Reducing plasma levels of low-density lipoprotein cholesterol (LDL-C) remains the cornerstone in the primary and secondary prevention of cardiovascular disease. However, lack of efficacy and adverse effects mean that a substantial proportion of patients fail to achieve acceptable LDL-C levels with currently available lipid-lowering drugs. Over the last decade, inhibition of proprotein convertase subtilisin/kexin type 9 (PCSK9) has emerged as a promising therapeutic strategy to reduce residual cardiovascular disease risk. Binding of PCSK9 to the LDL receptor targets the receptor for lysosomal degradation. The recognition that inhibition of PCSK9 increases LDL receptor activity has led to the development of a number of approaches to directly target PCSK9. Numerous monoclonal antibodies against PCSK9 are currently being evaluated in phase 3 trials, involving various patient categories on different background lipid-lowering therapies. Current evidence shows reductions in LDL-C levels of up to 70% may be achieved with PCSK9 inhibition, independent of background statin therapy. This review examines the most recent evidence and future prospects for the use of PCSK9 inhibitors in the prevention of cardiovascular disease.
The structure and function of PCSK9

Synthesis and structure

PCSK9, found at chromosome 1p32, is 22 kb in length, with 12 exons that encode a 692-amino acid protein [11]. It is a protease K-like enzyme, belongs to the secretory subtilase family and is primarily synthesised and secreted by hepatocytes [12, 13]. The synthesis of PCSK9 is up-regulated by sterol-regulatory-element-binding protein-2 (SREBP-2), a transcription factor that regulates PCSK9 expression by binding to the sterol-regulatory element in the promoter region of the gene [14]. SREBP-2 also increases LDL receptor and cholesterol synthesis, via the activation of genes encoding key enzymes involved in cholesterol homeostasis, including HMG-CoA reductase [15]. It is activated by low intracellular cholesterol concentrations. SREBP-2 and PCSK9 expression is suppressed in fasting mice fed a cholesterol-rich diet [16]. Prolonged fasting in animals and humans, however, also causes a decrease in PCSK9 and SREBP-2 activity [17]. In addition, in vivo evidence suggests a possible role for insulin in increasing the expression of PCSK9 [18].

The PCSK protein product is comprised of a N-terminal signal peptide, prodomain, catalytic domain, hinge region, and cysteine-rich C-terminal domain [13, 19]. Following the removal of the signal peptide domain, PCSK9 is synthesised as a ~74 kDa zymogen, which undergoes autocatalytic cleavage in the endoplasmic reticulum and Golgi body, to generate a pro-domain fragment and ~62 kDa mature protein, which remain strongly associated to one another [20–22].

LDL receptor cycling

The first 8 members of the PCSK family, PCSK 1–8, are serine proteases involved in the processing of inactive precursor proteins to generate functional and bioactive peptides, polypeptides and hormones, which play important roles in regulating growth and metabolism [23–25]. In contrast, PCSK9 plays a crucial role in the regulation of LDL receptor recycling [26]. The PCSK9 complex binds to the epidermal growth factor A (EGF-A) domain of the LDL receptor, leading to the lysosomal degradation of the latter and reduced clearance of circulating LDL-C. Extra-hepatic actions of PCSK9 include enhancement of chylomicron secretion and regulation of enterocyte cholesterol balance [13]. Moreover, data from experimental models suggest that the role of PCSK9 extends beyond lipid homeostasis; it is implicated as a regulator of glucose metabolism, liver regeneration and susceptibility to hepatitis C virus infection [27–30].

In mouse models, the accumulation of cholesteryl esters in aortic atherosclerotic lesions was markedly reduced by PCSK9 inactivation [31]. Conversely, overexpression of PCSK9 induced an excess burden of atherosclerosis. In LDLR-deficient mice, knockdown or overexpression of PCSK9 had no significant effects on cholesteryl ester accumulation or atheromatous plaque size. This study strongly suggested that the process by which PCSK9 enhances atherosclerosis is primarily mediated by its action on the LDLR [31]. Figure 1 displays normal, physiological LDLR recycling.

In humans studies, PCSK9 loss-of-function mutations have been associated with reductions in LDL-C and cardiovascular events [32]. Conversely, those with high levels of PCSK9 have higher level of plasma LDL-C and significantly increased lifetime CVD risk [32]. Gain-of-function mutations on PCSK9 are associated with a severe form of autosomal dominant hypercholesterolemia, phenotypically indistinguishable from FH due to LDL-receptor mutations [32].

Regulation

PCSK9 concentrations demonstrate a diurnal rhythm synchronous to cholesterol synthesis, with changes of ±15% from the mean value [33]. PCSK9 synthesis also induced by insulin and repressed by glucagon in rodents [18]. In healthy humans, PCSK9 levels are demonstrably reduced with fasting (decreasing 60 % over 36 h), and increase in the post-prandial period, suggesting a similar effect [33–35]. In addition, PCSK9 is positively controlled by the oxysterol-activated liver X receptor (LXR) [18, 36].

PCSK9 circulates in plasma in three main forms [37]. When secreted, PCSK9 exists as a monomer, but can self-
associate into di- and trimeric complexes, facilitated by the catalytic domain. It is present in free and protein-bound forms in human plasma, with 40% of circulating PCSK9 exclusively associated with LDL [16]. LDL-bound PCSK9 has diminished LDL receptor-binding activity. It has been proposed that this is a regulatory mechanism, by which higher plasma concentrations of LDL results in a greater proportion of LDL-bound PCSK9, thereby inhibiting PCSK9-mediated degradation of the LDL receptor [16]. In vitro evidence suggests that self-associated di-/trimers have enhanced LDL receptor-binding and degrading activity, compared with the monomer form [38]. PCSK9 also circulates as a 55 kDa furin-cleaved inactive fragment, resulting from the cleavage of the 62 kDa protein: mutations in the mature PCSK9 protein have been associated with increased or decreased susceptibility to furin cleavage, leading PCSK9 loss-of-function and gain-of-function phenotypes [22].

**Mechanism of action**

PCSK9 acts primarily as a soluble protein, targeting degradation of the membrane-bound LDLR by extracellular binding via rerouting to the lysosomal pathway [39]. At the molecular level, PCSK9 blocks the LDLR in an extended (open) conformation. This is achieved when the catalytic domain of PCSK9 (aa153–421) and the EGF-A domain of LDLR (aa314–355) bind [40]. This failure of the receptor to adopt a closed conformation results in a slowed recycling to the plasma membrane and subsequent degradation. LDL-receptors—like PCSK9—are particularly abundant in the liver, the primary organ responsible for clearance of plasma LDL. As the number of LDL-receptors on the surface of liver cells determines the rate of LDL removal from the bloodstream, PCSK9 presented an appealing target to beneficially modulate lipid homeostasis. Figure 2 illustrates the mechanism of action of PCSK9.

Impelled by promising pre-clinical evidence, the clinical development of therapeutic inhibitors of PCSK9 has progressed rapidly, with promising results reported from phase 2 and 3 clinical studies, in statin-intolerant and familial hypercholesterolemia patients, with sub-optimal LDL-C levels.

**PCSK9 inhibitors**

**Inhibition strategies**

Several strategies have been proposed for targeting PCSK9. Messenger RNA (mRNA) knockdown approaches, which include the use of PCSK9 antisense oligonucleotides, have been evaluated in animal models. Antisense oligonucleotides administered to mice reduced PCSK9 expression by >90% and lowered plasma cholesterol levels by 53% [41, 42]. A single intravenous injection of PCSK9 RNA interference (RNAi) delivered in lipidoid nanoparticles to cynomolgus monkeys reduced plasma PCSK9 and LDL-C levels (by 70 and 56%, respectively) [43]. However, the use of monoclonal antibodies (mAb), which interfere with the interaction of the PCSK9 catalytic domain and LDLR, is particularly promising [44].
nonhuman primates, intravenous infusion of mAb1 (3 mg kg\(^{-1}\)), which is specific for the catalytic domain of PCSK9, resulted in marked (80 %) reduction in plasma LDL-C [45].

PCSK inhibition may yield non-LDL-lowering, pleiotropic effects. High levels of lipoprotein(a) are an independent predictor of cardiovascular mortality, even in statin-treated patients with low LDL-C [46]. PCSK9 inhibitors reduce lipoprotein(a) by approximately 30 %. Such an effect is not observed with statin- or ezetimibe-mediated upregulation of LDL receptor activity (as lipoprotein(a) is not cleared by LDLR-dependent mechanisms, and is mainly regulated by hepatic secretion) [47]. Thus, PCSK9 inhibition as a therapeutic strategy has theoretical advantages beyond LDL-C lowering, raising the possibility that cardiovascular outcomes may be additionally favourable. Figure 3 displays the mechanism of action of PCSK9 mAb, in the presence of a statin.

In clinical studies, three monoclonal antibodies have demonstrated significant promise: evolocumab (AMG-145), alirocumab (SAR236553/REGN727) and bococizumab; the latter of which is in the early stages of development. Table 1 lists PCSK9 inhibitors in development.

**Evolocumab**

**Evolocumab in primary hypercholesterolemia**

Evolocumab is a fully human monoclonal antibody inhibitor of PCSK9. In the Monoclonal Antibody Against PCSK9 to Reduce Elevated LDL-C in Patients Currently Not Receiving Drug Therapy for Easing Lipid Levels (MENDEL) trial, 406 patients with hypercholesterolaemia and statin intolerance were randomly assigned to evolocumab 70, 105 and 140 mg every 2 weeks; evolocumab 280, 350 and 420 mg every 4 weeks; placebo every 2 weeks or every 4 weeks, or ezetimibe once-daily. Evolocumab reduced LDL-C concentrations in all dose groups, with the maximal effect for the regimen of 140 mg every 2 weeks (~51 %) and no reported treatment-related adverse events [48].

MENDEL-2 evaluated the efficacy, safety and tolerability of evolocumab compared with placebo and oral ezetimibe in 614 patients with hypercholesterolemia (LDL-C 100–190 mg dL\(^{-1}\) or 2.6–4.9 mmol L\(^{-1}\)) [49]. Patients 18–80 years of age with Framingham risk scores ≥10 % were randomised to one of six groups: (i) oral placebo and sub-cutaneous (SC) placebo fortnightly; (ii) oral placebo and SC placebo monthly; (iii) ezetimibe and SC placebo fortnightly; (iv) ezetimibe and SC placebo monthly; (v) oral placebo and evolocumab 140 mg fortnightly; or (vi) oral placebo and evolocumab 420 mg monthly. Evolocumab treatment produced greatest reductions in LDL-C from baseline, by 55–57 % more than placebo and 38–40 % more than ezetimibe (both \(p < 0.001\)).

In the LDL-C Assessment With PCSK9 monoclonal Antibody Inhibition Combined With Statin therapy (LAPLACE TIMI-57), 631 patients with hypercholesterolemia on statins were randomised to different regimens of evolocumab, with varying dosages and intervals of administration: 70 mg, 105 mg, and 140 mg or matching...
placebo every 2 weeks; or 280 mg, 350 mg, and 420 mg or matching placebo every 4 weeks [50]. At week 12, the mean LDL-C concentration reduction was dose-dependent, ranging from 41.8 to 66.1 % every 2 weeks, and from 41.8 to 50.3 % every 4 weeks [51]. The LAPLACE-2 trial assessed the response to addition of evolocumab (140 mg every 2 weeks or 420 mg monthly) vs. placebo, to moderate- or high-intensity statin therapy in 1896 patients with hyperlipidaemia [52]. The trial observed that evolocumab reduced plasma LDL-C concentrations by 66–75 %, vs. placebo, at the mean of weeks 10 and 12. Evolocumab added to statin therapy resulted in additional LDL-C lowering.

More recently, the Durable Effect of PCSK9 Antibody CompARed wiTh placEbo Study (DESCARTES) placed patients into one of four background lipid-lowering strategies (dietary changes alone, dietary changes plus atorvastatin 10 mg, dietary changes plus atorvastatin 80 mg, and dietary changes plus atorvastatin 80 mg and ezetimibe 10 mg) based on their LDL-C levels and cardiovascular risk [53]. Individuals with LDL-C ≥75 mg/dl were randomised to receive monthly SC evolocumab 420 mg or placebo. The mean reduction in LDL-C from baseline in the evolocumab group was 57.0 ± 2.1 % (p < 0.001 vs. placebo). The mean reduction was 55.7 ± 4.2 % among patients who underwent dietary

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**Table 1 | PCSK9 Inhibitors undergoing preclinical and clinical evaluation**

| Pharmaceutical company | Drug class | Agent | Phase |
|-------------------------|------------|-------|-------|
| Sanofi/Regeneron        | Human mAb  | Alicocumab (SAR236553/REGN727) | 3     |
| Amgen                   | Human mAb  | Evolocumab (AMG 145) | 3     |
| Pfizer/Rinat            | mAb        | Bococizumab (RN316) | 3     |
| Novartis                | mAb        | LGT-209 | 2     |
| Roche/Genetech          | mAb        | RG7652 | 2     |
| Alnylam Pharmaceuticals/The Medicines Company | siRNA oligonucleotide | ALN-PCS02 | 1     |
| Bristol-Myers Squibb/Adnexus | Monobody | BMS-962476 | 1     |
| Idera Pharmaceuticals   | Antisense Oligonucleotide | TBD | PC |
| Merck                   | mAb        | 1D05-IgG2 | PC |
| Schering-Plough         | Mimetic peptides | LDL EGF-AB peptide fragment | PC |

*mAb monoclonal antibody, PC pre-clinical, siRNA small interfering ribonucleic acid*
changes among those who received 10 mg of atorvastatin, 56.8 ± 5.3 % among those who received 80 mg of atorvastatin, and 48.5 ± 5.2 % among those who received a combination of 80 mg of atorvastatin and 10 mg of ezetimibe (p < 0.001 for all comparisons). Evolocumab treatment also significantly reduced levels of apolipoprotein B, lipoprotein(a) and triglycerides.

Evolocumab in familial hypercholesterolemia

In the Reduction of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder (RUTHERFORD) trial, 167 patients with heterozygous FH (HeFH) and poorly-controlled LDL-C (≥2.6 mmol L⁻¹ or 100 mg dL⁻¹) despite maximally-tolerated statin therapy, were randomised 1:1:1 to receive evolocumab 350 mg, 420 mg or matched placebo, every four weeks. A substantial reduction in LDL-C was observed (43 % for 350 mg vs. 55 % for 420 mg) in addition to that due to high-intensity statin therapy [54]. RUTHERFORD-2 subsequently evaluated evolocumab in combination with other lipid-lowering therapies in patients with HeFH [55]. In total, 331 HeFH patients unable to achieve target LDL-C (defined as per RUTHERFORD) despite maximally-tolerated statin alone, or in combination with ezetimibe, were randomised 2:1 to receive evolocumab 140 mg every 2 weeks, evolocumab 420 mg monthly, or matched placebo, for 12 weeks. Based on the Simon Broome criteria, 80 % of participants had definite FH; 20 % had probable FH. All patients received a statin; two-thirds received ezetimibe. Both schedules demonstrated significant reduction in mean LDL-C at week 12 (59.2 % for 140 mg every 2 weeks vs. 61.3 % for 420 mg monthly; both p < 0.0001).

Classically, homozygous FH (HoFH) patients were thought to have dual null mutations, conferring no LDL receptor activity, and thus would not be expected to respond to PCSK9 inhibition (which is LDL receptor-dependent). Indeed, a small proportion of FH patients are true genetic homozygotes, with identical null or loss-of-function mutations in both alleles of the affected gene. However, advanced genetic profiling has demonstrated that most patients with homozygous loss-of-function mutations are actually compound heterozygotes, with different receptor mutations. As such, HoFH patients may be phenotypically stratified using fibroblast culture; those with <2 % of LDL uptake are receptor negative; those with 2–25 % are receptor defective, compared to wild-type controls [56]. Thus, patients with HoFH may still have a degree of functional LDL receptor activity, which is associated with severity of LDL cholesterol elevation, and may be modulated via PCSK9 inhibition. Indeed, in the recent Trial Evaluating PCSK9 Antibody in Subjects With LDL Receptor Abnormalities (TESLA) Part B study, 50 patients with HoFH, on stable lipid-lowering therapy and not on lipoprotein apheresis, received evolocumab 420 mg monthly, in addition to statin therapy and other lipid-lowering medications [57]. Indeed, TESLA demonstrated that in the Evolocumab-treated HoFH patients, LDL-C was reduced by 31 % from baseline at week 12 compared with placebo (p < 0.0001); no serious adverse side effects were noted.

Evolocumab in statin-intolerant patients

With infrequent reports of adverse effects, PCSK9 inhibitors have been heralded as a potentially effective alternative treatment option for those who are statin-intolerant. Muscle-related side effects (MRSE) are the commonest reason given for discontinuation of statins. Worldwide, the incidence of myopathy is 1.5–5 % of statin-treated patients, although this is highly-dependent on the definition used [58]. One study found that mild-to-moderate muscular symptoms occurred more frequently in patients treated with high-dose statins in clinical practice (in 10.5 %), compared to randomised trials [59]. However, another reported that most patients discontinuing statins due to MRSE that are re-challenged demonstrate good tolerance long-term [60]. Despite the uncertainties regarding the true incidence of MRSE, there is a clear clinical need for alternative therapies in patients at high cardiovascular risk, with more severe degrees of myotoxicity [58].

The Goal Achievement After Utilizing an Anti-PCSK9 Antibody in Statin Intolerant Subjects (GAUSS) study aimed to establish whether there was an advantage to evolocumab over ezetimibe in this context [61]. In the GAUSS trial, 160 patients with statin intolerance were randomised to 5 groups: evolocumab alone at 280, 350, 420 mg, evolocumab at 420 mg with 10 mg ezetimibe once-daily, or 10 mg ezetimibe plus placebo once-daily. Statin intolerance was defined as the inability to tolerate at least one statin at any dose, or an increase in dose, because of intolerable myalgia (muscle pain, soreness, weakness, or cramps) or myopathy (myalgia plus elevated creatine kinase) and having symptom improvement or resolution with statin discontinuation. The administration of evolocumab was significantly associated with a reduction in LDL-C levels, ranging from 40 to 65 %, with good tolerability; myalgia was reported in: 7.4 % receiving evolocumab alone, 20 % receiving the evolocumab and ezetimibe combination, and 3.1 % receiving ezetimibe and placebo [61]. GAUSS-2 assessed statin-intolerant hyperlipidaemic patients [62, 63]. Intolerance was defined as inability to tolerate any dose, or increase the dose above the smallest tablet strength, because of intolerable muscle-related side effects. Evolocumab (140 mg every 2 weeks or 420 mg monthly) reduced LDL-C from baseline by 53 and
A total of 103 patients with LDL-C 2.6–4.9 mmol/L, otherwise receiving statins or other lipid-lowering therapy, were enrolled in the ODYSSEY-MONO trial evaluated the safety and efficacy of alirocumab as monotherapy in comparison with ezetimibe, over 24 weeks in patients with primary hypercholesterolemia and moderate cardiovascular risk, not otherwise receiving statins or other lipid-lowering therapy. The ODYSSEY-MONO trial demonstrated that alirocumab reduced the plasma concentration of LDL cholesterol by 61%, from a median of 120 mg dL\(^{-1}\) (3.1 mmol L\(^{-1}\)) to 48 mg dL\(^{-1}\) (1.2 mmol L\(^{-1}\); \(p < 0.001\)). The rate of a composite cardiovascular endpoint (defined as death, acute coronary syndrome, heart failure, stroke or a transient ischaemic attack) at 1 year was reduced from 2.18% in the standard-therapy group to 0.95% in the alirocumab group [Hazard Ratio (HR) 0.47; 95% confidence interval (95% CI) 0.28–0.78; \(p = 0.003\)]. A large proportion of these patients were receiving statin therapy at baseline (69.7% of alirocumab-treated patients vs. 70.9% of those receiving placebo), though no conclusions are drawn regarding the efficacy of alirocumab over and above statin therapy. Table 2 displays phase 2 studies evaluating evolocumab.

**Evolocumab and cardiovascular outcomes**

In the OSLER (Open Label Study of Long Term Evaluation Against LDL-C) trial, 4465 patients were randomised to receive either evolocumab 420 mg monthly, or 140 mg every two weeks, and followed up for a median of 11.1 months. The results demonstrated that evolocumab reduced mean LDL-C from a baseline of 80 mg dL\(^{-1}\) (5.2 mmol L\(^{-1}\)) to 70 mg dL\(^{-1}\) (4.5 mmol L\(^{-1}\); \(p < 0.001\)). The phase III, double-blind, double-dummy ODYSSEY-MONO trial evaluated the safety and efficacy of evolocumab as monotherapy in patients with primary hypercholesterolemia and additional CVD risk factors [66, 67]. In ODYSSEY-COMBO II, alirocumab lowered LDL-C levels significantly more than ezetimibe, at both week 24 (50.6% vs. 20.7% respectively; \(p < 0.0001\)) and 52 (49.5% vs. 18.3% respectively; \(p < 0.001\)). In addition, more alirocumab-treated than ezetimibe-treated patients achieved target LDL-C levels (≤1.8 mmol L\(^{-1}\), ≤70 mg dL\(^{-1}\)) by week 24 (77 vs. 45.6%; \(p < 0.0001\)). The ODYSSEY-OPTIONS studies demonstrated that the addition of alirocumab to statin regimens produced significantly greater LDL-C reductions than the addition of ezetimibe, doubling of statin dose, or switch to high-potency agent such as rosvastatin [68, 69].

**Alirocumab in familial hypercholesterolemia**

Alirocumab is a fully human monoclonal antibody to PCSK9. Phase II trials demonstrated that as monotherapy, alirocumab can reduce LDL-C as much as intensive statin treatment [64]. The phase III, double-blind, double-dummy ODYSSEY-MONO trial evaluated the safety and efficacy of alirocumab as monotherapy in comparison with ezetimibe, over 24 weeks in patients with primary hypercholesterolemia and moderate cardiovascular risk, not otherwise receiving statins or other lipid-lowering therapy [65]. A total of 103 patients with LDL-C 2.6–4.9 mmol L\(^{-1}\) (100–190 mg dL\(^{-1}\)), and 1–5% 10-year risk of fatal cardiovascular events (estimated via the Systematic Coronary Risk Evaluation [SCORE] tool) were randomised to receive either ezetimibe 10 mg or alirocumab, with the aim to achieve target HDL-C using the minimum effective dose of anti-PCSK9 antibody. Alirocumab was initially self-administered at a dose of 75 mg every 2 weeks, and uptitrated to 150 mg if LDL-C at week 8 was >1.8 mmol L\(^{-1}\) (70 mg dL\(^{-1}\)). Mean LDL-C reductions of 47% with alirocumab vs. 16% with ezetimibe were observed (intention-to-treat analysis; \(p < 0.0001\); 54 vs. 17%, on-treatment analysis; \(p < 0.0001\)). Prior to up-titration, alirocumab 75 mg every 2 weeks reduced LDL-C by 53%, indicating low-dose alirocumab is sufficient to provide 50% LDL-C reduction in the majority of patients.

The ODYSSEY-COMBO trials evaluated the efficacy of alirocumab in addition to maximally-tolerated daily statin therapy vs. ezetimibe, in patients with hypercholesterolemia and additional CVD risk factors [66, 67]. In ODYSSEY-COMBO II, alirocumab lowered LDL-C levels significantly more than ezetimibe, at both week 24 (50.6% vs. 20.7% respectively; \(p < 0.0001\)) and 52 (49.5% vs. 18.3% respectively; \(p < 0.001\)). In addition, more alirocumab-treated than ezetimibe-treated patients achieved target LDL-C levels (≤1.8 mmol L\(^{-1}\), ≤70 mg dL\(^{-1}\)) by week 24 (77 vs. 45.6%; \(p < 0.0001\)). The ODYSSEY-OPTIONS studies demonstrated that the addition of alirocumab to statin regimens produced significantly greater LDL-C reductions than the addition of ezetimibe, doubling of statin dose, or switch to high-potency agent such as rosvastatin [68, 69].
| Author, trial name (reference) | Year | Comparator | Study group | n  | Evolocumab dose(s) | Percentage change vs. placebo group |
|-------------------------------|------|------------|-------------|----|-------------------|-----------------------------------|
|                               |      |            |             |    |                   | LCL-C    | HDL-C     | Non-HDL | TG      | ApoB | Lp(a) |
| Giugliano et al., LAPLACE-TIMI 57 [50] | 2012 | Statin ± ezetimibe LDL-C > 85 mg mL<sup>-1</sup> on-treatment | 236 | 70 mg, 105 mg, 140 mg two-weekly | −41.8 to −66.1 | 6.6 to −8.1 | −38.4 to −61.4 | −18.1 to −33.7 | −34.7 to −56.4 | NA | NA |
|                               |      |            |             | 238 | 280 mg, 350 mg, 420 mg four-weekly | −41.8 to −50.3 | 1.6 to 5.5 | −37.8 to −47.6 | −13.4 to −19.4 | −34.4 to −42.0 | NA | NA |
| Koren et al., MENDEL [48]     | 2012 | Placebo only 100 ≤ LDL-C < 189 mg dL<sup>-1</sup> | 135 | 70 mg, 105 mg, 140 mg two-weekly | −37.3 to −47.2 | 4.2 to 10.2 | −35.1 to −45.2 | −7.4 to −12.0 | −32.3 to −44.2 | −11.1 to −29.3 |
|                               |      |            |             | 136 | 280 mg, 350 mg, 420 mg four-weekly | −43.6 to −52.5 | 3.3 to −5.8 | −37.7 to −47.1 | −1.7 to −5.3 | −33.2 to −42.5 | −21.6 to −29.2 |
| Raal et al., RUTHERFORD [54]  | 2012 | Statin ± ezetimibe HeFH; LDL-C ≥ 100 mg dL<sup>-1</sup> on-treatment | 111 | 350 mg, 420 mg four-weekly | −43.8 to −55.2 | 6.8 to 7.8 | −41.8 to −53.5 | −15.0 to −19.9 | −34.8 to −46.2 | −23.1 to −31.5 |
| Sullivan et al., GAUSS [61]   | 2012 | Statin, ezetimibe or other agent Statin intolerance, LDL-C ≥ 100 mg dL<sup>-1</sup> | 95  | 280 mg to 420 mg four-weekly | −26.0 to −35.9 | 6.6 to 8.5 | 24.8 to −33.6 | −8.7 to −13.8 | −21.4 to −29.9 | −12.4 to −18.0 |
|                               |      |            |             | 30  | 420 mg every four-weekly | −47.3 to −6.0 | 13.1 to −9.1 | −44.8 to −33.6 | −36.9 to −29.9 | −21.2 | NA |
| Hirayama et al., YUKAWA-1 [85] | 2014 | Statin ± ezetimibe High CVD risk, LDL-C ≥ 116 mg dL<sup>-1</sup> | 101 | 70 mg to 140 mg two-weekly | −52.9 to −68.9 | 4.4 to 9.1 | −49.5 to −62.6 | −14.3 to −16.6 | −46.8 to −60.7 | −41.5 to 50.6 |
|                               |      |            |             | 104 | 280 mg to 420 mg four-weekly | −58.2 to −63.9 | 13.2 to 16.3 | −53.5 to −58.1 | −17.1 to −20.2 | −32.3 to −31.3 | NA |

Values in table represent percentage (%) change in lipid parameters.

ApoB apolipoprotein B, HDL-C high-density lipoprotein cholesterol, HeFH heterozygous familial hypercholesterolaemia, LDL-C low-density lipoprotein cholesterol, Lp(a) lipoprotein (a), mg milligram, n number, NA not available, TG triglycerides.

See main text for full explanation of trial abbreviations. To convert stated LDL-C values from mg dL<sup>-1</sup> to mmol L<sup>-1</sup> divide presented value by 38.67.
Table 3 Completed phase II trials of alirocumab

| Author (reference) | Year | Comparator | Study group | n | Evolocumab dose(s) | Percentage change vs. placebo group |
|--------------------|------|------------|-------------|---|-------------------|-----------------------------------|
| McKenney et al. [78] | 2012 | Atorvastatin 10, 20 or 40 mg | LCL-C ≥ 100 mg dL⁻¹ on-treatment | 92 | 50 mg, 100 mg, 150 mg two-weekly | Percentage change vs. placebo group |
|                    |      |            |             |    |                   | LCL-C  HDL-C Non-HDL TG ApoB Lp(a) |
|                    |      |            |             |    | 50 mg, 100 mg, 150 mg | to to to to to |
|                    |      |            |             |    | two-weekly        | −34.5 5.1 to 7.7 −31.4 to −15.2 to −29.5 to −13.3 to |
|                    |      |            |             |    | 200 mg, 300 mg four-weekly | to to to to to |
|                    |      |            |             |    |                    | −38.1 7.3 to 9.5 −35.2 to −18.1 to −30.9 to −7.9 to |
|                    |      |            |             |    |                    | −42.6 to −38.5 to −20.5 to −35.3 to −16.7 to |
| Roth et al. [77]   | 2012 | Atorvastatin 10 or 80 mg | LCL-C ≥ 100 mg dL⁻¹ on-treatment | 60 | 150 mg two-weekly | Percentage change vs. placebo group |
|                    |      |            |             |    |                   | LCL-C  HDL-C Non-HDL TG ApoB Lp(a) |
|                    |      |            |             |    |                   | −48.9 9.4 to 12.8 −46.0 to −28.2 to |
|                    |      |            |             |    | 150 mg four-weekly | to to to to to |
|                    |      |            |             |    |                    | −55.9 9.4 to −12.8 to −46.0 to −28.2 to |
| Stein et al. [64]  | 2012 | Statin ± ezetimibe | HeFH; LCL-C ≥ 100 mg dL⁻¹ on-treatment | 31 | 150 mg two-weekly | Percentage change vs. placebo group |
|                    |      |            |             |    |                   | LCL-C  HDL-C Non-HDL TG ApoB Lp(a) |
|                    |      |            |             |    |                   | −18.2 4.3 to 7.8 −15.5 to −6.2 to −14.5 to −3.54 to |
|                    |      |            |             |    | 150 mg four-weekly | to to to to to |
|                    |      |            |             |    |                    | −31.9 7.8 to −27.6 to −22.0 to −11.4 to |
|                    |      |            |             |    | 300 mg four-weekly | to to to to to |
|                    |      |            |             |    |                    | −57.3 10.1 to −46.6 to −5.7 to −43.8 to −19.47 to |

Values in table represent percentage (%) change in lipid parameters

ApoB apolipoprotein B, HDL-C high-density lipoprotein cholesterol, HeFH heterozygous familial hypercholesterolaemia, LDL-C low-density lipoprotein cholesterol, Lp(a) lipoprotein (a), mg milligram, n number, NA not available, TG triglycerides. See main text for full explanation of trial abbreviations.

To convert stated LDL-C values from mg dL⁻¹ to mmol L⁻¹ divide presented value by 38.67

L⁻¹ or <100 mg dL⁻¹ and 81 vs. 9%; p < 0.0001 for LDL-C < 1.8 mmol L⁻¹ or <70 mg dL⁻¹). Most recently, these results have been reported to be maintained up to 78 weeks of treatment, with good tolerance [74]. Table 3 displays phase 2 studies evaluating alirocumab.

The safety of PCSK9 inhibition

So far, the clinical experience with monoclonal antibodies directed toward PCSK9 suggests that they are safe and well-tolerated, with no major safety issues and no evidence of serious drug-related adverse events [75]. The most common adverse events were nasopharyngitis, upper respiratory tract infections, influenza-like symptoms and back pain; injection site reactions were infrequent (<2 and <4 % of alirocumab and ezetimibe-treated patients, respectively) [76]. Isolated reports of adverse effects include: generalised pruritus after the first dose of alirocumab [64], delayed hypersensitivity-type reaction with rash, 12 days following the second injection of alirocumab [77], and a case of cutaneous leucocytoclastic vasculitis reported 9 days after initiation of alirocumab [78]. All of these patients responded well to withdrawal of the trial drug. Regarding completed phase III trials, in GAUSS-2, MRSE occurred in 12 % of evolocumab-treated, and 23 % of ezetimibe-treated patients [62]; in LAPLACE-2, adverse events were reported in 36, 40, and 39 % of evolocumab-, ezetimibe- and placebo-treated patients, respectively [52]. None of the evolocumab-treated patients developed serious adverse reactions. However, elevations in creatine kinase (CK) of 3–10 times the upper limit of normal have been reported in a total of 12 study drug-treated, and 4 placebo-treated patients. No deaths due to serious adverse events have been reported in PCSK9 clinical trials to date. Table 4 displays selected phase 3 studies of anti-PCSK9 mAbs.

One putative concern regarding this new class of cholesterol-lowering drugs is the potential for hypochondroplasia-associated adverse effects, such as cognitive impairment. Indeed, even allowing for the technical difficulties of accurate LDL-C measurement at severely low levels, many subjects in phase 2 trials reached very low cholesterol concentrations [80]. The identification of rare patients with double loss of function (LOF) mutations in the PCSK9 gene provides some reassurance, however. Such individuals, who have very low plasma PCSK9 and LDL-cholesterol concentrations, appear healthy and without cardiovascular or neurocognitive impairment [81]. Of these patients, who have very low plasma PCSK9 and LDL-cholesterol concentrations,
Table 4 Selected phase III clinical trials evaluating alirocumab and evolocumab

| Author, trial name (reference) | Year | n   | Agent          | Population and study design                                                                 | FU (w) | Percentage change vs. placebo group |
|-------------------------------|------|-----|----------------|-------------------------------------------------------------------------------------------|--------|------------------------------------|
|                              |      |     |                |                                                                                           |        | LDL-C                             |
|                              |      |     |                |                                                                                           |        | ApoB                              |
|                              |      |     |                |                                                                                           |        | Non-HDL-C                         |
|                              |      |     |                |                                                                                           |        | TG                                |
|                              |      |     |                |                                                                                           |        | HDL-C                             |
|                              |      |     |                |                                                                                           |        | Lp(a)                             |
| Farnier et al., ODYSSEY MONO  | 2014 | 103 | Alirocumab     | Patients with hypercholesterolemia on no statins vs. ezetimibe                             | 24     | -31.6 -25.8 -25.5 -1.2 4.4         |
| [65]                         |      |     |                |                                                                                           |        | -4.4                              |
| Kereiakes et al., ODYSSEY     | 2015 | 311 | Alirocumab     | Patients with hypercholesterolemia not adequately controlled and high CVD risk            | 24     | -45.9 -35.8 -37.5 -0.6 7.3         |
| COMBO I [66]                 |      |     |                |                                                                                           |        | -14.6                             |
| Colhoun et al., ODYSSEY COMBO| 2015 | 707 | Alirocumab     | Patients with hypercholesterolemia not adequately controlled and high CVD risk            | 24     | -29.7 -22.4 -22.9 -0.3 8.1         |
| II [67]                      |      |     |                |                                                                                           |        | -21.7                             |
| Robinson et al., ODYSSEY     | 2015 | 2341| Alirocumab     | Patients with hypercholesterolemia not adequately controlled and high CVD risk            | 24     | -61.9 -54.0 -52.3 -17.3 4.6        |
| LONG TERM [73]               |      |     |                |                                                                                           |        | -25.6                             |
| Blom et al., DESCARTES [53]  | 2014 | 901 | Evolocumab     | Patients with hyperlipidaemia had four-weekly 420 mg evolocumab in addition to diet alone, diet and atorvastatin or to diet plus atorvastatin plus ezetimibe | 52     | -57.0 -44.2 -50.3 -11.5 5.4        |
| Robinson et al., LAPLACE-2   | 2014 | 2067| Evolocumab     | Patients with hyperlipidaemia had either 140 mg fortnightly or 420 mg every 4 weeks evolocumab added to statin therapy compared with ezetimibe | 12     | -59.2 to -70.6 -70.0 -54.9 -9.3 to |
| [52]                         |      |     |                |                                                                                           |        | -71.4 to -53.6 -54.1 -50.2 -6.5 to |
| Stroes et al., GAUSS-2 [62]  | 2014 | 307 | Evolocumab     | Patients with statin intolerance given 140 mg fortnightly or 420 mg every 4 weeks evolocumab and were compared to those on ezetimibe | 12     | -68.8 to -69.7 -69.2 -32.9 NR       |
| [62]                         |      |     |                |                                                                                           |        | NR                                |
| Koren et al., MENDEL-2 [49]  | 2014 | 614 | Evolocumab     | Patients with hypercholesterolemia on no statins 140 mg fortnightly or 420 mg every 4 weeks evolocumab and were compared to those on ezetimibe | 12     | -54.8 to -57.1 -57.1 -49.8 to 5.9 to |
| [49]                         |      |     |                |                                                                                           |        | -17.8 to 9.3 -20.4                |
| Raal et al., RUTHERFORD-2    | 2015 | 329 | Evolocumab     | Patients with heterozygous FH given 140 mg fortnightly or 420 mg every 4 weeks evolocumab | 12     | -59.2 to -61.3 -61.3 -49.1 -11.6 to |
| [55]                         |      |     |                |                                                                                           |        | -19.6 -9.1 -28.2 to 9.2 -31.6      |
| Sabatine et al., OSLER-2     | 2015 | 4465| Evolocumab     | Hypercholesterolemia or mixed dyslipidaemia who had participated in the previous OSLER study | 12     | -59.2 to -61.0 -61.0 -47.3 -32.0 to |
| [79]                         |      |     |                |                                                                                           |        | -12.6 -7.0 -25.5                   |
| Raal et al., TESLA Part B    | 2015 | 49  | Evolocumab     | Patients with homozygous FH not on apheresis were given 420 mg every 4 weeks of evolocumab | 12     | -30.9 to -23.1 NR 0.3 -0.1 -11.8    |
| [57]                         |      |     |                |                                                                                           |        |                                   |

Values in table represent percentage (%) change in lipid parameters.

ApoB apolipoprotein B, FU follow-up, LDL-C high-density lipoprotein cholesterol, HeFH heterozygous familial hypercholesterolaemia, LDL-C low-density lipoprotein cholesterol, Lp(a) lipoprotein (a), mg milligram, n number, NA not available, TG triglycerides

See main text for full explanation of trial abbreviations.
physiological functions (e.g. synthesis of hormones and vitamins) and thus, such concerns may be misplaced.

**Future directions**

Impelled by the growing evidence-base regarding the safety and efficacy of monoclonal PCSK9 inhibitors, considerable momentum has accumulated in the translation of this novel pharmacotherapeutic paradigm to clinical practice. However, there is still a need to evaluate whether PCSK9 inhibition yields benefits on cardiovascular endpoints, for patients with primary hypercholesterolemia. Indeed, three large phase III programmes with anti-PCSK9 monoclonal antibodies are currently ongoing to offer definitive insights into their utilisation in preventing cardiovascular events and improving clinical outcome: the PROFICIO and FOURIER programmes evaluating evolocumab, and the ODYSSEY programme evaluating alirocumab. A list of currently ongoing clinical studies is presented in Table 5. These trials are due to report in 2017–2018, and will surely offer greater insights into the safety and efficacy of PCSK9 inhibition, particularly with regard to effects over and above statin therapy.

In July 2015, the United States Food and Drug Administration (FDA) approved alirocumab as a second-line treatment, for adults with HeFH, and those with proven CVD with hypercholesterolemia refractory to diet modification and maximally-tolerated statin therapy. One month later, the FDA similarly approved evolocumab for clinical usage. These approvals were conditional on the subsequent completion of planned phase III trials to determine efficacy in primary hypercholesterolemia. Both agents have also recently received marketing authorisation by the European Medicines Agency.

**Pharmacogenetic considerations**

Although the PCSK9 locus is polymorphic, evidence has not yet emerged to suggest that routine genetic testing would predict responsiveness to PCSK9 inhibition, in patients with primary hypercholesterolaemia. In patients with HoFH, there exists evidence of differential response to PCSK9 inhibition, dependent on the specific underlying causative gene mutation(s). Evolocumab was demonstrably effective in lowering LDL-C, only in patients with residual LDL receptor function (the receptor-defective phenotype; 2–25 % function), but not receptor negative patients [54, 55, 57]. Stratification of FH patients, via fibroblast culture or pharmacogenetic testing (which many candidates may have underwent as part of FH diagnosis), may allow

| Table 5 Major ongoing clinical studies of PCSK9 inhibitors |
|----------------------------------------------------------|
| **Title** | **Description** | **Study identifier** |
| Trial assessing efficacy, safety and tolerability of PCSK9 inhibition in paediatric subjects with genetic LDL disorders | 10–17 year olds with outcomes focused on cardiovascular risk | NCT02392559 |
| Effects of selective inhibition of cholesterol absorption with ezetimibe on intestinal cholesterol homeostasis in dyslipidemic men with insulin-resistance—a pilot study | Aged 18–60 and has metabolic syndrome | NCT01849068 |
| Evaluating PCSK9 binding antibody influence on cognitive health in high cardiovascular risk subjects | Testing spatial working memory in those aged 40 to 85 taking evolocumab | NCT02207634 |
| Further cardiovascular outcomes research with PCSK9 inhibition in subjects with elevated risk | 5 year cardiovascular death, myocardial infarction, hospitalization for unstable angina, stroke, or coronary revascularization | NCT01764633 |
| The evaluation of bococizumab (PF-04950615; RN316) in reducing the occurrence of major cardiovascular events in high risk subjects | Effect of bococizumab on number of Cardiovascular Events | NCT01975389 |
| A phase 1 study of an investigational drug, ALN-PCSSC, in subjects with elevated low density lipoprotein cholesterol (LDL-C) | Safety | NCT02314442 |
| A 2-part, phase 1, single and multiple ascending dose study to assess the safety, pharmacokinetics, and pharmacodynamics of CAT-2054 in healthy subjects | Frequency and severity of adverse events | NCT02374047 |
| Open label study of long term evaluation against LDL-C trial-2 | Incidence of adverse events | NCT01854918 |
| ODYSSEY outcomes: evaluation of cardiovascular outcomes after an acute coronary syndrome during treatment with alirocumab SAR236553 (REGN727) | To evaluate the effect of alirocumab on any adverse cardiovascular event | NCT01663402 |
personalised prediction of responsiveness to PCSK9 inhibition.

Pharmacoeconomic considerations

Since the approval of these agents by regulatory bodies, the uptake of PCSK9 inhibitors in US clinical practice has in large, been slow. This may be explained by several key factors. Priced over $14,000 per year before discounts ($14,100 for evolocumab, $14,600 for alirocumab), and with a paucity of definitive data regarding improvements in cardiovascular outcomes, insurers have been reluctant to fund the available PCSK9 inhibitors. Recent pharmacoeconomic analysis by the US Institute for Clinical and Economic Review, calculated the overall price, best representing the potential benefits to patients, would be between $3615 and 4811—a 67 % discount on the current list price [82]. However, when compared to apheresis (the current best-available, alternative treatment, following statin and second-line medical therapy for uncontrolled hypercholesterolaemia), which costs approximately $8000 per month ($96,000 per year), the price of evolocumab and alirocumab appear more attractive. Schulman et al. estimate that in a typical insurance pool, if 5 % of the estimated 27 % of US adults 40–64 years of age who have hypercholesterolaemia were eligible for a PCSK9 inhibitor, annual premiums would increase by approximately $124 per person; taxpayers would face the burden of similar increases in the cost of the Medicare Part D program [83].

Conclusions

A quarter century after approval of the first statin in 1987, reduction of LDL-C remains the best-validated treatment strategy in preventing cardiovascular disease. PCSK9 is a promising molecular target to reduce levels of LDL-C and other atherogenic lipoproteins, below levels achievable with statins. However, uncertainties remain regarding the long-term impact of therapeutic reduction of plasma LDL-C to very low concentrations (<1 mmol L⁻¹). Additionally, the increased risk of progression to diabetes seen with high-intensity statin treatment might also occur with PCSK9 inhibition, possibly resulting from the intracellular accumulation of lipids in insulin-secreting pancreatic beta cells [84]. However, more data are needed from large trials to exclude important emergent adverse effects of PCSK9 inhibitors. Although self-administered injections might not appear attractive for lifelong treatment, this route of administration may be acceptable to high-risk patients, unable to tolerate statins, or who need to achieve more stringent LDL-C targets. There seems little doubt that the advent of therapeutic PCSK9 inhibition heralds a change to the future of lipid management.

Compliance with ethical standards

Conflict of interest

None.

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