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INVITED REVIEW

Novel strategies to target proprotein convertase subtilisin kexin 9: beyond monoclonal antibodies

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Abstract

Since the discovery of the role of proprotein convertase subtilisin kexin 9 (PCSK9) in the regulation of low-density lipoprotein cholesterol (LDL-C) in 2003, a paradigm shift in the treatment of hypercholesterolaemia has occurred. The PCSK9 secreted into the circulation is a major downregulator of the low-density lipoprotein receptor (LDLR) protein, as it chaperones it to endosomes/lysosomes for degradation. Humans with loss-of-function of PCSK9 exhibit exceedingly low levels of LDL-C and are protected from atherosclerosis. As a consequence, innovative strategies to modulate the levels of PCSK9 have been developed. Since 2015 inhibitory monoclonal antibodies (evolocumab and alirocumab) are commercially available. When subcutaneously injected every 2–4 weeks, they trigger a ∼60% LDL-C lowering and a 15% reduction in the risk of cardiovascular events. Another promising approach consists of a liver-targetable specific PCSK9 siRNA which results in ∼50–60% LDL-C lowering that lasts up to 6 months (Phases II–III clinical trials). Other strategies under consideration include: (i) antibodies targeting the C-terminal domain of PCSK9, thereby inhibiting the trafficking of PCSK9-LDLR to lysosomes; (ii) small molecules that either prevent PCSK9 binding to the LDLR, its trafficking to lysosomes or its secretion from cells; (iii) complete silencing of PCSK9 by CRISPR-Cas9 strategies; (iv) PCSK9 vaccines that inhibit the activity of circulating PCSK9. Time will tell whether other strategies can be as potent and safe as monoclonal antibodies to lower LDL-C levels.

Keywords PCSK9 • LDL-C • Monoclonal antibodies • Gene silencing

1. PCSK9 biology

Proprotein convertase subtilisin kexin 9 (PCSK9) was discovered in 2003 as the last and unique inactive member of the family of subtilisin/kexin-like serine proteases.[1] In the same year, two gain-of-function (GOF) mutations in its gene (S127R and F216L) were associated with autosomal dominant hypercholesterolaemia[2] and, a few months later, its expression was shown to be downregulated by cholesterol in mice.[3,4] Although PCSK9 and low-density lipoprotein receptor (LDLR) mRNA levels were co-regulated by cholesterol, Maxwell *et al.*[5] established in 2004 the capacity of PCSK9 to trigger hepatic LDLR degradation, thus revealing a new level of regulation of hepatic LDLR levels. Subsequent studies confirmed and extended these data.[6,7]

Another breakthrough was the discovery in 2005 of loss-of-function (LOF) mutations in individuals with lifelong low levels of low-density lipoprotein cholesterol (LDL-C)[8] and reduced risk of coronary heart disease,[9] thereby making PCSK9 an attractive therapeutic target to reduce LDL-C levels.[10] PCSK9 is highly expressed in the liver[1] and secreted in the plasma. Following the binding of circulating PCSK9 to the EGF-A domain of the LDLR,[11–13] the complex is internalized and the LDLR is targeted to lysosomes for degradation,[14,15] thus resulting in a reduced LDLR expression on the hepatic cell surface, a reduced uptake of LDL particles from the blood, and a consequent rise in circulating LDL-C.[16,17] The most deleterious GOF mutation D374Y,[2,18] and others[19,20] are characterized by the early occurrence of cardiovascular events. These findings led to the development of monoclonal antibodies targeting circulating PCSK9, which reduce LDL-C levels by ∼60% and substantially improve cardiovascular outcomes in a variety of high-risk patients.[21–24]

In addition to monoclonal antibodies,[25] other strategies have been developed to target PCSK9, including the possibility to silence its mRNA expression, inhibit its mRNA translation, block the autocatalytic processing of proPCSK9 and alter the interaction between PCSK9 and the LDLR (*Figure 1*).[26] Moreover, a vaccine strategy is under evaluation. The aim of this review is to first summarize the ongoing strategies targeting PCSK9 and, second, to discuss other promising innovative strategies.

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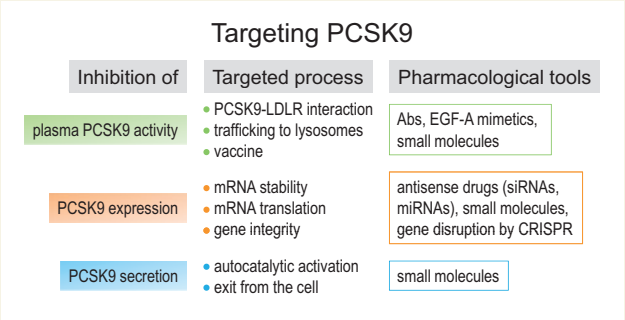

Figure 1 Strategies targeting PCSK9. PCSK9 activity can be inhibited at several levels. To date, only the PCSK9-LDLR interaction and PCSK9 mRNA stability are successfully targeted in humans with injectable mAbs (alirocumab, evolocumab) and siRNA (inclisiran), respectively.

2. Inhibition of the PCSK9-LDLR interaction by monoclonal antibodies

Anti-PCSK9 monoclonal antibodies (mAbs; alirocumab and evolocumab) represent the first pharmacological approach developed to target PCSK9 (*Figure 2*) and, so far, the only treatment approved by the regulatory agencies in many countries.

Following the positive results from Phase I and Phase II clinical trials,[27] the efficacy and safety of two anti-PCSK9 mAbs have been evaluated in two major programs (PROFICIO for evolocumab and ODYSSEY for alirocumab) including several Phase III trials. These studies have evaluated the effects of inhibitory mAbs to PCSK9 either as monotherapy or in combination with other lipid-lowering drugs across a broad patient population, including high cardiovascular risk patients, patients with heterozygous familial hypercholesterolaemia (HeFH) who cannot reach the

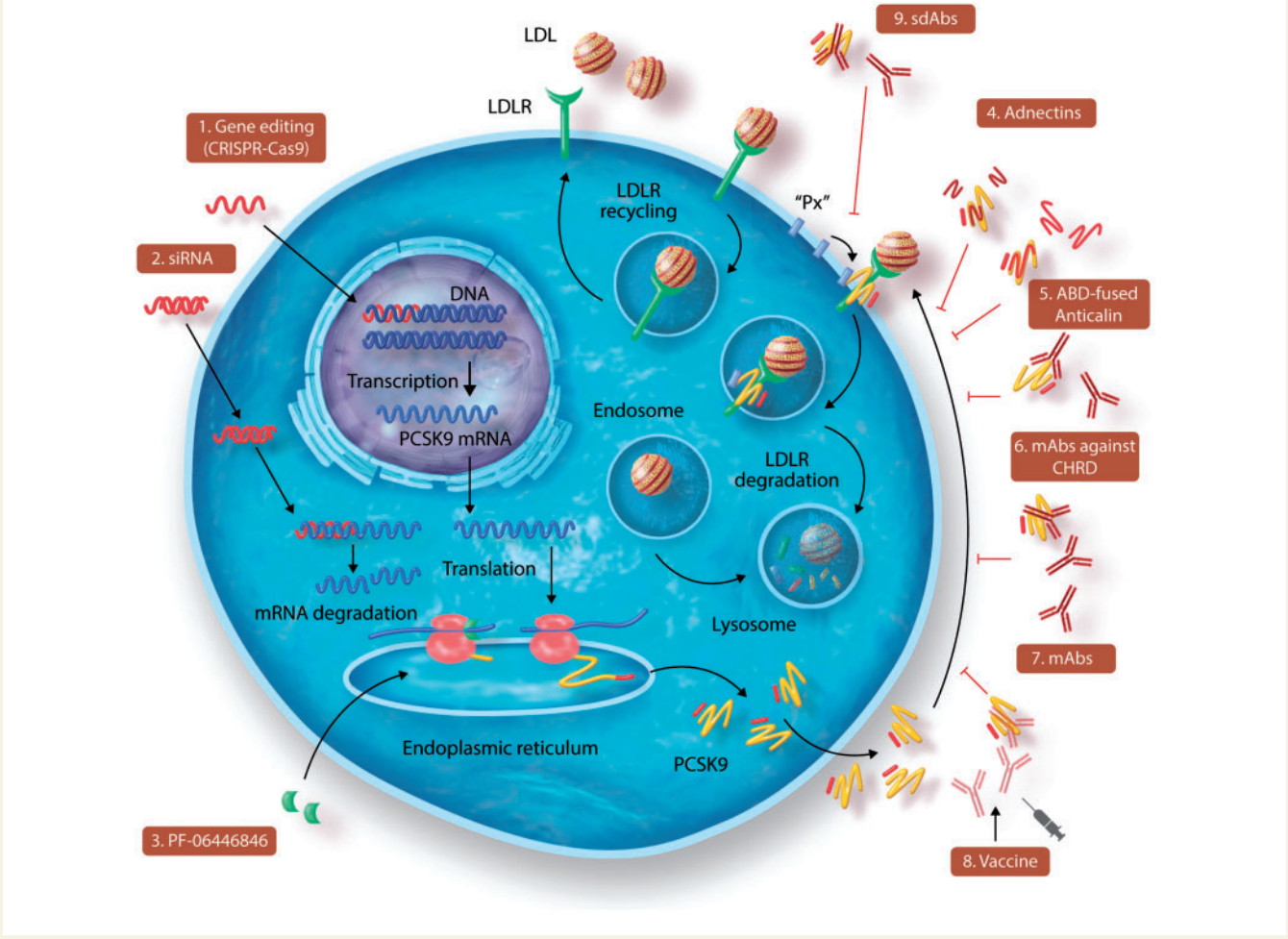

Figure 2 PCSK9 function and potential targets for inhibition. Following transcription and translation, PCSK9 is processed in the endoplasmic reticulum into the mature form and then secreted. In the absence of PCSK9, LDLR binds to circulating low-density lipoprotein (LDL) particle and the complex LDLR/LDL is internalized in the endosomes; LDL are shuttled in the lysosome for degradation while LDLR is recycled to the cell surface. When PCSK9 is present, it binds and escorts the LDLR/LDL complex for degradation in the lysosomes, with the net effect of reducing the number of LDLR on cell surface. PCSK9 can be inhibited at different levels including DNA gene editing (1); mRNA gene silencing (2); mRNA translational inhibition (3). In addition molecules targeting circulating PCSK9 are available or under development, these include adnectins (4), ABD-fused Anticalin (5), or selective antibodies (6–9) which, by binding PCSK9 prevent its interaction with LDLR (6–8) or the interaction of a hypothetical 'Px' protein to the complex LDLR-PCSK9 (9).

Table 1 LDL-C reduction through different PCSK9 inhibition approaches

Type of inhibition	LDL-C reduction (%)	Relative CV reduction	Status	References
mAbs targeting circulating PCSK9 (evolocumab and alirocumab)	55–60%	15–20%	Approved by FDA and EMA	[24,28]
Gene silencing (siRNA and inclisiran)	30–50%	Under evaluation in ORION-4	Phase III	[29,30]
Gene editing (CRISPR-Cas9)	~30% (TC)	–	Preclinical	[31]
Inhibition of PCSK9 mRNA translation (PF-06446846)	~58%	–	Preclinical (halted)	[32]
Adnectins	~50%	–	Preclinical (halted)	[33]
ABD-fused Anticalin	~50–60%	–	Preclinical	[34]
mAb against PCSK9 CHRD	~40%	–	Preclinical	[35]
Single domain antibodies (sdAbs)	~50%	–	Preclinical	[36,37]
Vaccine	13.3%	–	Phase I	Bauer *et al.*, ESC Congress, 2018 Munich

recommended LDL-C levels with current lipid-lowering therapies, as well as patients intolerant to statins. All these studies have indeed demonstrated that the inhibition of PCSK9 by the use of mAbs is overall safe; indeed, on top of maximally tolerated doses of statins, LDL-C levels are dramatically and significantly reduced (mean reduction: ~60%) (*Table 1*). In addition, the treatment with mAbs to PCSK9 is associated with significant reductions of Lp(a) levels by about 20–30%[38–40]; the clinical relevance of this effect, however, is still uncertain.[41] Finally, the addition of an anti-PCSK9 mAb to statin therapy results in the regression of coronary atherosclerosis[42] without affecting plaque composition,[43] with a continuous linear relation between achieved LDL-C levels and plaque regression. Although this study was not powered to assess clinical outcomes, a ~20% relative and ~3% absolute risk reduction for the first major adverse coronary event were observed.[42] Based on the results of these clinical trials, the two mAbs evolocumab (Repatha; https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125522s014lbl.pdf) and alirocumab (Praluent; https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/125559s002lbl.pdf) have been approved by the US FDA for subjects with HeFH or clinical atherosclerotic cardiovascular disease (ASCVD) under maximally tolerated statin doses and who still require additional lowering of LDL-C. Furthermore, evolocumab has received approval for use in homozygous FH. Similar indications were adopted by the European Medicines Agency (EMA) for the prescription of evolocumab (http://www.ema.europa.eu/docs/en_GB/document_library/Summary_of_opinion/human/003766/WC500246329.pdf) and alirocumab (http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2015/07/WC500190458.pdf). Outcome trials showed for evolocumab (FOURIER)[28] and alirocumab (ODYSSEY OUTCOMES)[24] that the reduction in plasma cholesterol levels translates into a significant reduction in the incidence of cardiovascular events (-15%). Based on the results of the FOURIER trial,[28] evolocumab has been approved also for the treatment of adults with established cardiovascular disease to reduce the risk of myocardial infarction, stroke, and coronary revascularization.

The clinical relevance of anti-PCSK9 mAbs administered as self-injection monthly or biweekly[44] may be also related to a higher adherence to treatment compared with a daily orally administered statin. The adherence to statin therapy may be negatively affected by muscle symptoms,[45] often leading to therapy discontinuation and consequent increase of cardiovascular risk. In addition, the variability in LDL-C levels during lipid-lowering treatment is a strong and independent predictor of cardiovascular events.[46] One- or two-year-long treatment with evolocumab[47,48] or alirocumab,[49] respectively, provides a sustained LDL-C lowering, although LDL-C level variability appears to be lower with every-2-week dosing than with every-4-week dosing.[50,51] A recent analysis of patients with statin-associated muscle symptoms and treated with evolocumab for up to 2 years showed that evolocumab provided persistent tolerability, adherence, safety, and efficacy in statin-intolerant patients.[52]. Of note, the treatment with mAbs targets circulating PCSK9, which exclusively originates from liver[53,54] without inhibiting intracellular PCSK9 in liver or other tissues. This might increase PCSK9 production in the liver as well as maintain PCSK9 intracellular activities which contribute to other processes.[1,10,55]

The therapeutic safety of anti-PCSK9 mAbs has been evaluated in several trials. Overall, adverse event rates did not differ between subjects treated with mAbs or control, which is reassuring in terms of the safety of very low LDL-C levels reached with these drugs. Creatine kinase and liver enzyme increases were infrequent and comparable between groups, and no drug-induced liver injury or renal impairment were observed.[56,57] A recent meta-analysis that investigated the effect of mAbs to PCSK9 on glycaemia and new-onset diabetes did not uncover drug-related changes in these parameters, independently of the mAb type, the characteristics of patients or treatment duration.[58] In addition, no evidence of side effects including neurocognitive adverse events were found to be associated with alirocumab or evolocumab treatment,[59,60] even in subjects who achieved very low levels of LDL-C. Noteworthy, no evidence for an increased risk of muscle-related adverse events was reported, despite the inclusion of statin-intolerant patients who experienced myalgia during statin therapy.[22] Finally, meta-analyses of various outcome trials revealed that the cost–benefit ratio for the use of PCSK9 mAbs together with statins over more than 2-years is highest for patients with baseline levels of LDL-C higher than 100–130 mg/dL.[61,62]

3. PCSK9 gene silencing and CRISPR editing

Gene silencing is a physiological post-transcriptional process by which cells regulate gene expression via turning off a selected gene. This approach has been translated into selective pharmacological targeting with the development of small interfering RNA (siRNA) controlling the expression of specific genes playing key roles under different physiopathological conditions, including those involved in lipid and lipoprotein metabolism.[63]

Inclisiran is a double strand siRNA of the latest generation (*Figure 2*), with a specific chemistry designed to increase its half-life[29] and a covalent linkage to a ligand containing three molecules of N-acetylgalactosamine (GalNAc). The latter confers liver specificity to inclisiran by binding the ASGR1/ASGR2 receptors, essentially localized at the hepatocyte surface.[64]

Results from Phase I study in healthy volunteers with LDL-C $\geq$ 100 mg/dL showed that a single inclisiran dose of 300 mg injected subcutaneously reduced PCSK9 levels by ~75% and LDL-C levels by 50% on average for 6 months.[29]

The ORION-1 Phase 2 trial further explored the impact of single dose (200, 300, or 500 mg) or two doses (100, 200, or 300 mg) at Days 1 and 90.[30] The two-dose regimen of inclisiran, 300 mg each, achieved a sustained 52.6% LDL-C reduction similar to that achieved by mAbs for 6 months (*Table 1*),[30] and the reduction was independent of the diabetic status presence.[65] Interestingly, although the siRNA inclisiran is expected to also reduce the intracellular levels of PCSK9, the generated drops in apoB, non-HDL-C, VLDL-C, and triglycerides were comparable to those obtained with mAbs, suggesting a limited impact of liver intracellular PCSK9 in cholesterol metabolism. Lp(a) levels were reduced by 26%, although not significantly, due to large inter-individual variations.[66]. A clinical Phase 3 trial is ongoing to evaluate the effects of inclisiran in hypercholesteraemic and heterozygous FH subjects, while the ORION-4 trial will examine clinical outcomes at 6 years or more.[67] Time will tell whether the reduction of both circulating and intracellular PCSK9 generates unexpected/unwanted effects. So far, inclisiran was shown to lack side effects.[29]

Another approach is the delivery of CRISPR-Cas9 to the liver for the *in vivo* base editing of PCSK9[31,68,69] (*Figure 2*) as an alternative to siRNA therapy in liver. So far, the results in animal models revealed a ~30% reduction in plasma cholesterol levels (*Table 1*), with no apparent evidence of off-target mutagenesis.[31] More recently, as a proof-of-concept for treating genetic diseases before birth, CRISPR-mediated gene editing *in utero* resulted in adult mice expressing a W159X LOF-PCSK9 that exhibit a substantial reduction in serum cholesterol.[70] Additional studies are needed to critically evaluate whether this approach might be translated into the clinic.

4. Inhibition of PCSK9 mRNA translation

A recent report reveals that translational inhibition of PCSK9 mRNA may represent an attractive approach to block PCSK9 synthesis[71] (*Figure 2*). An orally active compound (PF-06446846) efficiently interrupted PCSK9 translation around codon 34, within the Leu stretch of the signal peptide coding region[32]. Although leucine/hydrophobic stretches are not known to cause translation stalls, the string of 9 to 11 CUG leucine codons[72–74] present in the signal peptide coding region of PCSK9 was likely recognized by this inhibitor.[32] Unfortunately, even though such translational inhibitors were optimized,[75] the lack of PCSK9 specificity halted further development of this approach.

Another potential approach is represented by microRNA mimetics. Recently, miR-191, miR-222, and miR-224 were shown to post-transcriptionally down-regulate the levels of PCSK9 mRNA.[76] However, it needs to be taken into account that these miRNAs are not PCSK9-specific, as exemplified by the ability of miR-222 to also down-regulate the expression of CD4 receptor.[77]

5. Targeting the autocatalytic processing of proPCSK9

An additional strategy to target PCSK9 is to interfere with the autocatalytic processing of proPCSK9 into PCSK9. This is based on the original observation that PCSK9 can exit the endoplasmic reticulum (ER) only following the autocatalytic cleavage of the zymogen proPCSK9 and the generation of a heterodimer of mature PCSK9 with its prodomain which remains non-covalently bound to the catalytic domain.[1] Natural[78,79] and engineered[6,80,81] PCSK9 mutants were found unable to undergo autoprocessing and were retained in the ER. Interestingly, these zymogen forms act as dominant-negatives, as they can dimerize with wild type PCSK9 forcing its retention in the ER.[78–80] Of note the few heterozygous subjects carrying the LOF PCSK9-Q152H, which prevents zymogen processing and retains proPCSK9 in the ER, have all very low levels of circulating PCSK9 and LDL-C,[78] supporting the potential relevance of this approach. Seemingly, they are in good health and do not exhibit any overt pathology associated with the lack of circulating PCSK9, indicating that complete retention of proPCSK9 in the ER may not result in unwanted side effects. Indeed, our recent data revealed that PCSK9 is protective against the induction of ER stress and that the Q152H mutant is poised to protect cells from the unfolded protein response inducing ER stress.[82,83] Inhibition of the autocatalytic processing is thus an attractive approach to prevent PCSK9 secretion. However, engineering of a small molecule inhibitor turned out to be difficult because of the zero order kinetics of the autocatalytic processing of proPCSK9 into PCSK9 and the necessity to cross both plasma and ER membranes to reach the ER lumen.[84,85]

Finally, PCSK9 secretion may be reduced by inhibitors that would prevent the interaction of a recently reported ER resident cargo receptor, SURF4, with mature PCSK9, thereby facilitating its efficient exit from the ER into COP-II vesicles *en route* to the Golgi apparatus.[86]

6. Other inhibitors of PCSK9-LDLR binding

Within the strategies to inhibit the interaction between PCSK9 and the LDLR, a lot of interest is focused on EGF-A-like peptides or small molecule inhibitors. The first EGF-A-like peptide identified that effectively inhibited the PCSK9-LDLR binding was a Fc-fusion EGF66 that bound PCSK9 with a Kd of ~70 nM and inhibited the PCSK9-induced LDLR degradation in HepG2 cells and in mice.[87] Later on, shorter peptides able to bind PCSK9 with increased affinity have been generated, including the 13 amino acid (aa) Pep2-8, which however is 10-fold less active than EGF66.[88] Efforts to further improve the potency of Pep2-8 led to the discovery of a targetable pocket region in PCSK9 structure very close to the EGFA-PCSK9 interaction surface[89] that interacts with the N-terminal 10 aa P'

helix peptide (aa 153–162; SIPWNLERIT) of the catalytic domain of PCSK9. An intense engineering effort led to the design of a modestly active first generation 16-residue linear peptide MESFPGWNLV(homoR)IGLLR, which antagonizes PCSK9 activity.[89] Efforts are underway to improve this structure and generate a potent orally active small molecule inhibitor.[90]

Another approach to disrupt the extracellular PCSK9-LDLR interaction is the use of engineered adnectins (*Figure 2*). These are ∼11 kDa fragments of fibronectin type III domain (BMS-962476) that bind the catalytic subunit of PCSK9. A single injection in cynomolgus monkeys led to a ∼50% reduction in LDL-C (*Table 1*).[33] Although promising, the clinical development of this strategy has been abandoned as it could not favourably replace the mAb approach.

A recent alternative biologic approach was to use a ∼22 kDa albumin-binding domain (ABD)-fused Anticalin protein DS-9001a produced in bacteria (*Figure 2*).[34] In cynomolgus monkeys, a single subcutaneous injection of such ABD-fused Anticalin protein was reported to have a somewhat longer half-life in plasma (∼120h)[34] compared to mAbs (∼60–120 h)[91] and BMS-962476 (∼74–108 h).[33] Such treatment resulted in a sustained ∼50–60% reduction of LDL-C lasting up to 21 days (*Table 1*), and its effect was significantly potentiated by atorvastatin.[34] We have to wait until these compounds are tested in clinical trials before drawing firm conclusions on the efficacy and safety of this promising new class of biologics.

7. Blockade of PCSK9-LDLR sorting to lysosomes by CHRD antibodies

Antibodies against PCSK9 were designed to recognize the C-terminal cysteine–histidine rich domain (CHRD), which is critical for sorting the PCSK9-LDLR complex to lysosomes/endosomes for degradation.[10,15,92] Deletion of the CHRD does not impair PCSK9 folding and secretion, but results in an inactive secreted form of PCSK9 that still binds the LDLR but cannot sort it to degradation compartments.[15] This result suggested that a so far unidentified 'protein X (Px)' is required to bind the CHRD and/or LDLR and to escort the PCSK9-LDLR complex to lysosomes for degradation (*Figure 2*).[10,93] Thus, any approach that prevents the formation of the Px-PCSK9-LDLR trimeric complex could potentially inhibit PCSK9 function without necessarily preventing binding of PCSK9 to the LDLR. Indeed, a bulky Fab[94] that binds the CHRD was shown to inhibit ∼50% of the extracellular PCSK9's ability to enhance the degradation of the LDLR. Moreover, a CHRD-specific mAb also reduced LDL-C levels by ∼40% when injected to cynomolgus monkeys (*Table 1*).[35]

Recently, three single domain antibodies (sdAbs) raised in llamas that recognize exclusively the C-terminal M1/M3 domains[11] of the CHRD of PCSK9 have been generated (*Figure 2*).[36] When the antigen-binding nanobody domains are fused to a mouse Fc-sequence and injected in mice expressing exclusively human PCSK9,[37] a sustained ∼50% reduction of LDL-C that lasted more than 17 days was observed (*Table 1*).[36,37] Different from the mAbs that prevent the formation of the PCSK9-LDLR complex, the sdAbs did not inhibit such complex formation nor did they increase the levels of circulating PCSK9, but rather prevented PCSK9 activity on the LDLR. It could be hypothesized that the sdAb interfered with the binding of Px to the PCSK9-LDLR complex and hence prevented its intracellular sorting to lysosomes.[37] Although these sdAbs do not reach the efficacy of the mAbs, they represent a unique tool to dissect out the sorting mechanism of the PCSK9-LDLR complex to endosomes/lysosomes. Further experiments based on the crystal structure of the sdAb-CHRD complexes and site directed-mutagenesis of the CHRD domain are ongoing to identify critical residues regulating such trafficking, which might result in more effective sdAbs.[37]

8. PCSK9 vaccine

A completely different approach to interfere with PCSK9 is to instruct the immune system to eliminate endogenous circulating PCSK9. This could be achieved by using PCSK9-peptide-based vaccines (*Figure 2*).[95,96] So far preclinical studies in mice have shown that immunization induces a strong and long-lasting immune response resulting in reduced plasma levels of PCSK9, total cholesterol and non-HDL-C (VLDL-C and LDL-C), as well as systemic inflammation.[97] Moreover, immunization resulted in reduced atherosclerotic lesion area and aortic inflammation compared with control mice.[97] This vaccine has been tested in a Phase I clinical trial (https://clinicaltrials.gov/ct2/show/NCT02508896). Preliminary data showed that in healthy subjects immunization was safe and well tolerated; more than 90% of immunized subjects developed a PCSK9-specific antibody response that was reactivated after a second injection at Week 60; the mean LDL-C reduction was 13.3% at Week 70 (*Table 1*), and persisted for at least 30 weeks after the boost immunization (Bauer *et al.*, Communication at ESC Congress, 2018 Munich). Although this approach seems attractive and more permanent, similar to the CRISPR approach, it is crucial to exclude the possibility of any serious unsuspected side effects due to the absence of PCSK9 expression in adult livers, especially in situations where liver function is compromised, such as during regeneration or viral infections.[98]

9. Conclusions and perspectives

These last 15 years of experimental and clinical research have demonstrated that the LOF of PCSK9 towards the LDLR associates with reduced levels of LDL-C and overall lower rate of cardiovascular complications and all-cause mortality,[99] especially for patients starting with higher baseline levels of LDL-C.[62] The beneficial effects of the loss of PCSK9 are independent from other risk factors such as diabetes and hypertension. Lower PCSK9 levels/activity also associate with a reduced risk of complications in sepsis and/or inflammation.[100,101] Thus abolishing circulating PCSK9 seems to offer multiple advantages and minimal side effects.

PCSK9 is expected to have other functions in the developing liver and extrahepatic tissues, such as small intestine, cerebellum, pancreas, and kidney.[10,55,102,103] A recent paper reported an association between a PCSK9 LOF and a reduced risk of abdominal aortic aneurism,[104] an observation that may further extend the therapeutic indications for PCSK9 inhibition. Alirocumab and evolocumab reduce circulating PCSK9, which originates from the liver only.[53,54] Although a few individuals lack functional PCSK9,[79,105,106] only time will tell whether siRNA silencing of liver intracellular and secreted PCSK9 or lifelong deletion of PCSK9 in the above tissues by way of CRISPR/Cas9 is still as beneficial. It should be emphasized that, with regard to its impact on the LDLR, hepatocyte-derived PCSK9 mostly acts extracellularly after its secretion. This may not be the case for other functions or in other tissues where PCSK9 may act intracellularly, indeed some experimental evidence purports a role for intracellular PCSK9 in hepatic and non-hepatic cell metabolism.[107]

Although the mAb approach, that only targets circulating PCSK9, does not seem to enhance the onset of diabetes on the short

run,[23,58,108] it may on the long run increase the metabolic risk of developing a pre-diabetes state, possibly due to the increased ratio of apoB/PCSK9.[109] Indeed, a higher apoB/PCSK9 ratio is associated with higher postprandial white adipose tissue macrophage infiltration and priming of the NLRP3 inflammasome, whose role in the aetiology of type 2 diabetes is well established.[110]

While much work is needed to unravel PCSK9 functions in extrahepatic tissues,[103] its involvement in immune-inflammatory responses is emerging[111] thanks to its ability to modulate the innate immune response during sepsis[112] or to affect hepatitis C virus infectivity via the regulation of hepatic surface entry proteins.[113] Of note, anti-PCSK9 monoclonal antibodies reverse the pro-inflammatory profile of monocytes in familial hypercholesterolaemia[114] and improve vascular inflammation.[115]

Moreover, the observation that lower levels of PCSK9 are associated with reduced inflammation, especially in patients with the highest level of the inflammatory marker high sensitivity C-reactive protein (hs-CRP),[116] will need to be properly validated in specific clinical trials on inflammation-associated pathologies, as was done with the mAb canakinumab targeting interleukin-1β in the CANTOS anti-inflammatory thrombosis outcome study.[117] A recent meta-analysis reported a lack of effect of anti-PCSK9 therapy with mAbs on circulating hs-CRP levels, at least for the short-term treatment,[118] and an analysis of the FOURIER trial showed that changes in hs-CRP levels were similar between evolocumab and placebo, even in subjects with a higher baseline hs-CRP level.[119] Whether the combination of a PCSK9 inhibitor with canakinumab, or any other anti-inflammatory mAb,[120] is clinically beneficial in patients with an elevated atherogenic profile will have to be carefully evaluated.

In conclusion, the discovery of PCSK9 in 2003 and its powerful regulation of LDL-C *via* the enhanced degradation of the LDLR has led the way towards the development of powerful new strategies to significantly enhance the reduction of LDL-C over and above the levels achieved with the more commonly used orally active statins or statins + ezetimibe. While some of the injectable PCSK9-targeting drugs are rapidly evolving, we may still witness the development of safe, orally active PCSK9-inhibitors in the future.[89] Because of their anticipated lower cost, the latter may have a more widespread use worldwide in the treatment of various pathologies, benefiting from low levels of PCSK9.

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