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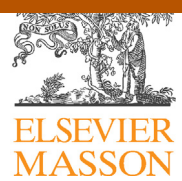

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REVIEW

PCSK9: From discovery to therapeutic applications

PCSK9 : de la découverte aux applications thérapeutiques

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Monoclonal antibodies

Summary The proprotein convertase subtilisin/kexin type 9 (PCSK9) regulates cholesterol metabolism mainly by targeting the low-density lipoprotein receptor (LDLR) for degradation in the liver. Gain-of-function mutations in PCSK9 are one of the genetic causes of autosomal dominant hypercholesterolaemia. Conversely, loss-of-function mutations are associated with lower concentrations of LDL cholesterol (LDL-C) and reduced coronary heart disease. As these loss-of-function mutations are not associated with apparent deleterious effects, PCSK9 inhibition is an attractive new strategy for lowering LDL-C concentration. Among the various approaches to PCSK9 inhibition, human data are only available for inhibition of PCSK9 binding to LDLR by monoclonal antibodies. In phase II studies, the two most advanced monoclonal antibodies in development (alirocumab and evolocumab) decreased atherogenic lipoproteins very effectively and were well tolerated. A dramatic decrease in LDL-C up to 70% can be obtained with the most efficacious doses. Efficacy has been evaluated so far in addition to statins in hypercholesterolaemic patients with or without familial hypercholesterolaemia, in patients with intolerance to statin therapy and in monotherapy. Large phase III programmes are ongoing to evaluate the long-term efficacy and safety of these very promising new agents.

Abbreviations: ADH, autosomal dominant hypercholesterolaemia; apo, apolipoprotein; CHD, coronary heart disease; FH, familial hypercholesterolaemia; GOF, gain-of-function; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; LOF, loss-of-function; Lp(a), lipoprotein (a); mAbs, monoclonal antibodies; PCSK9, proprotein convertase subtilisin/kexin type 9; Q2W, every 2 weeks; Q4W, every 4 weeks; siRNA, small interfering RNA; SREBP2, sterol-responsive element-binding protein 2.

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MOTS CLÉS
Proprotein convertase subtilisin/kexin type 9 (PCSK9) ;
Récepteur des LDL ;
LDL-cholestérol ;
Hypercholestérolémie familiale ;
Anticorps monoclonaux

Résumé PCSK9 ou proprotein convertase subtilisin/kexin type 9 est une protéine clé dans la régulation du métabolisme du cholestérol qui agit principalement en augmentant la dégradation du récepteur des lipoprotéines de basse densité (LDLR) dans le foie. Des mutations « gain-de-fonction » de PCSK9 sont l'une des causes génétiques de l'hypercholestérolémie autosomique dominante. À l'opposé, des mutations « perte-de-fonction » ont été associées avec des taux bas de LDL-cholestérol (LDL-C) et une réduction du risque de maladie coronarienne. Comme ces mutations « perte-de-fonction » n'induisent pas d'effets délétères, l'inhibition de PCSK9 est une nouvelle stratégie intéressante pour abaisser les taux plasmatiques de LDL-C. Parmi les diverses approches pour inhiber PCSK9, des données humaines sont seulement disponibles pour l'instant avec des anticorps monoclonaux qui inhibent la liaison de PCSK9 aux LDLR. Dans les études de phase II, les deux anticorps monoclonaux les plus avancés dans le développement (alirocumab et évolocumab) se sont révélés très efficaces pour diminuer les lipoprotéines athérogènes et ont été bien tolérés. Une diminution majeure du LDL-C jusqu'à 70 % a été observée avec les doses les plus efficaces. L'efficacité a été évaluée jusqu'alors en addition aux statines chez des patients hypercholestérolémiques avec ou sans hypercholestérolémie familiale, chez des patients avec intolérance à un traitement par statine, et en monothérapie. De larges programmes de phase III sont en cours pour évaluer l'efficacité et la sécurité d'emploi à long terme de ces nouveaux agents très prometteurs.

Background

High concentrations of low-density lipoprotein cholesterol (LDL-C) have consistently been associated with an increased risk of atherosclerotic cardiovascular disease, particularly coronary heart disease (CHD). Low-density lipoprotein (LDL) particles are removed from the circulation mainly by hepatic uptake *via* the LDL receptor (LDLR). LDL binds to the LDLR and the LDL/LDLR complex is internalized into clathrin-coated vesicles by endocytosis. Then, LDL is separated from its receptor in the endosomes and the LDLR is recycled for reuse. At the same time, LDL is degraded (Fig. 1a).

Both environmental and genetic factors regulate plasma concentrations of LDL-C. Among genetic causes, autosomal dominant hypercholesterolaemia (ADH) is associated with elevated LDL-C concentration and premature cardiovascular disease. In the majority of the cases, familial hypercholesterolaemia (FH) is related to mutations in the LDLR itself. A second and less frequent form of FH is caused by mutations in the ligand-binding domain of apolipoprotein (apo) B100, the protein component of LDL that interacts with the LDLR [1]. The identification of a third gene associated with FH, encoding proprotein convertase subtilisin/kexin type 9 (PCSK9), has generated intensive research into PCSK9, making this protein a key regulator for LDLR activity and an attractive target for the treatment of hypercholesterolaemia [2—5].

The discovery of PSCK9

In 2003, Seidah et al. identified the ninth member of the proprotein convertase family, PCSK9 [6]. In the same year, the involvement of PCSK9 in regulating cholesterol metabolism became evident, with the identification of two gain-of-function (GOF) mutations in PCSK9, in two French families with a clinical diagnosis of ADH and no detectable mutations in LDLR or *apoB100* genes [7]. Since this first report, several other GOF mutations have been reported [8], associated with mild to severe hypercholesterolaemia and an increased risk of CHD [9]. However, GOF mutations in PCSK9 are relatively rare and account for a small percentage of patients with ADH [1].

Conversely, the genetic evidence suggesting a potential role for PCSK9 inhibition in decreasing LDL-C concentration came from the identification of loss-of-function (LOF) mutations and common polymorphisms associated with lower LDL-C concentrations. The first LOF mutations were described in 2005 [10] and the effect of lifelong reductions in LDL-C induced by these LOF mutations was examined in the atherosclerosis risk in communities study [11]: the LOF mutations Y142X and C679X in African Americans were associated with a 28% reduction in LDL-C and an 88% reduction in the risk of CHD, whereas the R46L mutation in Caucasians was associated with a 15% reduction in LDL-C and a 47% reduction in the risk of CHD [11].

Numerous other LOF mutations or polymorphisms associated with decreased LDL-C concentrations have been identified [8]. The association between the R46L mutation and the risk of CHD has been extensively evaluated in three independent Danish studies [12]. In meta-analyses, R46L carriers had a 12% reduction in LDL-C and a 28% reduction in CHD risk [12]. The fact that CHD risk reduction was considerably larger than predicted with similar LDL-C reductions in statin trials [13] could be explained by the effect of long-term exposure to lower LDL-C beginning early in life. This is also in agreement with the results of a Mendelian randomization analysis, in which long-term exposure to lower LDL-C was associated with a three-fold greater reduction in CHD risk than that observed during treatment with a statin started later in life [14].

Structure and biosynthesis of PCSK9

The human *PCSK9* gene located on chromosome 1p32.3 encodes a 692-amino acid inactive glycoprotein. PCSK9 is expressed in several organs, particularly the liver and also the intestine and the kidney [5]. The 692-amino acid preproPCSK9 undergoes signal peptidase cleavage (domain

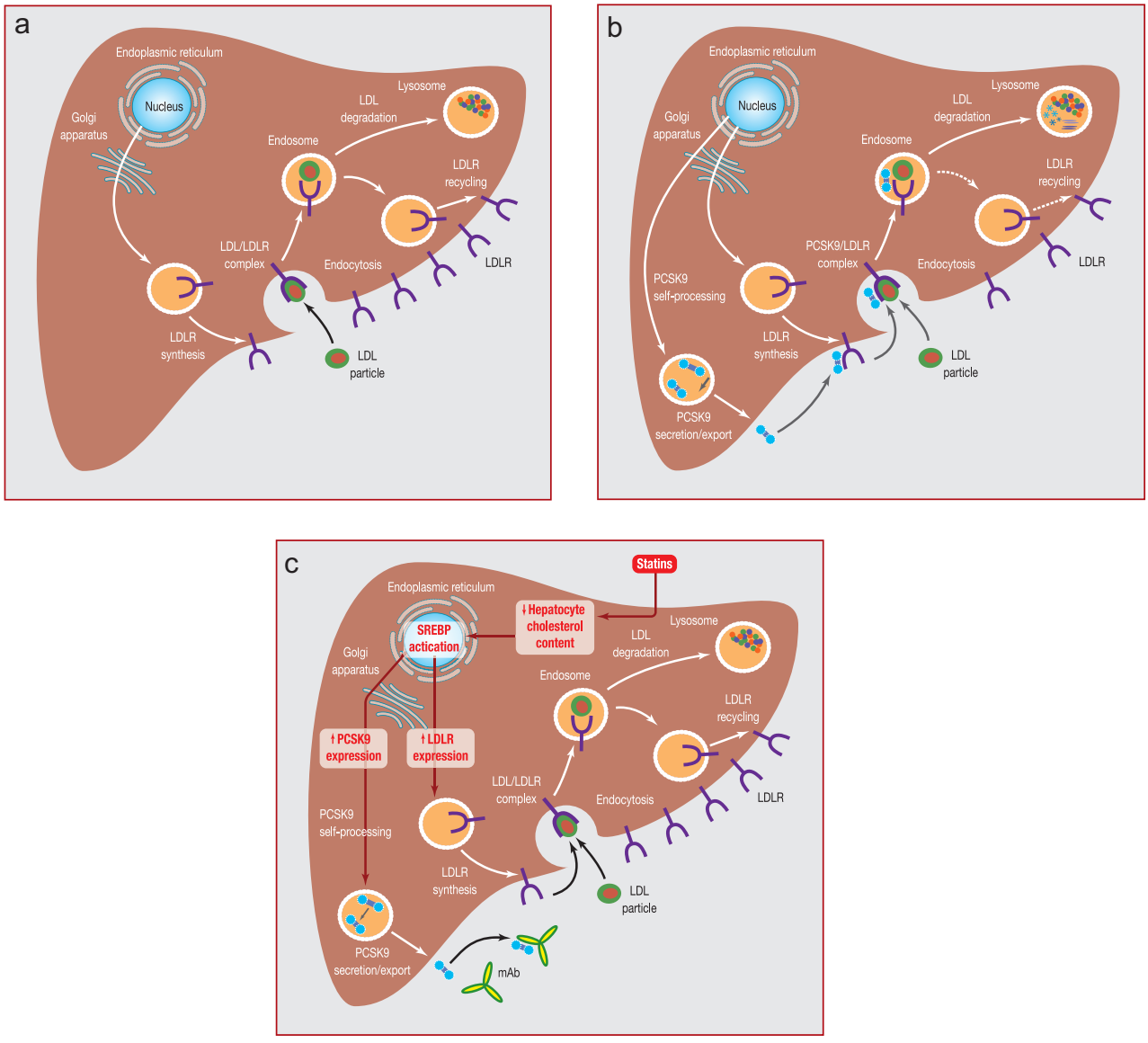

Figure 1. Role of PCSK9 in the regulation of the hepatocyte LDLR activity: a: synthesis and recycling of LDLR; b: synthesis, secretion of PCSK9 and effect on LDLR (PCSK9 binds to LDLR and, upon internalization, directs LDLR to lysosomal degradation, decreasing the number of LDLRs at the cell surface); c: AMG145) mechanisms of action of statins and mAb against PCSK9 (statin therapy *via* SERBP2 activation, stimulates both LDLR and PCSK9 expression; mAb prevents binding of PCSK9 to the LDLR/LDL complex).

1—30), then autocatalytic cleavage in the endoplasmic reticulum into two products: the prodomain and the mature PCSK9 containing the catalytic domain and the C-terminal domain. Cleavage of the prodomain is required for the maturation and secretion of PCSK9. Indeed, a recent LOF mutation due to an amino acid substitution within the cleavage site prevents autocatalytic processing and, by inhibiting PCSK9 secretion, is associated with a 48% reduction in LDL-C concentration [15].

After cleavage, the prodomain or prosegment remains associated by hydrogen bonds with the mature active form and the protein is finally secreted as an inactive dimer complex (Fig. 1b). Recent reviews have examined the role of this complex in preventing the access of other potential substrates to the catalytic domain of PCSK9 [16,17]. The activity of PCSK9 in promoting LDLR degradation seems independent of its catalytic activity. The catalytic domain of mature PCSK9 binds to the first epidermal growth factor-like repeat A domain of the LDLR, while the C-terminal domain binds to cell surface proteins, including Annexin A_2.

Functions of PCSK9

Role in the regulation of LDL-C concentration

The major function of PCSK9 is the degradation of the LDLR by complex mechanisms [4,5,16]: PCSK9 directly interacts with the LDLR both within the cell and at the surface of the plasma membrane [5,8,16]. However, evidence indicates that PCSK9 acts on the LDLR primarily as a secreted

factor and promotes the reduction of LDLR protein concentrations, mainly in the liver. LDLR protein concentrations are increased in the liver of PCSK9 knockout mice [18].

Secreted PCSK9 binds to the LDLR in a complex with its prosegment and is subsequently internalized together with the LDLR. The binding of PCSK9 to LDLR induces modification of LDLR conformation, avoiding normal recycling of LDLR to the plasma membrane and enhancing the LDLR lysosomal degradation [19] (Fig. 1b).

As a result, LDLR represents the main route of elimination of PCSK9 [20]. However, the mature secreted PCSK9 can be inactivated through cleavage by other proprotein convertases, particularly furin. The mature active form and the inactive form of PCSK9 circulate in the bloodstream.

Other functions of PCSK9

Although PCSK9 is a key player in cholesterol homeostasis through the regulation of LDLR concentrations, data suggest a role in triglyceride metabolism and triglyceride accumulation in visceral adipose tissue [21]. The function of PCSK9 in the intestine is not well known. It has been recently reported that PCSK9 can enhance chylomicron secretion and participate in the control of enterocyte cholesterol balance [22].

Beyond effects on lipid metabolism, animal data also suggest a role for PCSK9 in glucose homeostasis [23], liver regeneration and susceptibility to hepatitis C virus infection [2,17]. Although unexpected adverse effects cannot be excluded during PCSK9 inhibition, genetic variants of PCSK9 have given reassuring information. One of the most striking examples is that of a woman who carries two mutations in PCSK9 with no detectable circulating PCSK9, an LDL-C concentration of 14 mg/dL and normal hepatic, neuronal and renal functions [24].

Recently, it has been reported that absence of PCSK9 can be protective against melanoma invasion in mouse liver [25], suggesting that a PCSK9 inhibitor may be also useful in therapies against cancer metastasis. However, there is a large need for human data and, until now, PCSK9 inhibitors have been developed to treat hypercholesterolaemia and prevent atherosclerosis.

PCSK9 and atherosclerosis in animal models

In mice lacking PCSK9, the accumulation of cholesteryl esters in aortas was markedly reduced. By comparison, overexpression of PCSK9 induced an excess of atherosclerosis [26]. But in LDLR-deficient mice lacking or overexpressing PCSK9, no significant differences were observed in cholesteryl ester accumulation and plaque size, strongly suggesting that the process by which PCSK9 enhances atherosclerosis is primarily mediated through its action on the LDLR [26].

Cloned minipigs created by transposition of a human PCSK9 GOF mutant —a model for FH— had a significant increase in aortic atherosclerosis compared with wild-type minipigs [27].

Regulation of PCSK9 plasma concentrations

PCSK9 gene expression is mainly modulated by intracellular cholesterol concentrations and consequent activation of the transcription factor sterol-responsive element-binding protein 2 (SREBP2) [18], similarly to other genes involved in cholesterol homeostasis, such as LDLR. This concomitant regulation of both PCSK9 and LDLR by cholesterol *via* SREBP2 helps to explain the 'paradoxical effect' of statin therapy [2]: statins not only upregulate expression of the LDLR, but also expression of PCSK9, potentially limiting the pharmacological effect of reducing LDL-C concentration [28,29] (Fig. 1c). Statin administration to PCSK9 knockout mice enhanced LDL clearance from plasma [18]. These data support PCSK9 inhibition as a very attractive target for lowering LDL-C and enhancing the efficacy of statin treatment.

Strategies for PCSK9 inhibition

Several therapeutic approaches to the inhibition of PCSK9 have been proposed [30], including: inhibition of PCSK9 synthesis by gene silencing agents, such as antisense oligonucleotides or small interfering RNA (siRNA); inhibition of PCSK9 binding to LDLR by monoclonal antibodies (mAbs), small peptides or adnectins; and inhibition of PCSK9 autocatalytic processing by small molecule inhibitors. These strategies, targeting either extracellular or intracellular PCSK9, have been extensively described in recent reviews [3—5,31].

Preclinical studies on inhibition of PCSK9 synthesis by antisense oligonucleotides were promising, but the development of two antisense oligonucleotides by BMS/ISIS (BMS-84421) and Santaris Pharma (SPC5001) was stopped in phase I [3,31]. siRNA is another approach [32,33]: in a phase I trial of ALN-PCS —an siRNA developed by Alnylam Pharmaceuticals— a dose-dependent reduction in LDL-C was observed, with a 41% reduction at day 4 with the highest dose, associated with a 68% reduction in plasma PCSK9 concentrations [31,34]. Inhibition of PCSK9 binding to LDLR by small peptide inhibitors such as SX-PCK9 (Serometrix, East Syracuse, NY, USA) or adnectins such as BMS-962476 (BMS/Adnexus, Waltham, MA, USA) are in preclinical development or phase I [3,31]. On the basis of the discovery of an LOF mutation in the autocatalytic cleavage site of PCSK9 [15], inhibition of PCSK9 autocatalytic processing is the approach chosen by Cadila Healthcare and Shifa Biomedical [3,5], with molecules in preclinical development phase. Finally, mAbs are currently the most advanced approach in terms of clinical development, with published phase I and phase II human trials (Fig. 1c).

Efficacy of monoclonal antibodies targeting PCSK9

Several mAbs targeting PCSK9 have been tested in preclinical studies to assess their disruption of the PCSK9-LDLR interaction or inhibition of PCSK9 internalization [3]. Human data are available for three of these mAbs: SAR236553/

REGN727 (alirocumab) and AMG145 (evolocumab), two fully human mAbs developed by Sanofi/Regeneron and Amgen, respectively; and RN316/PF04950615, a humanized mAb developed by Pfizer/Rinat.

Alirocumab (SAR236553/REGN727)

Three phase I studies of alirocumab have been performed, two in healthy volunteers and one in patients with hypercholesterolaemia [35]. In the two single ascending-dose studies, alirocumab reduced LDL-C in a dose-dependent manner by 28—65% when given intravenously (0.3—12.0 mg/kg) and by 32—46% when given subcutaneously (50—250 mg). In the third placebo-controlled multiple-dose trial, alirocumab was administered subcutaneously at doses of 50, 100 or 150 mg in adults receiving atorvastatin with LDL-C > 100 mg/dL and at the 150 mg dose in adults on diet alone with LDL-C > 130 mg/dL. Alirocumab significantly reduced LDL-C by 38—65% in patients taking atorvastatin and by 57% in patients not taking atorvastatin. Alirocumab induced a maximum lowering of LDL-C within 2 weeks. It seems that the duration of action is longer in subjects who are not treated with atorvastatin, suggesting that the statin-stimulated production of PCSK9 might affect the duration of action of therapeutic mAbs.

The efficacy of alirocumab administered subcutaneously was examined in three phase II randomized double-blind placebo-controlled trials [36—38]. The efficacy on atherogenic lipid variables observed with the most efficacious dose is indicated in Table 1.

In 77 heterozygous FH patients on stable statin therapy (with or without ezetimibe) and LDL-C $\geq$ 100 mg/dL randomized into five study groups (placebo, alirocumab 150 mg every 4 weeks [Q4W], 200 mg Q4W, 300 mg Q4W and 150 mg every 2 weeks [Q2W] [38]), alirocumab dose dependently reduced LDL-C by 29—43% for 150—300 mg injected Q4W and by 68% for 150 mg injected Q2 W. With the 150 mg Q2W dose regimen, more than 80% of patients achieved an LDL-C concentration < 70 mg/dL. In addition, the 150 mg Q2W dose showed significant decreases in apoB and non-high-density lipoprotein cholesterol (non-HDL-C; Table 1) and increases in HDL-C (+6.5% versus baseline) and apoAI (+8.8% *versus* baseline).

In the second phase II trial, a 12-week study of 183 patients with LDL-C $\geq$ 100 mg/dL on stable-dose atorvastatin 10, 20 or 40 mg [36], dose-dependent and regimen-dependent reductions in LDL-C were observed: the dose of 150 mg Q2 W was found to be the most effective (72% reduction in LDL-C) and patients receiving 100 and 150 mg Q2W had greater reductions in LDL-C than those receiving 200 and 300 mg Q4W. LDL-C reductions were unaffected by atorvastatin dose. With the dose of 150 mg Q2W, apoB, non-HDL-C and lipoprotein (a) (Lp[a]) were also significantly reduced (Table 1) and all patients receiving this dose achieved targets of < 70 mg/dL, < 80 mg/dL and < 100 mg/dL for LDL-C, apoB and non-HDL-C, respectively [36].

In the last phase II trial [37], 92 patients with LDL-C $\geq$ 100 mg/dL on atorvastatin 10 mg were randomized to received 8 weeks of treatment with atorvastatin 80 mg plus alirocumab 150 mg Q2W, atorvastatin 10 mg plus alirocumab 150 mg Q2W or atorvastatin 80 mg plus placebo Q2W. Adding alirocumab to either atorvastatin 80 mg or atorvastatin 10 mg resulted in a significantly greater LDL-C reduction than that attained with atorvastatin 80 mg alone. Interestingly, the complementary LDL-C reduction was not significantly different between the groups receiving alirocumab added to atorvastatin 80 or 10 mg. All the patients assigned to alirocumab, compared with 52% in the group receiving atorvastatin 80 mg plus placebo, achieved the target LDL-C < 100 mg/dL. Alirocumab also induced significant decreases in ApoB, non-HDL-C and Lp(a) (Table 1).

Evolocumab (AMG145)

Two phase I studies have been published [39]. The phase Ia study is a single ascending subcutaneous or intravenous dose in healthy subjects. Single doses (7—420 mg administered subcutaneously; or 21 mg or 420 mg administered intravenously in a 1-hour infusion) of evolocumab induced dose-dependent reductions in LDL-C by up to 64% compared with placebo. The phase Ib study enrolled seven hypercholesterolaemic cohorts of subjects receiving stable statin therapy (five cohorts receiving low-to-moderate statin doses, one receiving high-dose statins and one with heterozygous FH) in multiple ascending subcutaneous doses with different dosing intervals (1—4 weeks). Evolocumab reduced mean LDL-C concentrations by up to 75% *versus* placebo at the end of the dosing interval.

The efficacy of evolocumab administered subcutaneously was evaluated in four 12-week phase II randomized placebo-controlled (or ezetimibe-controlled) trials [40—43]. The main efficacy results for atherogenic lipid variables obtained with the most efficacious doses are summarized in Table 1.

In 167 heterozygous FH patients on stable statin therapy (with or without ezetimibe) and LDL-C $\geq$ 100 mg/dL randomized to evolocumab 350 mg Q4W, 420 mg Q4W or placebo, evolocumab decreased LDL-C by 43.8% in the 350 mg group and 56.4% in the 420 mg group (*versus* placebo) [40]. Significant reductions were also observed for apoB, non-HDL-C and Lp(a) concentrations (Table 1). Seventy per cent of patients receiving 350 mg and 89% of those receiving 420 mg achieved LDL-C < 100 mg/dL; 44% and 65% achieved LDL-C < 70 mg/dL. The results obtained with both alirocumab and evolocumab in FH are impressive, taking into account the difficulty in reaching LDL-C goals for this category of patients [44].

In the LAPLACE-TIMI57 trial [40], 631 patients with LDL-C $\geq$ 85 mg/dL on stable statin therapy (with or without ezetimibe) were randomized into eight groups: six treatment arms with evolocumab at 70, 105 and 140 mg Q2W, or evolocumab at 280, 350 or 420 mg Q4W; and two control groups receiving placebo Q2W or Q4W. Evolocumab induced dose-dependent significant reductions in LDL-C (41.8—66.1% with Q2W regimen and 41.8—50.3% with Q4W regimen). The effects on other atherogenic lipoproteins observed with the most efficacious doses (140 mg Q2W and 420 mg Q4W) are listed in Table 1. Overall, 93.5% of patients receiving evolocumab 140 mg Q2W and 71.8% of patients receiving evolocumab 420 mg Q4W achieved the target LDL-C < 70 mg/dL. In a complementary analysis of LAPLACE-TIMI57 trial, evolocumab significantly reduced Lp(a) by up to 32% [45].

The GAUSS trial investigated the efficacy and safety of evolocumab in 160 statin-intolerant patients [43]. Treatment arms included evolocumab at 280 mg, 350 mg

Table 1 Efficacy of alirocumab 150 mg Q2W and evolocumab 140 mg Q2W or 420 mg Q4W monoclonal antibodies to PCSK9 on atherogenic plasma lipid and lipoprotein concentrations: data from phase 2 clinical trials[a].

Study population	Trial	Drug	LDL-C (%)	ApoB (%)	Non-HDL-C (%)	Lp(a) (%)
Heterozygous FH	RUTHERFORD [40]	AMG 420 mg Q4W	−56.4[c]	−46.2[c]	−53.5[c]	−31.5[c]
	Stein et al. [36]	SAR 150 mg Q2W	−57.3[d]	−43.8[d]	−46.6[d]	−19.5
Patients on stable statin therapy (± ezetimibe)	LAPLACE-TIMI57 [41,45]	AMG 140 mg Q2W	−66.1[d]	−56.4[d]	−61.4[d]	−32.3[c]
		AMG 420 mg Q4W	−50.3[d]	−42.0[d]	−47.6[d]	−23.1[c]
	Mc Kenney et al. [37]	SAR 150 mg Q2W	−67.3[d]	−58.3[d]	−60.3[d]	−28.6[d]
	Roth et al. [38][b]	SAR 150 mg Q2W	−66.2[g]	−54.4[g]	−58.3[g]	−34.7[g]
Statin intolerance	GAUSS [42][b]	AMG 420 mg Q4W	−50.7[e]	−42.1[e]	−48.6[e]	−23.6[f]
		AMG 420 mg Q4W + EZE 10 mg daily	−63.0[e]	−49.1[e]	−59.8[e]	−29.1[e]
Monotherapy	MENDEL [43]	AMG 140 mg Q2W	−47.2[d]	−44.2[d]	−45.2[d]	−29.3[d]
		AMG 420 mg Q4W	−52.5[d]	−42.5[d]	−47.1 [d]	−29.2[d]

AMG: AMG145or evolocumab; ApoB: apolipoprotein B; EZE: ezetimibe; Lp(a): lipoprotein(a); Q2W: every 2 weeks; Q4W: every 4 weeks; SAR: SAR236553/REG727 or alirocumab.

[a] Data expressed as % change *versus* placebo (except as indicated).

[b] % change *versus* baseline.

[c] $P < 0.001$ *versus* baseline.

[d] $P < 0.0001$ *versus* baseline.

[e] $P < 0.001$ *versus* ezetimibe + placebo.

[f] $P = 0.001$ *versus* ezetimibe + placebo.

[g] $P < 0.001$ *versus* atorvastatin 80 mg + placebo.

and 420 mg Q4W; a combined treatment group was given evolocumab at 420 mg Q4W plus ezetimibe 10 mg; and a control group received placebo subcutaneously Q4W plus ezetimibe 10 mg. *Versus* baseline, evolocumab induced a significant dose-dependent decrease in LDL-C from 40.8% to 50.7%. Furthermore, the combination of evolocumab and ezetimibe induced an almost additive 63% reduction in LDL-C.

The last phase II study (MENDEL) was a monotherapy trial evaluating the efficacy of evolocumab in 406 patients with LDL-C $\geq$ 100 mg/dL [41]. The treatment groups were identical to those for LAPLACE-TIMI57, with a complementary group receiving ezetimibe 10 mg. The dose-dependent reductions in LDL-C (37.3—47.2% with AMG Q2W and 43.6—52.5% with AMG Q4W) appeared similar to those observed in the LAPLACE-TIMI57 trial with the Q4W regimen, but smaller than those observed with the Q2W regimen. These data suggest that the Q4W regimen could be recommended without statin therapy and raises the question of the best regimen for those on statin therapy.

Finally, the efficacy of evolocumab in the rare population of homozygous FH patients has been tested in a pilot study conducted in eight patients (two receptor negative and six receptor defective). Evolocumab 420 mg Q2W and Q4W decreased LDL-C by 26.3 and 19.3%, respectively, in receptor-defective patients, with no reduction in receptor-negative patients [46]. These preliminary results need to be confirmed in a larger trial.

RN316/PF04950615

RN316, a humanized mAb with a pH-sensitive binding to the PCSK9 domain that interacts with the LDLR-EGFA domain, was developed to obtain longer serum half-life and duration for the LDL-C decrease in mice and monkeys [47]. In phase I studies, RN316 lowered LDL-C in hypercholesterolaemic subjects treated with single intravenous and subcutaneous doses, both as monotherapy and when added to atorvastatin [48]. In phase II trials, LDL-C concentrations were significantly reduced with 3.0 and 6.0 mg/kg doses of RN316 administered intravenously in addition to statin treatment [48]. The development of this drug is advancing with subcutaneous administration.

Safety of monoclonal antibodies targeting PCSK9

Overall, the mAbs tested so far have been generally safe and well tolerated, with no major safety issues from completed phase I and II studies. In each of the phase I studies for alirocumab and evolocumab, no serious adverse events were reported and no evidence of drug-related adverse events was observed [35,39].

In all of the phase II studies, alirocumab was generally well tolerated over the treatment period (8—12 weeks). Injection-site reactions were the most common adverse events in two of the phase II trials but were generally mild in severity and transient. However, in the phase II study assessing alirocumab for treatment of FH [38], one patient in the 300 mg dose Q4W group discontinued treatment after the first dose due to injection-site reaction and generalized pruritus. In another phase II trial [37], one patient receiving atorvastatin 80 mg plus alirocumab 150 mg Q2W discontinued treatment due to a hypersensitivity reaction and rash occurring 12 days after the second injection of mAb. There was a single case of cutaneous leukocytoclastic vasculitis reported in one patient, 9 days after initiation of alirocumab 300 mg [36]. The patient responded rapidly to withdrawal of the drug and initiation of steroid therapy.

Evolocumab was also generally well tolerated throughout the phase II trials, with a similar incidence of drug-related adverse events across treatment groups and no evidence of a relationship between the incidence of any adverse event and evolocumab dose [40—43]. Small numbers of serious adverse events occurred but none was considered related to the treatment. Injection-site reactions were generally infrequent and mild. In the specific trial conducted in statin-intolerant patients [43], myalgia was the most common treatment-emergent adverse event, but the frequency was low (3% in the placebo, evolocumab 350 mg and 420 mg groups; 20% in the evolocumab 420 mg/ezetimibe group); two patients in the evolocumab 350 mg group had creatine kinase concentrations greater than 10 times the upper normal limit during the study.

Finally, antibodies against alirocumab and evolocumab were detected at low titre in some patients. The use of fully human mAbs, such as alirocumab and evolocumab, will reduce the risk of immunosensitivity reactions [49].

PCSK9 inhibition with mAbs: evidence to date

Current data indicate that a fully human mAb targeting PCSK9 is very effective at lowering concentrations of atherogenic lipoproteins, with significant decreases in LDL-C, apoB, non-HDL-C and also Lp(a) concentrations. So far, efficacy has been demonstrated in addition to statins in hypercholesterolaemic patients with and without FH, in patients intolerant to statin therapy and in monotherapy. An additive effect between a PCSK9 inhibitor and ezetimibe has been observed for patients with statin intolerance.

Current data also have not revealed potential on-target effects of blocking PCSK9. It can be expected that mAbs targeting PCSK9 will exhibit fewer side effects than those associated with maximum statin doses. No short-term (12-week) safety issues have emerged from phase II trials and the mAbs tested so far have been well tolerated, apart from mild injection-site reactions.

PCSK9 inhibition: future perspectives

A number of important questions will need to be resolved before the approval of these new agents. Long-term efficacy and safety trials are critical, as patients will probably need life-long treatment. Even if anti-drug antibodies were rare in phase II trials, experience with other mAbs suggests that the development of anti-drug antibodies could reduce clinical efficacy and increase the incidence of adverse events [49]. Two large phase III programmes are ongoing: the PROFICIO programme with evolocumab; and the ODYSSEY

programme with alirocumab. Systematic monitoring of antibody development and adverse events will be needed in these programmes. Indeed, while genetic PCSK9 deficiency appears safe, this may not be relevant for mAb-based suppression of PCSK9 protein in individuals with genetically normal PCSK9. The safety of other mAbs and siRNA-based therapies against PCSK9 is still unknown.

As suggested by the reduced incidence of events in healthy patients with PCSK9 LOF mutations, the cardiovascular benefit in relation to the lowering effect of atherogenic lipoproteins will need to be evaluated in specific cardiovascular outcome trials. Two trials — FOURIER with evolocumab and ODYSSEY-OUCOMES with alirocumab — are ongoing.

Additional studies will also be needed to obtain a better understanding of the physiological role of PCSK9 and the effect of PCSK9 inhibition in other populations, such as patients with mixed hyperlipidaemia, diabetes or renal impairment.

Moreover, the place of PSCK9 inhibitors needs to be defined in comparison with other strategies that are either available or under development to reduce the residual risk linked to atherogenic lipoproteins. Undoubtedly, a PCSK9 inhibitor combined with statin therapy will be more effective than ezetimibe and resins in terms of LDL-C lowering. The use of a PCSK9 inhibitor combined with a low statin dose also seems an attractive strategy to avoid the side effects associated with high statin doses. The statin-fenofibrate combination therapy is only useful for high-risk patients with high triglycerides and low HDL-C on statin therapy. For this specific category of patients, the efficacy of PCSK9 inhibition still needs to be established. Among the new classes of agents that reduce LDL-C under development, the use of lomitapide (a microsomal transfer protein inhibitor) and mipomersen (an ApoB antisense oligonucleotide) will be limited to the rare population of patients with homozygous FH, while the putative benefit of new cholesteryl ester transfer protein inhibitors, such as anacetrapib and evacetrapib, remains controversial.

Among the candidate populations for PCSK9 inhibition, heterozygous FH should be considered as a priority. High-risk patients with documented statin intolerance are also candidates for PCSK9 inhibition. Other medical needs are high-risk patients not at goal on maximum lipid-lowering therapy, but the cost/benefit ratio will be an important issue.

Conclusion

PCSK9 is a key player in LDL metabolism, mainly by enhancing degradation of LDLR in the liver. The reduced incidence of cardiovascular disease in patients with PCSK9 LOF mutations provides a strong rationale for the development of PCSK9 inhibitors. The inhibition of PCSK9 is the most attractive new approach to reducing atherogenic lipoproteins and enhancing the efficacy of statins. Phase II trials have shown that fully human mAbs are effective and well tolerated. The ongoing phase III trials will determine long-term efficacy, safety and tolerability, including effect on cardiovascular disease.

Disclosure of interest

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