded that elevated LDL cholesterol is the prime driver of atherogenesis, whereas other risk factors worsen atherosclerosis or precipitate its complications. If this is true, treatment of these accelerating risk factors should probably be accompanied by cholesterol-lowering therapy.

PCSK9 inhibition as a lipid-lowering treatment

Description of two homozygous PCSK9 loss-of-function mutations leading to VLDL cholesterol but having no association with evident phenotypic abnormalities points to the beneficial and safe hypolipidemic approach of PCSK9 inhibition.[79,80] The ARIC study found a consistent reduction (−88%) of CAD risk in those with the PCSK9 loss-of-function mutation versus those without this mutation.[81] Following on from this finding, some researchers have started to study inhibition of PCSK9, primarily using monoclonal antibodies.

Some are the advantages of the use of monoclonal antibodies for inhibition of a protein, eg, the potency and specificity. In addition, the long-term inhibitory effect allows a broader dose frequency. Several monoclonal antibodies against PCSK9 have been developed, and some of them have already demonstrated encouraging clinical results, eg, alirocumab (Sanofi-Regeneron), evolocumab (Amgen), and bococizumab (Pfizer). Other strategies for modulating PCSK9 include small-molecule inhibitors and hepatic gene silencing.

The first approach consists of an adnectin-based engineered protein that prevents PCSK9-LDL receptor binding.[82] The reduced mass of this molecule, compared with monoclonal antibodies, makes it easier and cheaper to produce. However, it is characterized by rapid renal clearance and a short half-life. Serometrix LLC and Shifa Biomedical Corporation are now in the preclinical stages of developing two small chemical inhibitors for oral administration.[83] The first consists of an allosteric ligand of PCSK9 that disrupts normal protein folding to inhibit LDL receptor binding; the second blocks the autocatalytic cleavage of PCSK9, preventing its secretion from the cell.

The second approach consists of engineered antisense oligonucleotides or small interfering RNA that direct sequence-specific degradation of mRNA, suppressing synthesis of the corresponding proteins. ALN-PCS02, developed by Alnylam Pharmaceuticals have concluded Phase I trials that have had promising results.[84] Very recently, Pfizer has announced the development of a vaccine that stimulates the immune system to generate highly specific, long-lasting PCSK9 antibodies to overcome the relatively short life of monoclonal antibodies.[85]

Affiris AG anticipates promising preclinical results for its three vaccines, and clinical trials are expected to begin in 2015.[86] Table 1 gives a summary of PCSK9 inhibitors in development.

Antibodies against PCSK9 as additional therapy to statins

Use of PCKS9 as add-on therapy to statins is supported by some observations. One of these arises from the limit of statins in reducing LDL cholesterol as a paradoxical effect. The cholesterol reduction exerts by statin activates SREBP-2, and consequently the transcription of 3-hydroxy-3-methylglutaryl-coenzyme A reductase and PCSK9, as a feedback response,[17] with consequent downregulation of the LDL receptor. Careskey et al[87] observed that atorvastatin 40 mg/day significantly increased circulating PCSK9 levels by 34% versus baseline.[87] Other authors made the same observation for fibrates, although they affect these levels indirectly.[53] In a study conducted by Berge et al[88] reported the hyperresponsivity to statins of heterozygous PCSK9 loss of function carrier of heterozygous PCSK9 loss-of-function mutation. To date, many Phase III clinical trials, either concluded or ongoing, have demonstrated the efficacy and tolerability of PCSK9 monoclonal antibodies (Table 2).

The MENDEL-2 study that included 614 naive patients with moderate dyslipidemia (100 mg/dL ≤LDL cholesterol ≥190 mg/dL) showed that after 12 weeks of evolocumab, LDL cholesterol decreased by 55%–57% and by 38%–40% compared with ezetimibe.[89] A concomitant study, LAPLACE-2, evaluated the efficacy of evolocumab as add-on treatment to statins versus placebo and versus ezetimibe. In 2,067 dyslipidemic patients have been randomized to assume a dosage to moderate-intensity (atorvastatin 10 mg, simvastatin 40 mg, or rosuvastatin 5 mg) or high-intensity (atorvastatin 80 mg, rosuvastatin 40 mg). A significant lowering of LDL cholesterol occurred in all groups treated with statins and evolocumab. In patients treated with atorvastatin (10 mg or 80 mg), addition of ezetimibe resulted in a reduction in LDL cholesterol values of 17% until to 24% from baseline, while addition of evolocumab administered every 2 weeks reduced LDL cholesterol values range (61%–62%). Addition of evolocumab every 4 weeks reduced LDL cholesterol values (62%–65%) from baseline.[90] This was the first study to demonstrate that addition of evolocumab causes similar reductions in LDL cholesterol and achieved LDL cholesterol levels, regardless of type of statin being taken at baseline, its dosage, or its intensity.[90] The DESCARTES study enrolled 901 patients at high CV risk. Compared with placebo, the mean LDL cholesterol reductions from baseline on evolocumab at week 52 were 56% for the group with only dietary modifications, 62% for the group with diet changes and atorvastatin 10 mg, 57% for the group with diet and atorvastatin 80 mg, and 49% for diet and atorvastatin 80 mg plus ezetimibe 10 mg. The observed effect on PCSK9 levels and consequent reduction of LDL cholesterol levels were similar to those reported in patients receiving 10 mg or 80 mg of atorvastatin plus another PCSK9 monoclonal antibody, alirocumab (at a dose of 150 mg).[76] The authors suggested that patients who have been already treated with high-dose statins alone or in combinations with other lipid-lowering therapies may have less capacity to further upregulate the LDL receptor with PCSK9 inhibition or may require higher doses of antibody. Although statins upregulate PCSK9, this does not explain why the reduction in LDL cholesterol levels associated with a low dose of a

Table 1 PCSK9 inhibitors in development

Developer	Molecule	Description	Route of administration	Clinical stage
Regeneron/Sanofi	Alirocumab	Fully human IgG1 mAb	Subcutaneous	Phase III
Amgen	Evolocumab	Fully human IgG2 mAb	Subcutaneous	Phase III
Pfizer	Bococizumab	Humanized IgG2a mAb	Subcutaneous	Phase III
Roche	RG-7652	Humanized IgG1 mAb	Subcutaneous	Phase II
Eli Lilly Pharmaceuticals	LY-3015014	Humanized IgG mAb	Subcutaneous	Phase II
Alder Pharmaceuticals	ALD-306	Humanized IgG mAb	Subcutaneous	Preclinical
Pfizer	–	Vaccine	Subcutaneous	Preclinical
Affiris AG	ATH05, ATH06, ATH07	Vaccine	Subcutaneous	Preclinical
Alnylam	ALN-PCS02	Small interfering RNA	Subcutaneous	Phase I
Bristol-Myers Squibb	BMS-962476	Small protein (adnectin)	Subcutaneous	Phase I
Kowa Research Institute	K-312	Synthetic compound	Oral	Phase I
Serometrix LLC	SX-PCSK9	Small molecule	Oral	Preclinical
Shifa Biomedical Corporation	TBD	Small molecule	Oral	Preclinical
Santaris Pharma A/S	SPC-5001	Antisense oligonucleotide	Subcutaneous	Phase I
Idera Pharmaceuticals	–	Antisense oligonucleotide	Subcutaneous	Preclinical

Abbreviations: IgG, immunoglobulin G; mAb, monoclonal antibody; PCSK9, proprotein convertase subtilisin/kexin type 9.

Table 2 Phase III trials on the PCSK9 inhibitors, alirocumab, evolocumab, and bococizumab

Patients (n)	Population	Reference	Dose	Main results
Phase III trials on alirocumab				
103	Hypercholesterolemia (with or without statin therapy)	ODYSSEY MONO[95]	75 mg SC every 2 weeks	LDL-C, −47.2%; TC, −29.6%; HDL-C, +6.0%; Lp(a), −16.7%
251	Statin intolerance; primary hypercholesterolemia (heterozygous FH or non-FH); and moderate, high, or very high CVD risk (no statin therapy)	ODYSSEY ALTERNATIVE[113]	75–150 mg SC every 2 weeks	LDL-C, −52.2%; lower rate of muscle symptoms versus ezetimibe
183	Hypercholesterolemia (heterozygous FH or non-FH) not adequately controlled (atorvastatin with or without other lipid-modifying therapy), and high CVD risk	ODYSSEY OPTIONS I[113]	50–150 SC every 2 weeks or 200–400 mg every 4 weeks	LDL-C, −44% to −54%
415	Hypercholesterolemia not adequately controlled (rosuvastatin with or without other lipid-modifying therapy), and high CVD risk	ODYSSEY OPTIONS II[113]	75–150 mg SC every 2 weeks	LDL-C, −36% to −51%
316	Hypercholesterolemia not adequately controlled (with maximum dose of a statin with or without other lipid-modifying therapy), and high CVD risk	ODYSSEY COMBO I[113]	75–150 mg SC every 2 weeks	LDL-C, −48%
107	Heterozygous FH not adequately controlled with current lipid-modifying therapy (no specification regarding statin therapy)	ODYSSEY HIGH FH[113]	150 mg SC every 2 weeks	LDL-C, −46%
2,341	Hypercholesterolemia not adequately controlled with current lipid-modifying therapy, and high CVD risk (no specification regarding statin therapy)	ODYSSEY LONG TERM[100]	150 mg SC every 2 weeks	LDL-C, −62% After 78 weeks, event rate 1.7%
–	Hypercholesterolemia not adequately controlled (maximum dose of a statin with or without other lipid-modifying therapy), and high CVD risk	ODYSSEY COMBO II	Ongoing	
–	Hypercholesterolemia	ODYSSEY CHOICE I	Ongoing	
–	Heterozygous FH not adequately controlled with current lipid-modifying therapy (no specification regarding statin therapy)	ODYSSEY FH I	Ongoing	
–	Heterozygous FH not adequately controlled (with maximally tolerated statin with or without other lipid-modifying therapy)	ODYSSEY FH II	Ongoing	
–	Recent (in the past 4–16 weeks) acute coronary syndrome event requiring hospitalization	ODYSSEY OUTCOMES		
614	Framingham risk score ≤10% and LDL-C level ≥100 mg/dL (no specification regarding statin therapy)	MENDEL-2[89]	140 mg SC every 2 weeks or 420 mg every 4 weeks	LDL-C, −56.9% to 58.8%, HDL-C, +3.8% to 3.9%; Lp(a), −18.4% to 19.2%
307	Statin intolerance; hypercholesterolemia (no statin or low-dose statin)	GAUSS-2[109]	140 mg SC every 2 weeks or 420 mg every 4 weeks	LDL-C, −53% to 56%, lower rate of muscle symptoms versus ezetimibe
901	LDL-C level ≥85 mg/dL and either at ATP III target with background lipid therapy or taking maximum background lipid therapy (no specification regarding statin therapy)	DESCARTES[91]	420 mg SC every 4 weeks	LDL-C, −48.5% to 61.6%
2,067	Primary hypercholesterolemia or mixed dyslipidemia (taking statin therapy with or without ezetimibe)	LAPLACE-2[90]	140 mg SC every 2 weeks or 420 mg every 4 weeks	LDL-C, −63% to 75%
331	Heterozygous FH and LDL-C level ≥100 mg/dL with statin therapy (no specification regarding statin therapy)	RUTHERFORD-2[92]	140 mg SC every 2 weeks or 420 mg every 4 weeks	LDL-C, −55.7% to 59.2%, HDL-C, +5.4% to 8.1%, Lp(a), 21.6% to 22.9%
50	Homozygous FH and LDL-C level >130 mg/dL with stable lipid therapy (no specification regarding statin therapy)	TESLA[93]	420 mg SC every 4 weeks	LDL-C, −23.1%, HDL-C, 4.0%, Lp(a), −12.7%
4,465	Hypercholesterolemia or mixed dyslipidemia; completion of previous evolocumab study (no specification regarding statin therapy)	OSLER-2[99]	140 mg SC every 2 weeks or 420 mg every 4 weeks	LDL-C, −61.0%, 1-year event rate 0.95%

(*Continued*)

Table 2 (*Continued*)

Patients (n)	Population	Reference	Dose	Main results
–	Coronary heart disease; clinical indication for coronary catheterization; and LDL-C level ≥80 mg/dL or, with additional risk factors, ≥60 mg/dL and <80 mg/dL (no specification regarding statin therapy)	GLAGOV	Ongoing	
–	Clinical CVD, high risk of recurrent CVD event, and LDL-C level ≥70 mg/dL or non-HDL-C ≥100 mg/dL (no specification regarding statin therapy)	FOURIER	Ongoing	
–	Heterozygous FH; high or very high CVD risk; LDL-C level >70 mg/dL and TG level ≤400 mg/dL (with statin therapy)	SPIRE-HF		Ongoing
–	High or very high CVD risk; LDL-C level >70 mg/dL and TG level ≤400 mg/dL (with statin therapy)	SPIRE-HR		Ongoing
–	High or very high CVD risk; LDL-C level >70 mg/dL and TG level ≤400 mg/dL (with statin therapy)	SPIRE-LDL		Ongoing
–	High CVD risk; LDL-C level ≥70 mg/dL and <100 mg/dL, or non-HDL-C level ≥100 mg/dL and <130 mg/dL, with lipid-lowering therapy (no specification regarding statin therapy)	SPIRE-1		Ongoing
–	High CVD risk; LDL-C level ≥100 mg/dL or non-HDL-C level ≥130 mg/dL, with lipid-lowering therapy (no specification regarding statin therapy)	SPIRE-2		Ongoing

Abbreviations: ATP III, Third Report of the National Cholesterol Education Program-Adult Treatment Panel; CVD, cardiovascular disease; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Lp(a), lipoprotein(a); SC, subcutaneous; FH, familial hypercholesterolemia; TC, total cholesterol; PCSK9, proprotein convertase subtilisin/kexin type 9; TG, triglycerides.

statin is relatively large in comparison with the additional 6% reduction observed when the dose of statin is doubled.[91] In all studies, the most common adverse events were flu-like symptoms, nasopharyngitis, upper respiratory tract infection, and back pain. The doubt about the efficacy of PCSK9 inhibition in FH was dispelled in the RUTHERFORD-2 study of 331 subjects with heterozygous FH, in whom there was an LDL cholesterol reduction of approximately 60%.[92] Another Phase III trial conducted in 49 patients with homozygous FH (TESLA Part B) showed that evolocumab reduced LDL cholesterol by 30.9% when compared with placebo, with no occurrence of serious adverse events.[93] The efficacy of alirocumab was initially tested in addition to atorvastatin in patients with primary hypercholesterolemia. After 12 weeks of treatment, LDL cholesterol decreased (by 39.6%) on 50 mg of alirocumab every 2 weeks and by 72.4% on 150 mg every 2 weeks versus placebo. There was not much difference between the dose of 200 mg and 300 mg every 4 weeks (–43.2% and –47.7%, respectively).[94] The ODYSSEY MONO study showed a similar profile of evolocumab in naive patients with moderate CV risk. LDL cholesterol was reduced by 47% with alirocumab 75 mg every 2 weeks versus 16% with ezetimibe. Doubling the dose of the PCSK9 inhibitor, administered once a month, patients achieved a LDL cholesterol reduction of 54% [95] Gaudet et al[96] investigated the effect of alirocumab on Lp(a) and found a significant reduction of about 30% from baseline versus a reduction of 0.3% with placebo. Pooled analysis of more than 1,300 patients in four evolocumab trials confirmed the efficacy of PCSK9 inhibition in reduction of Lp(a); a significant dose-related effect was observed, ie, 29.5% and 24.5% with 140 mg and 420 mg, respectively. While the mean percentage reduction was significantly greater in patients with lower Lp(a) values at baseline, the absolute reduction was substantially greater in those with high baseline levels.[97] Phase III trials with bococizumab are in progress. The first study, on heterozygous FH with high CV risk, is expected to be completed in January 2016.[98] Endpoint trials, ie, ODYSSEY for alirocumab, FOURIER for evolocumab, and SPIRE for bococizumab, are currently in progress to evaluate reduction in CV risk. Preliminary data are available, and show a reduction of the rate of CV events at 1 year from 2.18% in the standard therapy group to 0.95% in the evolocumab group (hazard ratio for evolocumab group 0.47; 95% confidence interval 0.28–0.78, P=0.003).[99] Similar results came out of a post hoc analysis of alirocumab, where the incidence of major adverse CV events was lower when compared with placebo (1.7% versus 3.3%, respectively; hazard ratio for alirocumab 0.52; 95% confidence interval 0.31–0.90, P=0.02).[100]

Clinical trials on evolocumab and alirocumab have reported a small but significant number of adverse neurocognitive effects in patients taking these drugs. Therefore, the

US Food and Drug Administration has recently asked for an assessment of potential neurocognitive side effects, such as memory loss and confusion, and a new larger trial is underway to provide a more definitive assessment of safety over the longer term (ClinicalTrials.gov NCT02207634). The US Food and Drug Administration said it could not discuss specific development programs, but is "aware of concerns raised with neurocognitive adverse events and other lipid-lowering therapies, including statins, and as part of our oversight of new drug development, we are carefully monitoring these events". However, researchers have had difficulty finding a mechanism to explain these effects, since lipoproteins and monoclonal antibodies do not cross the blood-brain barrier. Furthermore, PCSK9 loss-of-function variants have not been associated with impaired cognitive performance.[101]

Alternative therapy in statin intolerance

The most common symptoms limiting statin use are muscular.[102,103] Non-statin therapies are available, but the reduction of LDL cholesterol concentrations is very modest (15%–20%) compared with those obtained with a maximum statin regimen.[104,105] Dosages intermittent to statin allow reductions from 12% to 38% of LDL cholesterol.[104,106] Thus, patients at high CV risk, who are unable to tolerate effective doses of statins because of muscle-related side effects, represent a difficult population to treat. This population is steadily increasing, and needs alternative strategies to reduce CV risk.

An estimated 10%–20% of patients cannot tolerate statins or adequate doses of these agents to achieve treatment goals. However, this figure is not a reliable indicator of prevalence, because most patients with a clinical history of statin toxicity are excluded from the statin trials. The Effect of Statin Medications on Muscle Performance study reported that subjects treated with atorvastatin 80 mg daily had more muscle symptoms than those treated with placebo (9.4% versus 4.6%, respectively).[107] The GAUSS study assessed the efficacy and safety of evolocumab in patients with a documented history of adverse muscular effects from use of statins. Statin intolerance was defined as inability to tolerate at least one statin at any dose or an increase of the dose because of intolerable myalgia or myopathy, and having symptom improvement or resolution with discontinuation of the statin. A consistent dose-dependent reduction in LDL cholesterol levels versus baseline was found with evolocumab alone administered every 4 weeks (–40.8% on 280 mg, –42.6% on 350 mg, and –50.7% on 420 mg). Addition of ezetimibe 10 mg to the maximum dose of evolocumab produced an additional 12.3% reduction (–63% versus baseline). Ezetimibe achieved a reduction of –14.8%. In early trials, Lp(a) was reduced by a mean of 23.3% versus 7.9% with ezetimibe. Evolocumab increases HDL cholesterol modestly, from a minimum of about 6% to a maximum of 12% in combination to ezetimibe. Myalgia was the most common side effect (7.4% with evolocumab versus 3.1% with ezetimibe). Patients on evolocumab/ezetimibe reported muscle side effects in 20% of cases.[108] The GAUSS-2 study included patients intolerant to at least two statins, reflecting a population with a true unmet need. After 12 weeks of treatment, about 80% of evolocumab-treated patients and less than 10% of ezetimibe-treated patients at high risk achieved an LDL cholesterol $<$100 mg/dL.

In two Phase II trials of alirocumab with a stable dose of statin, muscle disorders were reported in 6% of patients treated with the PCSK9 inhibitor versus 7% of patients on placebo. Further studies confirm a larger number of muscle symptoms in patients treated with alirocumab plus atorvastatin 80 mg versus alirocumab plus atorvastatin 10 mg (19% versus 6%, respectively). Almost all patients tolerated the PCSK9 inhibitor well, with a low incidence of myalgia.[109] In two Phase II trials (of alirocumab and a stable dose of statin), muscle disorders were reported in 6% of patients treated with the PCSK9 inhibitor versus 7% of placebo patients.[94,110] Further studies confirm a larger number of muscle symptoms in patients treated with alirocumab plus atorvastatin 80 mg versus alirocumab plus atorvastatin 10 mg (19% versus 6%, respectively).[111] The ODYSSEY ALTERNATIVE trial tested the efficacy and safety of alirocumab versus ezetimibe in patients blindly rechallenged with atorvastatin 20 mg. The study included a 4-week period in which patients received only single-blind placebo. Patients who reported muscle symptoms during this period were not randomized, assuming that the symptoms were not statin-related. These controls were lacking in previous anti-PCSK9 trials that included the same population; considering that these allow more rigorous identification of patients intolerant to statins.[112] Preliminary data presented by Moriarty et al at the 2014 annual meeting of the American Heart Association showed a significant 52.2% reduction of LDL cholesterol with alirocumab versus 17.1% with ezetimibe after 24 weeks of treatment, with a lower rate of skeletal muscle adverse effects requiring discontinuation of treatment (15.9% with alirocumab versus 20.2% and 22.2% with ezetimibe and atorvastatin, respectively).[113] Treatment with evolocumab produced LDL cholesterol reductions of 41%–63% without significant muscle-related side effects. These reductions are comparable with those achieved using maximal doses of the most efficacious statins.[108] The

incidence of muscle-related adverse effects reported with evolocumab appears similar to that seen with alirocumab in patients who were able to tolerate background statins.[94] Inhibition of PCSK9 using monoclonal antibody technology allows some advantages versus statins, potentially enabling the same results to be achieved.

Some statins are highly lipophilic whereas others are highly hydrophilic, so their kinetic profile is variable.[114] Statins are substrates for several membrane transporters that can modify plasma concentrations of these drugs. Polymorphisms of cytochrome P450 enzymes and the *SLCO1B1, ABCB1*, and *COQ2* genes may produce different and unpredictable statin metabolism and in some cases statin intolerance.[115,116] While their metabolism is susceptible to some gene polymorphisms, monoclonal antibody concentrations are predictable, not being influenced by drug–drug interactions and possibly not causing muscle intolerance due to high concentrations.[117] Few alternatives are available to date, and do not lower achieve the target LDL cholesterol concentrations expected by the European guidelines. If data from these short-term trials can be confirmed in larger cohorts, the muscle tolerability of these two PCSK9 inhibitors provides an alternative therapy in high-risk patients who have a poor possibility of treatment.

Conclusion

CV disease is a leading cause of death globally, and lipid modification, particularly lowering of LDL cholesterol, is one of the cornerstones of its prevention and treatment. However, even after lowering of LDL cholesterol to conventional goals, a sizeable number of patients continue to suffer CV events. More aggressive lowering of LDL cholesterol and optimization of other lipid parameters is required, given that triglycerides, HDL cholesterol, and Lp(a) are part of the residual CV risk, particularly in individuals with severe FH. In this regard, it is necessary to consider new therapeutics, such as PCSK9 inhibitors, because LDL cholesterol cannot be lowered beyond a certain extent using traditional lipid-lowering therapy. In patients without known CV disease, there is conflicting evidence as to the benefits of aggressive pursuit of numerical lipid targets, particularly with respect to all-cause mortality. Given the poor lipid control and problems related to the use of statins in patients at high CV risk, evaluation of new classes of lipid-lowering drugs that contribute to the reduction of adverse events becomes relevant. The results of the Phase II and III studies indicate that PCSK9 inhibitors are important innovative agents for reducing CV risk. It is important to consider using alternative agents with a low side effect profile in patients who are intolerant of statins, those with FH, and those with CV events, considering that such patients have to take therapy lifelong. Such drugs are potentially usable for primary and secondary prevention in subjects at high CV risk in addition to statin therapy or as an alternative treatment in patients intolerant to statins, and have shown a good safety and tolerability profile; above all, they have shown good efficacy in achieving LDL cholesterol concentrations below 70 mg/dL, as recommended the by European guidelines. The currently available data from Phase III studies confirm the safety, tolerability, and adherence to treatment with this new class of drugs. In particular, a future goal will determine whether, and how addict, to add these drugs to existing treatment or use them in place of statin therapy. Finally, PCSK9 can reduce the residual CV risk and could be a valid alternative to conventional treatment in patients intolerant or poorly responsive to statins. A meta-analysis by Sattar et al reported a higher risk of developing diabetes among patients taking statins (odds ratio 1.09; 95% confidence interval 1.02–1.17).[118] This increased risk is probably correlated with statin dose and potency, as suggested by other authors.[119] Even if the risk is low when compared with the reduction of coronary events, association with PCSK9 inhibitors is likely to be useful in terms of reducing the statin dose and consequently the risk of developing diabetes.

Use of PCSK9 inhibitors is likely to be limited by cost factors. If the cost of other monoclonal antibodies is any indication, PCSK9 inhibitors will not be cheap. Pharma market experts have estimated an annual price that ranges from US$7,000–$12,000,[120] which represents a huge expenditure considering the number of potentially eligible patients and the long-life duration of treatment. These new drugs may be more likely limited to patients with FH and those who are intolerant or not responsive to statins.

Another issue involves the new American College of Cardiology/American Heart Association guidelines for prevention of primary and secondary atherosclerotic disease, which recently suggested that a management to a specific LDL cholesterol goal is not appropriate, recommending that patients should be treated with a high-dose statin to lower cholesterol as far as possible.[121] This "statin-centric" view of CV prevention could threaten the use or even approval of this new class of experimental cholesterol lowering drugs. In contrast, the European Society of Cardiology/European Atherosclerosis Society guidelines defining an LDL cholesterol target and leave it to clinicians to decide how to reach it.

Disclosure

The authors report no conflicts of interest in this work.

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