PCS19 inhibitors and cardiovascular disease: heralding a new therapeutic era

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Purpose of review
The first monoclonal antibodies targeting proprotein convertase subtilisin/kexin type 9 (PCSK9) have been approved for clinical use. This timely review highlights recent developments.

Recent findings
Low-density lipoprotein cholesterol (LDL-C) is the primary driver of atherosclerosis and the key target for intervention. Yet despite best treatment including statins, attaining sufficient LDL-C lowering can be problematic for high cardiovascular risk patients. The development of PCSK9 inhibitors, driven by novel genetic and mechanistic insights, offers an answer. Removal of circulating PCSK9 increases LDL receptor availability, and thus markedly decreases plasma LDL-C levels (by ~50–60%), and is additive to the lipid lowering effects of statins and ezetimibe. PCSK9 inhibition also reduces (by 25–30%) plasma levels of lipoprotein(a), a causal factor in atherosclerotic vascular disease, suggestive of partial catabolism of lipoprotein(a) by LDL receptors. The ODYSSEY and PROFICIO (Programme to Reduce LDL-C and Cardiovascular Outcomes Following Inhibition of PCSK9 In Different Populations) clinical trial programmes involving a wide range of high-risk patients, including statin intolerant patients, have confirmed the consistency of the LDL response, even with concomitant high-intensity statin or nonstatin therapy. Extensive evidence to date attests to a favourable safety and tolerability profile for these innovative agents.

Summary
The new pharmacotherapeutic era of PCS19 inhibition is upon us, promising major reduction in cardiovascular events across a wide spectrum of high-risk patients.

Keywords
alirocumab, cardiovascular risk, evolocumab, low-density lipoprotein cholesterol, proprotein convertase subtilisin/kexin type 9 inhibitors

INTRODUCTION
Preventing cardiovascular disease (CVD) is the insurmountable challenge for clinicians worldwide. CVD is already the leading cause of death and disability; by 2030, global CVD events are projected to exceed 23.3 million with an estimated cost exceeding US$ one trillion, unless urgent action is taken [1,2]. A two-pronged attack is needed, not only targeting lifestyle but also ensuring that modifiable cardiovascular risk factors are managed successfully, and in a coordinated manner, in individuals at high risk.

There is indisputable evidence that low-density lipoprotein cholesterol (LDL-C) is a principal driver of atherosclerotic vascular disease, the underlying cause of the majority of clinical manifestations of CVD, and thus the key target for intervention [3\textsuperscript{*}]. Insights from Mendelian randomization studies have clearly shown that the magnitude of clinical benefit in preventing CVD events relates to the extent of LDL-C lowering, and not the mechanism itself [4\textsuperscript{*}]. Lowering LDL-C is equally critically related to improved plaque stability and decreased atheroma volume [3\textsuperscript{*}].

Lowering LDL-C with statin treatment is the cornerstone of lipid lowering therapy. Yet attaining
the minimum guideline recommended LDL-C target is an issue for most patients at high cardiovascular risk (Fig. 1) [5]. This unmet clinical need is best exemplified by familial hypercholesterolaemia, in which genetic mutations, typically in the gene encoding the LDL receptor (LDLR), result in high cumulative LDL-C burden and premature coronary heart disease (CHD) [6*]. Even with high-intensity statin treatment, most patients do not attain LDL-C targets, leading to earlier onset of coronary events, disability, and death [7,8*]. Furthermore, it is less well recognized that genetic variability in the organic anion transporting polypeptide (OATP or SLCO1B1) transporter on the hepatocyte surface, whose action is key to ensure that statins gain access to their intracellular target enzyme, 3-hydroxy-3-methyl-glutaryl-CoA reductase, frequently underlies marked variability in statin response [9]. Statin intolerance, predominantly involving muscle symptoms, referred to as statin-associated muscle symptoms, is equally an issue which impacts therapeutic efficacy and CVD outcomes. Indeed, the European Atherosclerosis Society Consensus Panel has focused attention on the unmet needs of these high cardiovascular risk groups [6*,10*]. Clearly, efficacious novel LDL-C lowering treatments are needed to ensure attainment of LDL-C goal in such patient populations.

PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9: A NEW ERA IN LOW-DENSITY LIPOPROTEIN CHOLESTEROL LOWERING

Proprotein convertase subtilisin/kexin type 9, better known as PCSK9, broke dramatically onto the scene of lipid and cholesterol metabolism in 2003, when a collaborative discovery in Paris and Montreal identified families in whom autosomal dominant hypercholesterolaemia and premature CVD was because of rare gain-of-function mutations in this gene [11].

**FIGURE 1.** Despite statin treatment, high-risk patients remain at residual risk of cardiovascular events, including recurrent events. Although nonmodifiable risk factors, such as age and sex, are key factors contributing to this residual risk, failure to attain LDL-C targets, as recommended in the European Society of Cardiology/European Atherosclerosis Society Guidelines for Management of Dyslipidemia [5], is also a critical component. Furthermore, modifiable lipid-related risk factors, including elevated levels of lipoprotein(a), triglyceride-rich lipoproteins and remnants, together with subnormal HDL-C concentration, all contribute to the residual cardiovascular risk frequently observed on a background of statin treatment. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride.
Subsequent studies showed that individuals with loss-of-function mutations or variants in the PCSK9 gene not only displayed lifelong lower plasma levels of LDL-C but also were at lower risk of CVD [11,12,13]. These key findings drove the quest to elucidate PCSK9 biology with the ultimate hope of developing PCSK9-targeted therapeutics.

Proprotein convertase subtilisin/kexin type 9 biology
Intracellular levels of cholesterol in hepatocytes primarily reflect the combination of uptake of cholesterol contained in LDL and other lipoproteins, endogenous cholesterol synthesis, cholesterol conversion to bile acids, excretion of bile acids and biliary cholesterol, and secretion of nascent lipoproteins (principally very low-density lipoprotein). Circulating LDL binds to the LDL receptor on the hepatocyte surface, is endocytosed within clathrin-coated vesicles, trafficked intracellularly in the endosomal pathway, and subsequently degraded by lysosomes. The LDL receptor dissociates from the LDL particle at acid lysosomal pH, and then recycles back to the plasma membrane to bind additional LDL. Ultimate control of circulating LDL-C levels is exerted via two pathways: the sterol regulatory element binding protein-2 (SREBP-2) pathway, which is subject to regulation by intracellular cholesterol concentration and regulates expression of both the LDLR gene and the gene encoding PCSK9[3], and the inducible degrader of the LDL receptor (IDOL) pathway, which is LDL receptor-specific and under control of the liver X receptor transcription factor [14].

PCSK9 is a 692-amino acid serine protease, synthesized as an inactive zymogen (proPCSK9, about 72kDaltons); it is transformed by autocatalytic cleavage of the prodomain in the endoplasmic reticulum, thereby allowing entry into the secretory pathway. Whereas upregulation of LDLR by SREBP-2 increases LDL receptor availability and plasma clearance of LDL-C, upregulation of PCSK9 by the same transcription factor has the reverse effect, resulting in elevation of plasma LDL-C levels because of attenuated LDL receptor recycling (the reader is referred to recent reviews) [13*,15]. Upregulation of PCSK9 expression by SREBP-2 is equally detrimental for patients with primary hypercholesterolaemia and heterozygous familial hypercholesterolaemia [16]; importantly, enhanced PCSK9 expression counteracts the beneficial upregulation of LDL receptors by statin to a significant degree [13*,15].

In 2015, the fully human monoclonal antibodies alirocumab and evolocumab were the first PCSK9 therapeutics approved in Europe and the USA; a third, bococizumab, a humanized antibody, is in Phase III development, and has shown comparable LDL-C lowering response [17]. These injectable treatments are administered as either a 2-weekly or monthly regimen; the monthly dose for evolocumab is three-fold higher than the 2-weekly dose for equivalent LDL-C lowering [18]. Other approaches, including recombinant adnectins and RNA interference therapeutics [19], are at earlier stages of development. Antisense inhibition of PCSK9 has raised issues of safety [20]. This timely review aims to highlight the latest developments in the ongoing PCSK9 story.

TARGETING UNMET CLINICAL NEEDS
Familial hypercholesterolaemia
As discussed, familial hypercholesterolaemia is poorly managed even with best available treatment, and thus the likely highest patient priority for PCSK9 inhibitor therapy. Both alirocumab and evolocumab are highly effective in the setting of heterozygous familial hypercholesterolaemia (Table 1) [21*,22*,23]. In RUTHERFORD-2 (Reduction of LDL-C With PCSK9 Inhibition in Heterozygous Familial Hypercholesterolaemia Disorder Study-2) [21*], treatment with evolocumab (140 mg every 2 weeks or 420 mg monthly) against a background of statin ± ezetimibe resulted in placebo-corrected mean decreases in LDL-C of ~60–65%, with more than 60% of patients attaining LDL-C goal (<1.8 mmol/l or 70 mg/dl). Importantly, treatment response was similar irrespective of LDLR mutation status. Pooled data from the ODYSSEY familial hypercholesterolaemia I and II studies with alirocumab (75 mg titrating to 150 mg every 2 weeks depending on LDL-C response) showed a similar, sustained LDL-C lowering response [22*]. Even in severe familial hypercholesterolaemia (LDL-C levels >5 mmol/l or 200 mg/dl on maximally tolerated lipid-lowering therapy), ODYSSEY HIGH familial hypercholesterolaemia showed that 57% of these difficult-to-treat patients attained LDL-C goal (<2.6 mmol/l or 100 mg/dl) on alirocumab [23].

The treatment of homozygous familial hypercholesterolaemia, which is characterized by onset of symptomatic atherosclerotic vascular disease as early as the second decade of life, continues to present major challenges [24*]. In a proof-of-concept study with evolocumab, a wide range in LDL-C reduction was observed in six LDL receptor-defective patients (4–48% with 2-week dosing of 420 mg), whereas no reduction was seen in two LDL receptor-negative patients [25]. These findings were entirely
consistent with those in a statin-induced skin fibroblast system in vitro, in which alirocumab upregulated LDLR expression in receptor-defective homozygous familial hypercholesterolaemia fibroblasts, whereas no effect was seen in receptor-negative cells [26]. Subsequently, TESLA (Trial Evaluating PCSK9 Antibody in Subjects With LDL Receptor Abnormalities) Part B in homozygous familial hypercholesterolaemia (n = 49), showed that evolocumab (420 mg every 4 weeks for 12 weeks) lowered LDL-C by 30.9% (placebo-corrected) against a background of maximally tolerated lipid-lowering therapy, but excluding lipoprotein apheresis [27**]. In individuals with two different LDLR defective alleles, mean LDL-C lowering was higher (46.9%) [27**]. Yet even in responders, LDL-C levels remained markedly elevated (mean 7.0 mmol/l or 270 mg/dl), underlining the inherent difficulties in managing these severely affected patients [28]. Most recently, TAUSSIG (Trial Assessing long term USE of PCSK9 Inhibition in Subjects with Genetic LDL Disorders) showed sustained LDL-C lowering (by 20–25%) over 48 weeks of treatment with evolocumab in homozygous familial hypercholesterolaemia patients (95% with LDLR mutations). Lipoprotein apheresis slightly reduced the LDL-C lowering response [29]. Clearly, and given the excellent tolerability of PCSK9 inhibitors in these studies, PCSK9 inhibition presents a new therapeutic option in this serious disorder, provided that at least one allele is not LDLR negative.

To prevent the high risk of premature CHD associated with familial hypercholesterolaemia, early identification and initiation of optimal treatment, ideally in children and adolescents, is crucial [30**]. Yet to date, studies of PCSK9 inhibitors have excluded these key groups. HAUSER-RCT (Trial Assessing Efficacy, Safety and Tolerability of PCSK9 Inhibition in Paediatric Subjects With Genetic LDL Disorders), currently enrolling familial hypercholesterolaemia patients aged 10–17 years, is pivotal to assessment of the role of PCSK9 inhibition (evolocumab) in younger patients [31].

Other high cardiovascular risk patients
Both alirocumab and evolocumab have been extensively investigated in patients with established CVD or other cardiovascular risk factors, in whom LDL-C was inadequately controlled on statin therapy ± other lipid lowering treatment (Table 2) [32–44]. Overall, the studies show consistent LDL-C lowering (by on average ~50–65%) across the spectrum of high cardiovascular risk patients, with a durable response over at least 12 months. Moreover, a series of studies from the ODYSSEY programme showed that in statin-treated patients, adding alirocumab

| Reference | Trial lipid inclusion criteria | Treatment | Comparator | N (treated) | % LDL-C reduction versus placebo | % at LDL-C goal* |
|-----------|-------------------------------|-----------|------------|-------------|-------------------------------|-----------------|
| [21*]     | RUTHERFORD-2 (stable LLT including statin for ≥4 weeks) | Evolocumab 140 mg/2 weeks or 420 mg/month | Placebo | 329 | Mean at week 12: 59% (140 mg/2 weeks) 61% (420 mg/month) | Mean, weeks 10 and 12 60% (140 mg/2 weeks) 66% (420 mg/month) |
| [22]      | ODYSSEY FH I (maximally tolerated statin ± other LLT) | Alirocumab 75/150 mg every 2 weeks* | Placebo | 486 | LS mean at week 24: 58% 60% |
| [23]      | ODYSSEY FH II (LDL-C > 4.0 mmol/l or 160 mg/dl on maximally tolerated statin ± other LLT) | Alirocumab 150 mg/2 weeks | Placebo | 106 | LS mean at week 24: 46% |

FH, familial hypercholesterolaemia; LDL-C, low-density lipoprotein cholesterol; LLT, lipid-lowering therapy; LS, least squares; N, number of patients; RUTHERFORD-2, Reduction of LDL-C With PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder Study-2.

*LDL-C goal was defined as <1.8 mmol/l or 70 mg/dl except for ODYSSEY FH II where LDL-C goal was defined as <2.6 mmol/l or 100 mg/dl.

*Alirocumab dose was increased to 150 mg every 2 weeks at week 12 if LDL-C >1.8 mmol/l or 70 mg/dl at week 8.
| Reference | Trial | Patient population | N   | MAb dose regimen | Comparator regimen | % LDL-C reduction |
|-----------|-------|---------------------|-----|------------------|--------------------|-------------------|
| [32]      | LAPLACE-2 | Primary hypercholesterolaemia and mixed dyslipidaemia. Moderate to high intensity statin | 2067 | 140 mg/2 weeks or 420 mg/month | Ezetimibe 10 mg daily or placebo | Mean at weeks 10 and 12: 66–75% (140 mg/2 week) 63–75% (420 mg/month) 17–21% (ezetimibe) |
| [33]      | DESCARTES | Hyperlipidaemia, on diet with or without LLT | 901  | 420 mg/4 weeks | Placebo | Mean at week 52: 57%; 56% on diet alone 62% on ATOR 10 mg 57% on ATOR 80 mg 49% on ATOR 80 mg/EZE 10 mg |
| [34,35]   | OSLER | Primary hypercholesterolaemia, mixed dyslipidaemia or HeFH with or without LLT (70% on statin) | 4465 | 140 mg/2 weeks or 420 mg/month | Placebo | Mean week 12: 61% |
| [36]      | ODYSSEY COMBO I | Hypercholesterolaemia, on maximally tolerated statin ± other LLT | 316  | 75/150 mg every 2 weeks\(^a\) | Placebo | LS mean week 24: 46% |
| [37]      | ODYSSEY COMBO II | Hypercholesterolaemia, on maximally tolerated statin | 720  | 75/150 mg every 2 weeks\(^a\) | Ezetimibe 10 mg/day | LS mean week 24: 51% [alirocumab] 21% [ezetimibe] |
| [38]      | ODYSSEY OPTIONS I | Hypercholesterolaemia, on ATOR 20 or 40 mg | 355  | 75/150 mg every 2 weeks\(^a\) | Ezetimibe 10 mg/day or Switching to ROS 40 (ATOR 40 only) | LS mean week 24: 44–54% [alirocumab], 21–23% [ezetimibe], 4.8–5.0% [Switching to ROS], 21% [Ezetimibe] |
| [39]      | ODYSSEY OPTIONS II | Hypercholesterolaemia, on ROS 10–20 mg | 305  | 75/150 mg every 2 weeks\(^a\) | Ezetimibe 10 mg/day doubling ROS dose | LS mean week 24: 38–51% [alirocumab], 11–14% [ezetimibe], 16% [Ezetimibe] |
| [40]      | ODYSSEY CHOICE I | Hypercholesterolaemia, on maximally tolerated statin therapy or statin naïve or intolerant | 803  | 75/150 mg every 2 weeks\(^a\) or 300 mg/4 weeks | Placebo | LS mean week 24: statin-naïve 52%, on statin 59% (300 mg/4 week) |
| [41]      | ODYSSEY CHOICE II | Hypercholesterolaemia, on EZE, FEN or diet alone | 233  | 75/150 mg every 2 weeks\(^a\) or 150 mg/4 weeks | Placebo | LS mean week 24: 56% [150 mg/4 week] |
| [42]      | ODYSSEY LONG TERM | Hypercholesterolaemia, on maximally tolerated statin ± other LLT | 2341 | 150 mg/2 weeks | Placebo | Mean week 24: 62% |

\(^a\)Alirocumab dose was increased to 150 mg every 2 weeks at week 12 if LDL-C >1.8 mmol/l or 70 mg/dl at week 8, or not achieving LDL-C goal at week 8 (OPTIONS I and II).

ATOR, atorvastatin; EZE, ezetimibe; FEN, fenofibrate; HeFH, heterozygous familial hypercholesterolaemia; LDL-C, low-density lipoprotein cholesterol; LLT, lipid-lowering therapy; LS, least squares; MAb, monoclonal antibody; N, number of patients; ROS, rosuvastatin.

Trial acronyms: LAPLACE-2: LDL-C Assessment With Proprotein Convertase Subtilisin Kexin Type 9 Monoclonal Antibody Inhibition Combined With Statin Therapy 2; DESCARTES: Durable Effect of PCSK9 Antibody Compared with Placebo Study; OSLER: Open Label Study of Long Term Evaluation Against LDL-C Trial.

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resulted in significantly superior LDL-C lowering compared with adding ezetimibe [37–39], doubling the dose of statin [38,39], or switching to a higher-dose intensity statin [38]. In ODYSSEY CHOICE II, alirocumab added to nonstatin therapy (fenofibrate or ezetimibe) resulted in incremental LDL-C lowering compared with placebo [41]. Evidence of comparable LDL-C lowering in high cardiovascular risk patients with mixed dyslipidaemia [elevated LDL-C together with elevated triglycerides and/or low high-density lipoprotein cholesterol (HDLC)] is also available [45].

PCSK9 levels are known to be upregulated by both statins and fibrates [46]. Therefore, it is feasible that concomitant higher-intensity statin treatment may reduce the LDL-C lowering efficacy of PCSK9 inhibitors. However, exhaustive subgroup analysis of more than 4000 patients enrolled in the ODYSSEY study programme has demonstrated that concomitant high-intensity statin or nonstatin therapy did not reduce the response to alirocumab [47]; similarly the LAPLACE-2 (LDL-C Assessment With Proprotein Convertase Subtilisin Kexin Type 9 Monoclonal Antibody Inhibition Combined With Statin Therapy 2) study showed no difference in LDL-C reduction with evolocumab in low or high-dose statin groups [32]. Mechanically, this observation suggests that, under steady state conditions, there is a residual potential of up to ~50% of additional upregulation of LDL receptor activity with concomitant statin-mediated upregulation of PCSK9, and independently of statin dose. This residual potential appears accessible when plasma PCSK9 levels are reduced to less than 5% of baseline concentrations [48], even in the presence of a mutated allele of the LDLR gene as noted above for heterozygous familial hypercholesterolaemia.

**Statin intolerance**

Statin-intolerant patients represent a major high-risk group of concern. Two key studies showed benefit of PCSK9 monoclonal antibody therapy over ezetimibe, currently the primary option for this group [49,50]. Both studies were similar in the definition of patient population (unable to tolerate at least two statins) but differed in design. ODYSSEY ALTERNATIVE (alirocumab) included both a blinded 4-week placebo run-in period to exclude patients reporting muscle symptoms, and also a rechallenge atorvastatin arm, in addition to ezetimibe. Not surprisingly given the possibility of allocation to statin, myalgia rates were higher compared with those reported in GAUSS-2 (Goal Achievement After Utilizing an Anti-PCSK9 Antibody in Statin Intolerant Subjects -2) with evolocumab, although in long-term follow-up of ODYSSEY ALTERNATIVE, there was virtually no myalgia reported with alirocumab. Irrespective of these differences in protocol design, both studies showed a consistent LDL-C lowering response with the PCSK9 inhibitor as reported in other high-risk groups (55–60%), which was significantly superior to that for ezetimibe.

**MECHANISTIC INSIGHTS – IS IT ALL LOW-DENSITY LIPOPROTEIN CHOLESTEROL?**

*In vitro* and animal studies show that inhibition of PCSK9 both decreases lysosomal degradation of the LDL receptor and enhances receptor recycling, resulting directly in increased LDL receptor availability on the hepatocyte surface and upregulation of plasma clearance of LDL-C and apolipoprotein (apo) B [13*]. This mechanism has now been corroborated in human studies showing that PCSK9 inhibitor-mediated reduction in apo B levels in both intermediate-density lipoprotein and LDL is because of their enhanced fractional catabolic rate, particularly for LDL [51].

Elevated levels of lipoprotein a [Lp(a)] constitute a causal factor for accelerated CVD, as well as myocardial infarction, aortic valve stenosis, and ischaemic stroke [52,53*]. Given that Lp(a) is essentially refractory to both lifestyle intervention and statin treatment, we lack efficacious pharmacotherapeutic interventions that specifically target this atherothrombogenic particle. Importantly, PCSK9 inhibitors have been shown to reduce Lp(a) by 25–30% as a function of baseline level [54*,55*]. Although the mechanism of Lp(a) catabolism *in vivo* and the impact of PCSK9 on this process has been contentious, recent *in vitro* studies have overturned previous thinking that the LDL receptor does not play any role in Lp(a) catabolism. Indeed, under the supraphysiological LDL receptor availability and low circulating LDL-C levels resulting from PCSK9 inhibition, Lp(a) is a ligand of this receptor, and thus may contribute to Lp(a) catabolism and clearance [56]. Definitive proof is, however, awaited from *in vivo* studies. Finally, it is relevant that markedly low plasma levels of small dense LDL acceptor particles possessing apo B 100 in an optimal conformation for linkage to apo(a) may limit formation of Lp(a), thereby contributing to PCSK9 inhibitor-mediated reduction in Lp(a) levels.

**SAFETY**

Beyond efficacy, safety and tolerability are key considerations underpinning any novel therapy. Extensive evidence to date with alirocumab and evolocumab attests to a favourable safety profile, with similar low injection site reaction rates (~5%)
Importantly, data from ODYSSEY LONG TERM showed no increase in the incidence of adverse events in patients attaining very low LDL-C levels (<25 mg/dl or 0.65 mmol/l) [42]; correspondingly, OSLER (Open Label Study of Long Term Evaluation Against LDL-C Trial) showed no increase in the frequency of adverse events with decreasing LDL-C levels [34]. However, it should be emphasized that long-term exposure data are needed to definitively assess the benefit versus risk profile of these novel agents.

The possibility that PCSK9 inhibitors may induce neurocognitive effects has been queried ever since the US Food and Drug Administration directed their developers to monitor such effects and to consider neurocognitive testing in at least a subset of patients in ongoing trials [59]; EBBINGHAUS (Evaluating PCSK9 Binding antiBody Influence on coGnitive HeAlth in high cardiovascular risk Subjects), a substudy of FOURIER (Further cardiovascular OUtcomes Research with PCSK9 inhibitors In subjects with Elevated Risk), the evolocumab outcomes study, is addressing this [60]. The justification for this action probably dates from the statin era, although even here there is no definitive prospective evidence of any neurocognitive risks associated with statins [61]. Moreover, it is important to bear in mind that the PCSK9 monoclonal antibodies are large molecules and, therefore, do not normally cross the blood brain barrier.

Another issue involves possible effects on glucose homeostasis, as PCSK9 monoclonal antibody treatment will be coadministered with statins in most high cardiovascular risk patients. Evidence to date suggests a lack of detrimental effects. In the DESIR (Data from an Epidemiological Study on the Insulin Resistance syndrome) study, carriage of the PCSK9 p.R46L loss-of-function variant was not associated with any significant change in markers of glucose homeostasis in nondiabetic patients [62]; furthermore, these carriers were not at increased risk of new onset type 2 diabetes during a 9 year follow-up period. One year follow-up data from DESCARTES (Durable Effect of PCSK9 Antibody Compared with Placebo Study) showed no increased incidence of new onset type 2 diabetes in patients treated with evolocumab compared with placebo [63]. Clearly, further data both from ongoing trials and from postmarketing surveillance are required.

**UNANSWERED QUESTIONS**

**Cardiovascular disease outcomes**
The key question is whether PCSK9 monoclonal antibody therapy reduces composite endpoints for cardiovascular outcomes in high-risk patients receiving concomitant statin therapy. Indeed, it is pertinent that regulatory approval was given for alirocumab and evolocumab despite the lack of definitive outcomes evidence. There are encouraging findings from exploratory analyses of ODYSSEY LONG TERM (alirocumab, n=2341) and OSLER (evolocumab, n=4465), which indicate about 50% reduction in cardiovascular outcomes (using the same definitions as in the ongoing ODYSSEY OUTCOMES and FOURIER trials) over a treatment period of up to 78 weeks [34,42], although the possibility of positive bias because of failure to exploit the maximal potential of statins has been suggested [64]. Additionally, meta-analysis of 24 trials of PCSK9 monoclonal antibody therapy, including more than 10 000 patients, showed a 55% reduction in all-cause mortality, (odds ratio =0.45, 95% confidence interval =0.23–0.86, \( P =0.015 \)), as well as 50% reduction in cardiovascular mortality, and 51% reduction in myocardial infarction [65]. These findings are not, however, definitive and we eagerly await completion of the ongoing outcomes studies. Moreover, evidence from genetic studies of lifelong exposure to low LDL-C levels reaffirms the urgent need to start treatment early to optimize CVD benefit. Finally, these studies may also provide insights into the impact of PCSK9 inhibition in cerebrovascular disease and other high cardiovascular risk groups, as defined by guidelines (Fig. 2) [5], as well as definitive evaluation of the benefit/risk ratio.

**How will proprotein convertase subtilisin/kexin type 9 therapeutics be used in real clinical practice?**
The integration of PCSK9 monoclonal antibody therapy into clinical practice will undoubtedly be driven by the severity of cardiovascular risk. For patients with homozygous familial hypercholesterolaemia or severe familial hypercholesterolaemia and established CVD who fail to meet LDL-C targets with current therapy, there is a clear role for PCSK9 therapeutics; patients who are already at LDL-C goal, but remain at high risk because of plaque progression should also be considered. Additionally, there is a potential role for these novel agents in high-risk patients with established CVD who cannot tolerate statin therapy. The extent of clinical risk and the ability to meet cost constraints will no doubt exert major influence on clinical management.

**Benefits beyond low-density lipoprotein cholesterol lowering?**
Emerging data suggest future potential for PCSK9 inhibition in other clinical settings, most notably
sepsis [66], given that pathogen lipids, such as lipopolysaccharide, are cleared in part by LDL receptor-mediated hepatic uptake. In support of this hypothesis is evidence that PCSK9 loss-of-function genetic variants are associated with improved survival in septic shock patients, as well as decreased inflammatory cytokine response in both septic shock patients and in healthy volunteers after lipopolysaccharide administration. The opposite effects were observed in septic shock patients with PCSK9 gain-of-function variants [67]. Evaluation of the clinical efficacy of PCSK9 inhibition in the setting of sepsis, therefore, appears warranted.

**CONCLUSION**

PCSK9 inhibitors undoubtedly represent an effective strategy to address the unmet clinical needs of high cardiovascular risk patients. These agents efficaciously lower LDL cholesterol, but equally Lp(a); moreover, potential effects on triglyceride-rich lipoproteins, atherogenic remnants and even HDL particles cannot be excluded at this time [45]. There is a clear case for the use of PCSK9 inhibitors in severe familial hypercholesterolaemia, given obvious practical advantages over lipoprotein apheresis, although homozygous familial hypercholesterolaemia patients will continue to need the armamentarium of LDL-C lowering treatments. Definitive answers relating to impact on cardiovascular events, safety and tolerability await the conclusion of ongoing outcomes studies and post-marketing surveillance. Findings of these studies will be needed to inform health economics modelling, as ultimately, accessibility to such treatments will depend on budgetary constraints.

Finally, emerging evidence suggests potential for PCSK9 inhibition in other clinical settings such as infectious disease. The unique PCSK9 story looks set to continue for some time yet.

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