PREVENTION OF CVD

PCSK9 inhibitors: clinical evidence and implementation

Marc S. Sabatine

Abstract | The gene encoding PCSK9 was first identified and linked to the phenotype of familial hypercholesterolaemia approximately 15 years ago. Soon after, studies uncovered the role of PCSK9 in the regulation of LDL-receptor recycling and identified loss-of-function variants of *PCSK9* that were associated with low circulating levels of LDL cholesterol (LDL-C) and a reduced risk of coronary artery disease. With amazing rapidity, monoclonal antibodies against PCSK9 were developed and studied in large clinical programmes. These PCSK9 inhibitors lowered plasma LDL-C levels by approximately 60%, even in patients already receiving maximum-dose statin therapy. In the past year, three cardiovascular outcome trials were completed and showed that PCSK9 inhibitors significantly reduce the risk of major vascular events. Reassuringly, this benefit comes with no major offsetting adverse events, such as an excess of myalgias, elevation of hepatic aminotransferases levels in the plasma, incident diabetes mellitus or neurocognitive adverse events. The clinical benefit of PCSK9 inhibitors seen in these trials occurred in the setting of reducing LDL-C levels to unprecedentedly low levels, suggesting that more aggressive LDL-C targets should be adopted. New technologies to inhibit PCSK9 are now being harnessed and might further revolutionize our treatment of dyslipidaemia.

The story of PCSK9 inhibition is rooted in genetics, in light of the observation that some families presented with the clinical phenotype of autosomal dominant hypercholesterolaemia (commonly called familial hypercholesterolaemia or FH) — a disorder characterized by elevated levels of plasma LDL cholesterol (LDL-C) and precocious development of atherosclerosis and its attendant complications — although these individuals did not harbour mutations in the genes encoding the LDL receptor or apolipoprotein B (APOB). Using classic Mendelian genetics and linkage analysis, investigators identified a region on chromosome 1 that was linked to the presence of this phenotype[1]. Abifadel and colleagues went on to show that mutations in the *PCSK9* gene caused autosomal dominant hypercholesterolaemia[2].

Eventually, studies showed that PCSK9 has a crucial role in the lifecycle of the LDL receptor[3]. LDL receptors are clustered in clathrin-coated pits on the surface of hepatocytes. After binding to LDL, the pit pinches off from the surface of the cell, which creates an endocytic vesicle. In the endosome, the LDL receptor can undergo a conformational change leading to the release of the LDL particle; the LDL receptor is then recycled back to the surface of the hepatocyte to extract more LDL from the circulation. Of note, LDL receptors can be recirculated approximately 100 times. Meanwhile, the LDL particle is shuttled to the lysosome where it is degraded. PCSK9 is secreted by hepatocytes and binds to the epidermal growth factor-like repeat A (EGF-A) domain of the LDL receptor. When the PCSK9–LDL receptor complex is internalized, the presence of PCSK9 prevents the conformational change in the LDL receptor, and the LDL receptor then travels together with LDL to the lysosome, where it is destroyed[3].

Sequencing analysis revealed that some families with the phenotype of autosomal dominant hypercholesterolaemia had gain-of-function mutations in *PCSK9*, leading to greater LDL-receptor destruction and, therefore, less hepatic removal of LDL-C from the circulation[4,5]. Conversely, investigators reasoned that individuals with loss-of-function variants in *PCSK9* should have more recirculation of LDL receptors back to the surface of the hepatocyte, more removal of LDL-C from the circulation and, therefore, be protected from atherosclerosis. Cohen and colleagues identified individuals with nonsense mutations in *PCSK9* who had circulating levels of LDL-C approximately 40% lower than individuals who did not carry the mutations[6]. The research team went on to study the incidence of coronary heart disease (CHD) in patients from the ARIC study according to the presence or absence of *PCSK9* loss-of-function variants that were associated with lowered plasma levels of LDL-C[7]. Variants that lowered LDL-C levels by 15% were associated with a 47% reduction in the risk of CHD,

TIMI Study Group, Division of Cardiovascular Medicine, Brigham and Women's Hospital and Harvard Medical School, Hale Building for Transformative Medicine, Boston, MA, USA.

e-mail: msabatine@bwh.harvard.edu

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Key points

- Monoclonal antibodies targeting PCSK9 can lower plasma LDL-cholesterol (LDL-C) levels by approximately 60%.
- In dedicated cardiovascular outcome trials, PCSK9 inhibitors significantly reduced the risk of major adverse cardiovascular events.
- This benefit was consistent in patients with a baseline LDL-C level <70 mg/dl, who achieved an LDL-C level of approximately 20 mg/dl, which is well below the current guideline-recommended targets.
- No offsetting safety concerns were reported in these trials over the timeframes studied.

and variants that lowered LDL-C levels by 28% were associated with an 88% reduction in the risk of CHD[7]. Similar data have been shown in multiple other cohorts[8]. In addition to supporting PCSK9 inhibition as a potentially valuable clinical target, these data underscore the value of prolonged, in this case, lifelong, LDL-C lowering compared with the effect observed over the shorter timescale of a clinical trial.

A Mendelian randomization study compared the effect of LDL-C-lowering variants located in *HMGCR*, the gene encoding the target of statins, or in *PCSK9* on the risk of cardiovascular disease[9]. Data were available for a total of 112,772 participants from 14 studies, with 14,120 cardiovascular events. Variants in *PCSK9* and *HMGCR* were associated with nearly identically lower risk of cardiovascular events (myocardial infarction (MI) or death from CHD) per 10 mg/dl decrease in plasma LDL-C level, with odds ratios of 0.81 (95% CI 0.74–0.89) for *PCSK9* variants and 0.81 (95% CI 0.72–0.90) for *HMGCR* variants[9]. Similar effects were seen for all individual components of the composite outcome, including death from CHD. These data suggest that PCSK9 inhibition should yield the same clinical benefit per unit decrease in plasma LDL-C level as statin therapy.

This Review describes the pharmacological effects of the PCSK9 inhibitors and provides an evaluation of efficacy and safety data from the dedicated cardiovascular outcome trials published in the past 18 months, with discussion of the implications for targets of plasma LDL-C levels and selection of patient subgroups for treatment. Lastly, the future directions of PCSK9 inhibition therapy, including potential new patient populations and therapeutic interventions, are examined.

Monoclonal antibodies against PCSK9

Among the several monoclonal antibodies developed against PCSK9, two — evolocumab and alirocumab — have been approved for clinical use. Both are fully-human monoclonal antibodies that are administered subcutaneously.

Evolocumab. Evolocumab can be given at a dose of 140 mg every 2 weeks or 420 mg monthly. These two doses produce similar degrees of plasma LDL-C level lowering[10]. In phase III clinical trials, evolocumab at those doses reduced plasma LDL-C levels by approximately 60% whether given as a monotherapy, added to statin therapy, given to patients who were statin-intolerant or given to patients with heterozygous FH[11–15] (TABLE 1). Overall, lowering of plasma LDL-C levels was highly consistent across the different clinical subgroups[16]. Moreover, evolocumab equally reduced LDL-C levels regardless of baseline plasma PCSK9 levels[17].

In patients with homozygous FH, the picture was more nuanced. Overall, in a double-blind, placebo-controlled trial, evolocumab reduced plasma LDL-C levels by 31%[18]. As expected, when receiving evolocumab, patients with two defective *LDLR* alleles (defined as 2–25% of normal LDL uptake) had a 47% reduction in plasma LDL-C level, patients with one defective and one negative allele (defined as <2% of normal LDL uptake) had a 25% reduction in plasma LDL-C level, and patients with two negative alleles did not respond to the therapy[18]. An open-label study of evolocumab in 106 patients with homozygous FH showed similar results[19]. Patients treated with 420 mg of evolocumab per month showed a 20% reduction in LDL-C level, and a greater reduction of 28% with uptitration to 420 mg of evolocumab every 2 weeks. Similarly, genotype mattered in this study, with a 20% reduction in LDL-C level observed in patients with one or more *LDLR* defective alleles, no benefit in LDL-C level reduction in patients with two negative alleles, a 47% reduction in patients with an *APOB* mutation, and a 6% reduction in patients who were compound heterozygotes with a *PCSK9* gain-of-function mutation and an *LDLR*-negative allele[19]. Therefore, additional therapeutic strategies are needed for patients with homozygous FH, especially those with *LDLR*-negative alleles.

Alirocumab. A variety of alirocumab dosing regimens have been used in phase III clinical trials. One regimen was a titrated dose that was based on achieved reductions in plasma LDL-C levels: patients would start at 75 mg of alirocumab every 2 weeks and then the dose would be increased to 150 mg every 2 weeks if the LDL-C level was ≥70 mg/dl. In general, this approach yielded LDL-C level reductions of 45–50% whether given as monotherapy, added to statin therapy or given to patients who were statin-intolerant[20–23] (TABLE 1). The maximum dose of alirocumab in these trials, 150 mg every 2 weeks, reduced plasma LDL-C levels by approximately 60%[24], which is similar to what has been shown with evolocumab. In another trial, an alirocumab regimen of 300 mg every 4 weeks lowered plasma LDL-C levels by 55–60%[25]. Data showed that in patients with heterozygous FH, treatment with alirocumab lowered LDL-C levels by approximately 40–60%[26,27]. In patients with homozygous FH, the changes in LDL-C levels observed with alirocumab treatment ranged from a 7% rise to a 64% decrease depending on the genotype of the patient[28].

PCSK9 inhibitors and statins. PCSK9 inhibitors and statins have some similarities and a few important differences (TABLE 2). At the highest doses, both statins and PCSK9 inhibitors reduce plasma LDL-C level by approximately 60%. Statins tend to lower plasma triglyceride levels more than PCSK9 inhibitors (25–35% versus approximately 15%)[29–31]. However, the reduction in plasma APOB levels, which integrate all atherogenic particles, is similar (approximately 50%) with either therapy[29–31]. PCSK9 inhibitors reduce the levels of lipoprotein (a) (Lp(a)) in plasma by approximately 25% whereas statins do not decrease, and might even

increase, plasma Lp(a) levels[32]. By contrast, statins seem to have an anti-inflammatory effect, as evidenced by reduced levels of C-reactive protein (CRP) in plasma after statin therapy, whereas PCSK9 inhibitors had no effect on CRP levels measured by high-sensitivity assay[33]. Whether these different effects of PCSK9 inhibitors and statins on other parameters besides LDL-C levels would translate into differences in clinical risk reduction remains an unresolved question.

Cardiovascular outcome trials

Three randomized, double-blind, placebo-controlled cardiovascular outcome trials have evaluated PCSK9 inhibitor therapy to date (TABLE 3).

FOURIER trial. The FOURIER trial[34] of evolocumab was the first successfully completed cardiovascular outcome trial of a PCSK9 inhibitor. A total of 27,564 patients with clinically evident atherosclerotic cardiovascular disease (ASCVD) — specifically, either a history of MI, non-haemorrhagic stroke or symptomatic peripheral artery disease (PAD) — were enrolled in the trial. Patients were eligible for inclusion if they had an LDL-C level ≥70 mg/dl or a non-HDL-cholesterol (non-HDL-C) level ≥100 mg/dl and were receiving optimized, stable lipid-lowering therapy, preferably high-intensity statin at least as potent as 20 mg of atorvastatin daily, with or without ezetimibe. Patients were randomly assigned to receive evolocumab (either 140 mg every 2 weeks or 420 mg every month, according to the patient's preference) or matching placebo injections. Plasma LDL-C levels were estimated using the Friedewald equation unless the level was <40 mg/dl, in which case preparative ultracentrifugation was performed. The primary efficacy end point was major cardiovascular events, defined as the composite of cardiovascular death, MI, stroke, coronary revascularization or hospitalization for unstable angina (requiring electrocardiogram (ECG) changes, evidence of inducible ischaemia or angiographic evidence of clinically significant disease with or without revascularization). The main secondary efficacy end point, for which the trial was powered, was the composite of cardiovascular death, MI or stroke[34].

The mean age of patients was 63 years, and 25% were women. A total of 81% of patients had a history of MI, 19% had a prior nonhaemorrhagic stroke and 13% had symptomatic PAD. The median time from qualifying MI or stroke to the time of randomization was approximately 3 years. The baseline plasma LDL-C level was 92 mg/dl, and 69% of individuals were receiving high-intensity statin therapy. Evolocumab lowered LDL-C levels by 59% at 48 weeks compared with placebo, with a mean absolute reduction of 56 mg/dl to a median level of 30 mg/dl, and this reduction was maintained over time[30]. The median follow-up was 2.2 years, which was shorter than originally planned (approximately 4 years), owing in part to faster accrual of events than anticipated in this high-risk patient population.

Over the course of the trial, evolocumab therapy significantly reduced the risk of the primary end point by 15% (HR 0.85, 95% CI 0.79–0.92) and the main secondary end point by 20% (HR 0.80, 95% CI 0.73–0.88) compared with placebo[30]. Significant reductions (21–27%) were observed in the risks of fatal or nonfatal MI, fatal or nonfatal stroke, and coronary revascularization compared with placebo[30]. As shown in other trials, urgent coronary revascularization seemed more modifiable in response to LDL-C lowering than elective coronary revascularization (27% versus 13% relative risk reductions)[31,35]. No effect was observed on cardiovascular death or hospitalization for unstable angina[30].

As demonstrated in clinical trials of statins, the translation of LDL-C lowering into clinical benefit takes time. Specifically, the clinical risk reduction per mmol/l of LDL-C lowering is far less in the first year of statin therapy than in subsequent years[36]. The same phenomenon was observed in the FOURIER trial[30]. The event curves for the evolocumab and placebo groups seemed to start diverging at approximately 6 months, and then the curve separation progressively widened. In landmark analyses of the trial, the reduction in cardiovascular death, MI or stroke with evolocumab was 16% in the first year and 25% after the first year compared with placebo[30].

The clinical benefit of evolocumab was largely consistent across demographic subgroups. Importantly, the benefit was also consistent across baseline LDL-C level subgroups, including in patients who had a baseline LDL-C level <70 mg/dl, in whom evolocumab lowered LDL-C to 21 mg/dl and reduced the risk of cardiovascular death, MI or stroke by 30% compared with placebo (HR 0.70, 95% CI 0.48–1.01)[37].

No significant differences were seen in the rates of adverse events or serious adverse events between

Table 1 | **Percentage reduction in plasma LDL-C level with PCSK9-inhibitor therapy**

PCSK9 inhibitor	Dosing	Reduction in plasma LDL-C level with PCSK9 inhibition				
		As monotherapy	Added to statin therapy	In patients with statin intolerance	In patients with heterozygous FH	In patients with homozygous FH
Evolocumab	140 mg every 2 weeks or 420 mg monthly	55–57%[11]	63–75%[12]	55–56%[13,14]	60–66%[15]	31%[18]
Alirocumab	75 mg every 2 weeks, increased to150 mg if LDL-C level ≥70 mg/dl	47%[20]	46–51%[21,22]	45%[23]	58%[26,27]	ND
	300 mg every 4 weeks	59%[25]	56%[25]	ND	ND	ND
	150 mg every 2 weeks	ND	62%[24]	ND	39%[26,27]	ND

FH, familial hypercholesterolaemia; LDL-C, LDL cholesterol; ND, no data.

evolocumab and placebo. In addition, no imbalances in any of the prespecified adverse events ascribed by some studies to statin therapy or to low plasma LDL-C level in general were reported, including myalgias, cataracts, new-onset diabetes mellitus, neurocognitive events or elevations in plasma levels of aminotransferases[30]. Specifically, no excess risk of diabetes could be observed with evolocumab compared with placebo even in patients with prediabetes at baseline, which is the subgroup of patients in whom the excess risk of diabetes with statin therapy seems to be largely confined[38,39]. Moreover, patients with diabetes, those with prediabetes or individuals with normoglycaemia showed no differences in glycaemic parameters (glycated haemoglobin (HbA_{1c}) and fasting plasma glucose) over the duration of the trial[39].

In terms of neurocognitive events, the EBBINGHAUS trial[40] was a dedicated, neurocognitive substudy conducted in 1,974 patients from the FOURIER trial[30]. Cognitive function was assessed using the Cambridge Neuropsychological Test Automated Battery. The primary end point was the score on the spatial working memory strategy index of executive function. Secondary end points were the scores for working memory, episodic memory and psychomotor speed. Assessments of cognitive function were performed at baseline, at week 24, yearly and at the end of the trial[41]. No significant between-group differences in cognitive function were observed. Moreover, an exploratory analysis found no associations between LDL-C levels and cognitive changes[41].

The only adverse event that was imbalanced was injection-site reactions, which were slightly more frequent with evolocumab than with placebo (excess of 0.2% per year). However, the vast majority of these events were mild and no between-group differences were reported in allergic reactions. Lastly, given that evolocumab is a fully-human antibody, no patients developed neutralizing antibodies[30].

SPIRE-1 and SPIRE-2 trials. The results of the SPIRE-1 (REFS[42–44]) and SPIRE-2 (REFS[42–44]) trials of bococizumab were presented at the same time as the results of the FOURIER trial[30]. These trials were terminated prematurely by the sponsor after the realization that the effect of bococizumab, a humanized but not fully-human monoclonal antibody, on the reduction of plasma LDL-C level was not sustained owing to the development of neutralizing antibodies[42].

A total of 27,438 patients with either a previous cardiovascular event or a history of diabetes, chronic kidney disease or peripheral vascular disease were enrolled in the SPIRE-1 and SPIRE-2 trials[43]. Patients were eligible for inclusion if they had an LDL-C level ≥70 mg/dl or non-HDL-C level ≥100 mg/dl (SPIRE-1 trial[43]) or an LDL-C level ≥100 mg/dl or non-HDL-C level ≥130 mg/dl (SPIRE-2 trial[43]). Patients had to be receiving high-intensity statin therapy unless they could not take those statin doses without adverse effects, in which case lower-intensity regimens were permitted. Patients were randomly assigned to receive 150 mg of bococizumab every 2 weeks or matching placebo injections subcutaneously. Protocol-mediated dose-reductions of the treatment were triggered if LDL-C levels were persistently <10 mg/dl. The primary efficacy end point was the composite of cardiovascular death, MI, stroke or urgent coronary revascularization[43].

The mean age of the patients was 63 years, 30% were women, and a total of 15.5% were high-risk, primary prevention patients. Baseline plasma LDL-C level of the participants was 109 mg/dl, and 85% of the individuals were receiving high-intensity statin therapy. Bococizumab lowered plasma LDL-C levels by 59% at 14 weeks compared with placebo, but this effect was attenuated over time, with reductions of only 42% at 1 year and 38% at 2 years[44].

Given that the trial was terminated early, the median follow-up was shorter than planned: only 7 months for the SPIRE-1 trial[44] and 12 months for the SPIRE-2 trial[44]. Over the course of the combined trials, the HR for the primary end point with bococizumab was 0.88 (95% CI 0.76–1.02, $P=0.08$)[44]. In the SPIRE-2 trial[44], which had a longer follow-up, and in which patients had a larger absolute reduction in LDL-C levels than in the SPIRE-1 trial[44], bococizumab significantly reduced the risk of the primary end point by 21% compared with placebo (HR 0.79, 95% CI 0.65–0.97, $P=0.02$). In the SPIRE-2 trial[44], the effects of bococizumab on the individual components of the composite end point, including cardiovascular death, were directionally consistent. The overall rate of adverse events was similar with bococizumab and placebo, but the rate of injection-site reactions was more than eightfold higher in the group treated with bococizumab[44].

ODYSSEY Outcomes trial. The results of the ODYSSEY Outcomes trial[45,46] presented at the ACC Scientific Sessions in March 2018 should be considered as preliminary given that the results have not yet been published. A total of 18,924 patients were enrolled in the ODYSSEY trial[45] 1–12 months after hospitalization for MI or unstable angina. Inclusion criteria were LDL-C level ≥70 mg/dl, non-HDL-C level ≥100 mg/dl or APOB level ≥80 mg/dl. Patients were required to be receiving high-intensity statin therapy (atorvastatin ≥40 mg or

Table 2 | **Effect of high-intensity statin therapy and PCSK9-inhibitor therapy on levels of clinical plasma parameters**

Therapy	LDL-C level	HDL-C level	Triglyceride level	Apolipoprotein B level	Lipoprotein (a) level	High-sensitivity CRP level
High-intensity statin	↓ 50–60%	↑ 5–10%	↓ 25–35%	↓ 40–50%	↔/↑	↓ ~35%
Ezetimibe	↓ 20–25%	↔	↓ 5–10%	↓ 15–20%	↓ ~10%	↓ 15–20%
PCSK9 inhibitor	↓ ~60%	↑ 5–10%	↓ ~15%	↓ ~50%	↓ ~25%	↔

CRP, C-reactive protein; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol.

Table 3 | **Summary of the placebo-controlled cardiovascular outcome trials on PCSK9 inhibitors**

PCSK9 inhibitor	Trial name	Number of patients	Type of patients	Time from MI or stroke	Patients receiving high-intensity statin therapy (%)	Baseline LDL-C level (mg/dl)	Dosing of PCSK9 inhibitor	Mean absolute reduction in plasma LDL-C level (mg/dl)	Median follow-up	Outcomes (primary end point and main secondary end point)	Refs
Evolocumab	FOURIER	27,564	Patients with MI, stroke or PAD	~3 years	69	92	140 mg every 2 weeks or 420 mg every 4 weeks	56	2.2 years	CV death, MI, stroke, hospitalization for unstable angina or coronary revascularization: HR 0.85 (95% CI 0.79–0.92); CV death, MI or stroke: HR 0.80 (95% CI 0.73–0.88)	30,34
Alirocumab	ODYSSEY Outcomes	18,924	Patients with history of ACS	4–52 weeks (median 2.6 months)	89	87	75 mg or 150 mg every 2 weeks (titrated to achieve LDL-C 25–50 mg/dl)	37–48[a]	2.8 years	CHD death, MI, ischaemic stroke or hospitalization for unstable angina: HR 0.85 (95% CI 0.78-0.93)	45,46
Bococizumab	SPIRE-1	16,817	Patients in secondary prevention of CVD (84%) or high-risk patients in primary prevention of CVD (16%)	NA	92	94	150 mg every 2 weeks (titrated down if plasma LDL-C level <10 mg/dl)	54	7 months	CV death, MI, stroke or urgent revascularization: HR 0.99 (95% CI 0.80–1.22)	43,44
Bococizumab	SPIRE-2	10,621	Patients in secondary prevention of CVD (84%) or high-risk patients in primary prevention of CVD (16%)	NA	73	134	150 mg every 2 weeks (titrated down if plasma LDL-C level <10 mg/dl)	67	12 months	CV death, MI, stroke or urgent revascularization: HR 0.79 (95% CI 0.65–0.97)	43,44

ACS, acute coronary syndrome; CHD, coronary heart disease; CV, cardiovascular; CVD, cardiovascular disease; MI, myocardial infarction; NA, not available; PAD, peripheral artery disease. [a]From 12 to 48 months.

rosuvastatin ≥20 mg daily) or receiving the maximum tolerated dose of one of those statins. Patients were randomly assigned to receive alirocumab (75 mg or 150 mg every 2 weeks) or matching placebo injections subcutaneously. The dose of alirocumab was titrated to achieve an LDL-C level of 25–50 mg/dl, and alirocumab was stopped if the LDL-C level was persistently <15 mg/dl. The primary efficacy end point was the composite of CHD death, MI, ischaemic stroke or hospitalization for unstable angina (requiring ECG changes and angiographic evidence of clinically significant disease)[45].

The mean age of the patients was 58 years, and 25% were women. The median time from qualifying acute coronary syndrome (ACS) to randomization was 2.6 months. Baseline plasma LDL-C level was 87 mg/dl among the participants, and 89% of the individuals were receiving high-intensity statin therapy. Alirocumab lowered LDL-C levels by 57% at 4 weeks compared with placebo, but this effect was attenuated over time, with reductions of 50% at 1 year and 36% at the end of the study, presumably owing to the down-titration scheme[46]. The median follow-up was 2.8 years.

Over the course of the trial, alirocumab significantly reduced the risk of the primary end point by 15% compared with placebo (HR 0.85, 95% CI 0.78–0.93, $P = 0.003$). The effects of alirocumab on the individual components of the composite end point, including coronary heart death, were directionally consistent. A nominal 15% reduction (HR 0.85, 95% CI 0.73–0.98) in all-cause mortality with alirocumab treatment compared with placebo was also reported, which was not statistically significant given that it was tested after two nonsignificant outcomes (CHD death and cardiovascular death) in the testing hierarchy[46].

As seen in other lipid-lowering trials, the event curves for the treatment and placebo groups did not

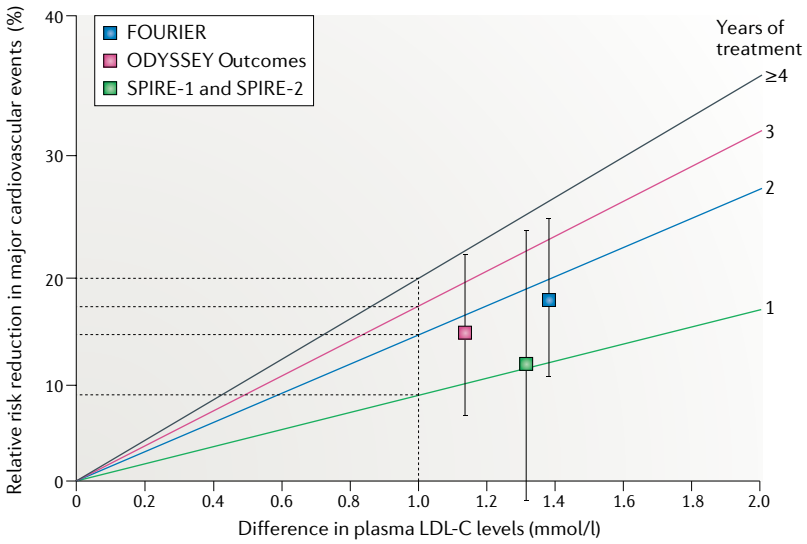


Fig. 1 | **Clinical benefit of PCSK9 inhibition relative to the benefit of statin therapy over time.** Regression lines represent the clinical benefit of statin therapy as a function of LDL-cholesterol (LDL-C) lowering for different durations of treatment, on the basis of data from the Cholesterol Treatment Trialists' Collaboration[77]. The boxes represent the effect estimate of PCSK9 inhibitor therapy on major cardiovascular events consisting of coronary heart (FOURIER and ODYSSEY Outcomes trials) or cardiovascular (SPIRE-1 and SPIRE-2 trials) death, myocardial infarction, stroke and either urgent coronary revascularization (FOURIER and SPIRE trials) or hospitalization for unstable angina (ODYSSEY Outcomes trial). The vertical lines represent the 95% CI. Adapted with permission from REF.[49], Oxford University Press.

diverge immediately and, in this trial, not until close to 1 year. Of note, the time interval between the ACS and the randomization was much shorter in the ODYSSEY Outcomes trial[45] than in the FOURIER trial[30]. Some evidence suggests that the early divergence of event curves seen in previous lipid-lowering trials of patients after ACS might be more closely related to the lowering of CRP levels than to the lowering of LDL-C levels[47]. PCSK9 inhibitors robustly reduce LDL-C level but have no effect on CRP level[33].

No significant heterogeneity in LDL-C level reduction was reported in any of the subgroups. With regard to the analysis by baseline LDL-C level, the data show a non-monotonic pattern of risk reduction of the primary efficacy end point with alirocumab, with a HR 0.86 (95% CI 0.74–1.01) in the groups of patients with a baseline LDL-C level <80 mg/dl, HR 0.96 (95% CI 0.82–1.14) in patients with a baseline LDL-C level 80–100 mg/dl and HR 0.76 (95% CI 0.65–0.87) in patients with a baseline LDL-C level ≥100 mg/dl. However, given the protocol-mediated down-titration of therapy on the basis of achieved LDL-C levels, patients allocated to alirocumab therapy who had a lower LDL-C level at baseline were more likely to have their alirocumab dose reduced or discontinued, thereby attenuating the clinical benefit of alirocumab in these patients[46]. The safety data looked equally reassuring as in the FOURIER trial[30], with no excess in elevations of creatine kinase or aminotransferase levels in the plasma and no excess in the incidence of new-onset diabetes, cataracts or neurocognitive disorders. The excess of injection-site reactions in the ODYSSEY trial[46] was nearly twice that in the FOURIER trial[30], with an excess of approximately 0.6% per year.

Putting PCSK9 inhibition in perspective

The relative risk reduction in cardiovascular disease with LDL-C lowering has been shown to be a function of two important variables: the absolute amount of LDL-C lowering and the duration of therapy. Our group has shown previously that for both statin and non-statin therapies associated with an upregulation of LDL-receptor levels (including diet, bile-acid sequestrants, ileal bypass surgery and ezetimibe), the relative risk reduction in cardiovascular disease was strongly related to the absolute decrease in plasma LDL-C level[48]. Specifically, the relative risk of major vascular events per 1 mmol/l reduction in LDL-C level was 0.77 (95% CI 0.71–0.84, $P<0.001$) for statins and 0.75 (95% CI 0.66–0.86, $P=0.002$) for the aforementioned non-statin therapies compared with control[48]. Indeed, the absolute reduction in LDL-C level in these studies accounted for 98% of the between-study variability in relative risk reduction[48].

The other important factor is time. Significant heterogeneity ($P<0.0001$) can be seen in the benefit of statin therapy depending on the length of the treatment. Whereas an overall 20% relative risk reduction per 1 mmol/l reduction in plasma LDL-C level has been observed, this estimate is based on statin trials that had a median duration of approximately 5 years. In the first year of therapy, the relative risk reduction per 1 mmol/l reduction in plasma LDL-C level was only 9%. Subsequently, this relative risk reduction grew to 22%, 24% and so on for each additional year of therapy[36]. Therefore, in the trials on PCSK9 inhibitors, the clinical benefit needs to be put into the context of the fairly limited duration of these trials (median follow-up of 2.2 years in FOURIER[30], <1 year in SPIRE-1 (REF.[44]) and SPIRE-2 (REF.[44]) and 2.8 years in ODYSSEY Outcomes[46]). By doing so, Ference and colleagues showed that the clinical benefits of PCSK9 inhibitors are largely consistent with what one would expect from the relationship seen between LDL-C lowering and risk of cardiovascular disease in the trials on statin therapy[49] (FIG. 1).

Likewise, absolute risk reductions and numbers needed to treat have to be put into the context of duration of therapy. For a series of trials of statin therapy, such as CARE[50], LIPID[51], HPS[52] and TNT[53], the absolute risk reductions in major adverse cardiovascular events (MACE) were approximately 2–4% over 5 years. The absolute risk reductions in the FOURIER[30] and ODYSSEY Outcomes[46] trials were approximately 1.5–2.0% over 2–3 years. Extrapolated to 5 years, the absolute risk reduction would be around 3% and, therefore, quite similar to that in the trials on statins (TABLE 4).

Evolocumab had no effect on the risk of cardiovascular death. However, inspection of the statin trials that have shown a reduction in the risk of death (for example, the 4S[54] and LIPID[51] trials) reveals that the event curves for each group did not start to diverge appreciably until 1.5–2.0 years. Given the fairly short follow-up in the FOURIER trial[30], the lack of a reduction in the risk of cardiovascular death was not surprising. The ODYSSEY Outcomes trial[46], which had a longer follow-up, showed

a nominal reduction in mortality with alirocumab therapy, which, as in the statin trials, did not start to emerge until approximately 1.5 years. Moreover, as noted above, data from Mendelian randomization studies showed similar reductions in the risk of CHD death per unit reduction in plasma LDL-C level, whether the LDL-C level reduction was genetically mediated through *PCSK9* or *HMGCR*[9].

Of note, to date, the safety profile of the PCKS9 inhibitors seems to be excellent. Other than a slightly higher rate of injection-site reactions than with placebo, most of which were mild, no imbalances were seen in other adverse events, including incidence of new-onset diabetes. The latter point is interesting given that the Mendelian randomization studies showed an association between LDL-C lowering via *PCSK9* genetic variants and an increased risk of diabetes[9]. Long-term, placebo-controlled studies of PCSK9 inhibition will, therefore, be important to provide further reassurance regarding the safety profile of this therapy.

A new target for plasma LDL-C level?

When combined with data from other trials on LDL-C-lowering therapies, the results of the trials on PCSK9 inhibitors support strategies to achieve far lower LDL-C targets than the ones currently endorsed by the guidelines (70 mg/dl). As noted above, in patients from the FOURIER trial[30] who had a baseline LDL-C level <70 mg/dl (median level of 66 mg/dl), evolocumab lowered LDL-C level to 21 mg/dl and reduced the risk of cardiovascular death, MI or stroke by 30% compared with placebo (HR 0.70, 95% CI 0.48–1.01)[37]. Likewise, the IMPROVE-IT trial[31] of ezetimibe (with an LDL-C level of 70 mg/dl in the control group) and HPS3/TIMI55-REVEAL trial[55] of the cholesteryl ester transfer protein inhibitor anacetrapib (with an LDL-C level of 63 mg/dl in the control group) both showed that further LDL-C lowering, even in patient populations with a median level of LDL-C <70 mg/dl, results in a significant reduction in the risk of cardiovascular disease.

Moreover, an observational analysis nested in the FOURIER trial showed a highly significant monotonic relationship between low achieved levels of LDL-C and lower risk of cardiovascular outcomes, extending down to patients with an LDL-C level <10 mg/dl[56]. Conversely, the analysis showed no significant associations between achieved LDL-C levels and multiple prespecified safety outcomes, including elevation in plasma levels of hepatic transaminases or creatine kinase and incidence of new or recurrent cancer, cataracts, haemorrhagic stroke, neurocognitive adverse events, new-onset diabetes mellitus and non-cardiovascular death[56]. Given that the reductions observed with PCSK9 inhibitor therapy in LDL-C levels and in the rate of major vascular events are not associated with an offsetting risk of adverse effects, targeting LDL-C levels to ≤20 mg/dl would be a reasonable strategy.

Targeting the optimal patients

PCSK9 inhibitors are currently expensive; therefore, a reasonable strategy for health-care systems would be to target the use of this therapy to patients who are most likely to experience the largest absolute risk reductions[57–59]. In parallel, reducing the cost of PCSK9 inhibitors would improve access to this therapy, especially in poor regions and countries. Our group has previously published a simple nomogram that uses baseline LDL-C level and baseline 10-year predicted major vascular events to create isopleths of predicted absolute risk reduction[60]. Given that the reduction in cardiovascular risk is directly proportional to the absolute amount of LDL-C lowering, a simple mathematical function shows that patients who start with higher levels of LDL-C will experience greater cardiovascular risk reduction for any given percent reduction in LDL-C level. However, a 2018 analysis suggested that more intensive LDL-C lowering would not convey any benefit in reducing mortality in individuals with baseline LDL-C levels <100 mg/dl[61]. This analysis was problematic for multiple reasons, and has been disproven by the results of the

Table 4 | **Absolute cardiovascular risk reduction in the trials on statins and PCSK9 inhibitors**

Trial name	Setting	Absolute reduction in MACE (%)	Follow-up (years)	Estimated absolute reduction in MACE at 5 years (%)	Estimated number needed to treat over 5 years	Refs
CARE	Statin versus placebo in patients with average plasma LDL-C level	3.0–4.2	5.0	3.0–4.2	24–34	50
LIPID	Statin versus placebo in patients with average plasma LDL-C level	3.6–4.4	6.1	3.0–3.6	28–34	51
HPS	Statin versus placebo in patients with average plasma LDL-C level	3.1–4.5	5.0	3.1–4.5	22–32	52
TNT	High-intensity versus moderate-intensity statin therapy	2.2	4.9	2.2	45	53
FOURIER	PCSK9 inhibitor versus placebo in patients receiving statin therapy	2.0[a]	3.0[a]	3.3	30	30
ODYSSEY Outcomes	PCSK9 inhibitor versus placebo in patients receiving statin therapy	1.6	2.8	2.9	35	46

LDL-C, LDL cholesterol; MACE, major adverse cardiovascular events (composite of coronary, cardiovascular or all-cause death, myocardial infarction or stroke; range provided when trials did not report the triple composite). [a]Event reduction based on Kaplan–Meier rates at 3 years.

Trial	Starting LDL-C levels (mmol/l)	Cardiovascular events		RR (95% CI) in major vascular events per 1 mmol/l reduction in LDL-C levels	
		Experimental group	Control group		
Statin therapy					
CTTC (subgroup LDL-C level <2 mmol/l)	1.7	910	1,012	0.78 (0.65–0.94)	
Non-statin LDL-C-lowering therapy					
IMPROVE-IT	1.8	2,455	2,649	0.79 (0.67–0.93)	
FOURIER (subgroup LDL-C level <1.8 mmol/l)	1.7	81	103	0.80 (0.61–1.04)	
HPS3/TIMI55–REVEAL	1.6	2,068	2,214	0.77 (0.63–0.96)	
Summary of non-statin therapy	1.6–1.8	4,604	4,966	0.79 (0.70–0.88)	*P* = 0.00005
Overall summary of LDL-C-lowering therapy	1.6–1.8	5,514	5,978	0.79 (0.71–0.87)	*P* = 0.000001

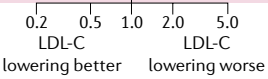


Fig. 2 | **Efficacy of LDL-C-lowering therapy in patients with very low baseline levels of LDL-C.** The reduction in the risk of major cardiovascular events per mmol/l reduction in plasma LDL-cholesterol (LDL-C) level is consistent even in patient populations with very low levels of LDL-C at baseline. Risk ratios (RRs) were generated from fixed-effects models. Combined RRs were also calculated for all non-statin LDL-C-lowering therapies and for all LDL-C-lowering therapies. CTTC, Cholesterol Treatment Trialists' Collaboration. Adapted with permission from REF.[62], American Medical Association.

HPS3/TIMI55-REVEAL trial[55], in which patients started the trial with an LDL-C level of 63 mg/dl and benefited from consistent reductions in MACE and in CHD death of a magnitude entirely expected from the reduction in LDL-C level. In a study published this year, our group has shown that the reduction in cardiovascular risk — MACE and its components, including CHD death — per mmol/l reduction in plasma LDL-C level is consistent even in patient populations with a baseline level of LDL-C ≤70 mg/dl[62] (FIG. 2).

Secondary analyses of the FOURIER trial have suggested that certain patients might be particularly attractive candidates for PCSK9-inhibition therapy. We have previously shown that, within the broad group of patients with a history of MI, several readily ascertainable features from the history of coronary artery disease — such as the time from the latest MI, the number of previous MIs and the presence of residual multivessel coronary artery disease — identified patients at high risk of cardiovascular disease who would show the greater relative and/or absolute risk reduction with the therapies[63–66]. The efficacy of evolocumab was analysed in those subgroups of patients in the FOURIER trial. These subgroups indeed identified patients at high risk of cardiovascular disease, with a baseline risk that was 40–100% higher than in patients without these risk factors. In addition, the relative risk reductions in these groups tended to be twice those in patients without these risk factors, for example, 21–30% versus 11–16% reductions in cardiovascular death, MI or stroke. The combination of the two factors of higher baseline risk and greater relative risk reduction led to greater absolute risk reductions at 3 years, with >3% reductions in the high-risk groups and approximately 1% reductions in the low-risk groups[67]. Subgroups with greater absolute risk reductions driven by PCSK9 inhibition will, by definition, need smaller numbers to treat to prevent an event and, therefore, represent more cost-effective populations.

Patients with PAD are another subgroup in which PCSK9-inhibitor therapy has demonstrated particularly notable benefits[68]. A total of 3,642 patients in the FOURIER trial (13.2%) had PAD. These patients were at substantially higher risk of cardiovascular death, MI or stroke than patients without PAD (2.5-year Kaplan–Meier rate 13.0% versus 7.6%), which persisted after adjustment for other clinical factors (HR 1.81, 95% CI 1.53–2.14, $P < 0.001$)[68]. Although the relative risk reductions in cardiovascular death, MI or stroke with evolocumab in patients with or without PAD did not significantly differ (27% versus 19%), given their higher baseline risk, patients with PAD had greater absolute risk reductions in cardiovascular death, MI or stroke than patients without PAD (3.5% versus 1.4%). Moreover, evolocumab significantly reduced the risk of major adverse limb events (acute limb ischaemia, major amputation or urgent peripheral revascularization for ischaemia) in all patients by 42% compared with placebo (HR 0.58, 95% CI 0.38–0.88, $P = 0.0093$). Furthermore, as had been shown for MACE, a consistent relationship was seen between lower achieved levels of LDL-C and lower risk of major adverse limb events ($P = 0.026$ for the β coefficient) that extended down to LDL-C levels <10 mg/dl[68].

Future directions

Among the additional therapeutic approaches that are being explored for PCSK9 inhibition, one using small interfering RNAs (siRNAs) is the most clinically developed. As an example, inclisiran is a chemically synthesized siRNA that is covalently linked to a ligand containing three *N*-acetylgalactosamine (GalNAc) residues. These GalNAc residues bind to the asialoglycoprotein receptor on hepatocytes, leading to inclisiran uptake. When inside the hepatocyte, inclisiran engages the natural pathway of RNA interference by binding to the RNA-induced silencing complex (RISC), enabling RISC to cleave specifically the *PCSK9* mRNA, thereby reducing PCSK9 synthesis[69]. A single

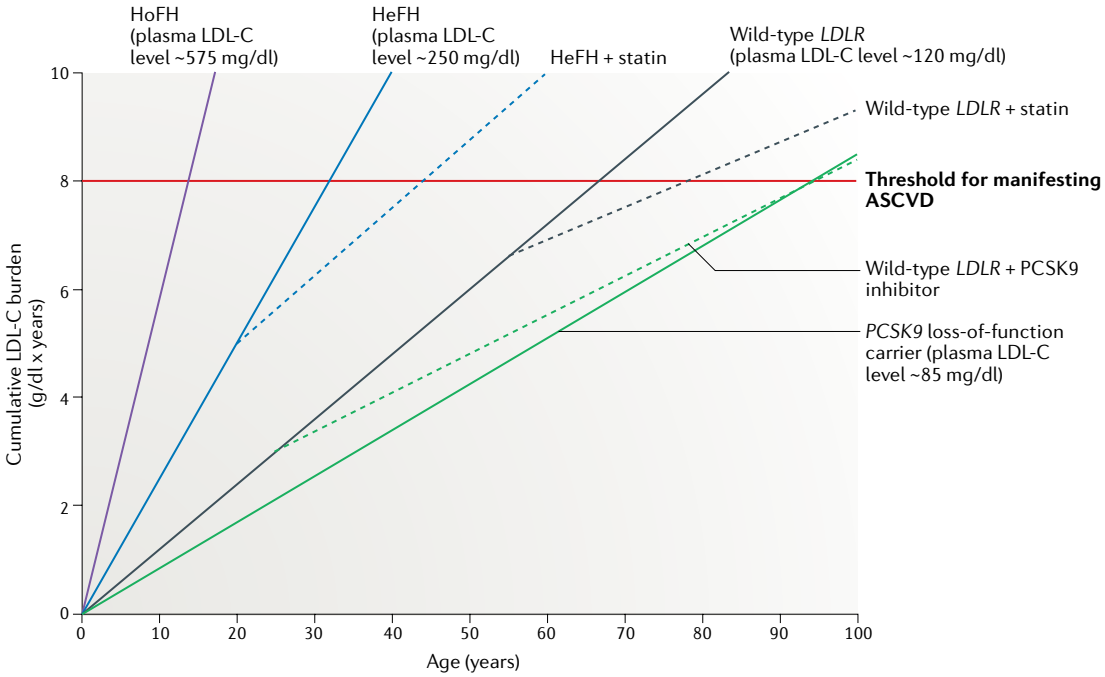


Fig. 3 | **Cumulative burden of LDL-C.** The lines represent cumulative LDL-cholesterol (LDL-C) levels (y-axis) as a function of circulating LDL-C levels (slope) and time (x-axis). The red line representing the threshold for manifesting atherosclerotic cardiovascular disease (ASCVD) varies from individual to individual depending on the presence of other risk factors. HeFH, heterozygous familial hypercholesterolaemia; HoFH, homozygous familial hypercholesterolaemia. Adapted with permission from REF.[3], American Society for Biochemistry and Molecular Biology.

siRNA-bound RISC complex is catalytic and cleaves many mRNA transcripts. Moreover, the effects of a single dose of inclisiran last for many months, allowing for dosing every 6 months, which is a major logistical advantage over the PCSK9 monoclonal antibodies. On the basis of data from the phase II ORION-1 trial[69], an inclisiran dose of 300 mg every 6 months should yield an average reduction in plasma LDL-C level of approximately 50%[69,70]. Through 210 days, inclisiran did not increase the rate of serious adverse events, and injection-site reactions occurred in 7% of patients receiving a 300 mg dose of inclisiran. Several phase III clinical trials are ongoing, and a 15,000-patient, dedicated cardiovascular outcome trial with a planned duration of 5 years has started (the HPS-4/TIMI 65–ORION-4 trial[71]).

The capacity to reduce LDL-C level easily and safely with PCSK9 inhibition opens the possibility for early intervention to yield greater clinical benefit. Similar to the notion of 'pack-years' associated with cigarette smoking, the concept of lifelong cholesterol burden has been introduced and represents the cumulative burden of circulating LDL-C level, defined as circulating LDL-C level multiplied by years[3]. At a certain threshold, individuals will start to manifest clinical coronary artery disease. Naturally, that threshold will vary from individual to individual, depending on other risk factors for coronary artery disease. Individuals with FH have high circulating levels of LDL-C and, therefore, cross this threshold and manifest atherosclerosis far earlier in life than individuals without FH (FIG. 3). Conversely, individuals who were fortunate enough to inherit a loss-of-function *PCSK9* variant have lower circulating levels of LDL-C and have a much lower likelihood of developing CHD than individuals without defective *PCSK9*.

Pharmacological interventions to reduce circulating LDL-C levels can decrease the slope of the line of cumulative LDL-C burden and, therefore, delay the onset of ASCVD. Typically, trials of primary prevention of cardiovascular disease using statin therapy have enrolled patients aged approximately 50–60 years and have shown that statin therapy reduces the incidence of ASCVD. However, earlier intervention, for instance when an individual is aged approximately 25 years, would magnify the benefit of the LDL-C lowering and delay the onset of clinically overt ASCVD more effectively. Daily statin use is possible but logistically challenging. If proven to be effective and safe, periodic injection of a *PCSK9* siRNA (such as 300 mg of inclisiran given once a year, which should yield a 40% reduction in plasma LDL-C levels) might offer, at a reasonable cost, an attractive option to largely eradicate ASCVD (FIG. 3).

Going even a step further, researchers are exploring a strategy that consists of immunizing mice with peptides that mimic PCSK9 to induce antibodies against native PCSK9, with the aim of decreasing circulating levels of both PCSK9 and total cholesterol and to reduce the atherosclerosis burden[72,73]. Other researchers are targeting *Pcsk9* using CRISPR–Cas9 gene editing and have shown reductions in plasma cholesterol levels in mice[74–76]. If these approaches can be successfully translated into humans and proven to be safe, they could one day give us an option for switching permanently to a state of low plasma LDL-C level with a single intervention.

Conclusions

PCSK9 inhibitors are now proven to be valid additions to the clinicians' armamentarium for the treatment of dyslipidaemia. These drugs reduce plasma LDL-C level by approximately 60%, significantly reduce the risk of major vascular events and have no adverse effects except for injection-site reactions. Patients with a high burden of ASCVD are at increased risk of major vascular events and benefit from greater relative and/or absolute risk reductions with PCSK9 inhibition. The clinical benefit seen in trials on PCSK9 inhibition occurred with lowering of plasma LDL-C down to levels of approximately 20 mg/dl, suggesting that more aggressive LDL-C targets should be adopted. If the clinical efficacy and safety of *PCSK9* siRNA inhibitors is validated, these therapeutics could offer the opportunity to intervene earlier and more easily to treat dyslipidaemia and potentially to largely eradicate coronary disease.

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Competing interests
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