Lipid lowering with PCSK9 inhibitors

Razvan T. Dadu and Christie M. Ballantyne

Abstract | Statins are the most-effective therapy currently available for lowering the LDL-cholesterol (LDL-C) level and preventing cardiovascular events. Additional therapies are necessary for patients who cannot reach the target LDL-C level when taking the maximum-tolerated dose of a statin. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is an enzyme with an important role in lipoprotein metabolism. Rare gain-of-function mutations in *PCSK9* lead to a high LDL-C level and premature coronary heart disease, whereas loss-of-function variants lead to a low LDL-C level and a reduced incidence of coronary heart disease. Furthermore, the PCSK9 level is increased with statin therapy through negative feedback, which promotes LDL-receptor degradation and decreases the efficacy of LDL-C lowering with statins. PCSK9 inhibition is, therefore, a rational therapeutic target, and several approaches are being pursued. In phase I, II, and III trials, inhibition of PCSK9 with monoclonal antibodies has produced an additional 50–60% decrease in the LDL-C level when used in combination with statin therapy, compared with statin monotherapy. In short-term trials, PCSK9 inhibitors were well tolerated and had a low incidence of adverse effects. Ongoing phase III trials will provide information about the long-term safety of these drugs, and their efficacy in preventing cardiovascular events.

Dadu, R. T. & Ballantyne, C. M. *Nat. Rev. Cardiol.* **11**, 563–575 (2014); published online 24 June 2014; doi:10.1038/nrcardio.2014.84

Introduction

Cardiovascular disease (CVD) is currently the leading cause of death in developed countries.[1] Atherogenic lipoproteins, which are clinically assessed by measurement of LDL cholesterol (LDL-C), non-HDL cholesterol (non-HDL-C), or apolipoprotein B (apoB) levels, have been identified as independent risk factors for CVD.[2,3] Statins have been used for many years to lower the levels of atherogenic lipoproteins, with a primary focus on LDL-C lowering, and have been successful in reducing the incidence of CVD events.[4,5] Although statins are the most-effective therapy for preventing CVD events, a need exists for additional therapies for LDL-C lowering and CVD prevention. Some patients at high risk of CVD who are already receiving a maximum dose of a statin still have a residual CVD risk. For example, in the PROVE-IT study,[6] the group receiving intensive statin therapy (atorvastatin 80 mg daily) had a substantial residual risk of cardiac events, with an incidence of 22.4% over the 2 years of the study. Another category of patients with an increased residual lifetime risk of CVD is those with inherited disorders that cause a markedly elevated LDL-C level, such as patients with familial hypercholesterolaemia, for whom maximal doses of the most-potent statins are not sufficient to achieve the target LDL-C level in >50% of individuals.[7] Furthermore, although statins are generally well tolerated, adverse effects might prevent some patients from taking adequate doses. Some patients are unable to tolerate statin therapy at all, and others are able to tolerate only small doses because of adverse effects, such as myalgia and rhabdomyolysis.[8] In a large survey performed by the National Lipid Association in the USA, about 12% of patients discontinued statin therapy, 62% of whom because of adverse effects.[8] Finally, meta-analyses have indicated that intensive-dose statin therapy is associated with an increased incidence of new-onset diabetes mellitus,[9,10] which might further limit the use of the highest doses of statins. Given these important issues associated with statin therapy, novel therapies that are safe and effective for the treatment of dyslipidaemia and that prevent CVD events are required. In this Review, we describe inhibitors of proprotein convertase subtilisin/kexin type 9 (PCSK9), which are a new class of therapeutic agents that have shown promising results in phase I and II studies, and which are now being evaluated in large, phase III trials of CVD outcomes.

Sections of Cardiovascular Research and Cardiology, Department of Medicine, Baylor College of Medicine, 6565 Fannin Street, MS A601, Houston, TX 77030, USA (**R.T.D.**, **C.M.B.**).

Correspondence to: C.M.B.
cmb@bcm.edu

Competing interests
C.M.B. is a consultant for: Abbott, Aegerion, Amarin, Amgen, Arena, Cerenis, Esperion, Genentech, Genzyme, Kowa, Merck, Novartis, Omthera, Pfizer, Regeneron, Resverlogix, Roche, and Sanofi-Synthelabo; and a member of the speakers' bureau for Abbott. C.M.B.'s institution has received grants or research support from: Abbott, Amarin, Amgen, Eli Lilly, Genentech, GlaxoSmithKline, Merck, Novartis, Pfizer, Regeneron, Roche, Sanofi-Synthelabo, and Takeda, and from the AHA and the NIH. R.T.D. declares no competing interests.

Clinical effects of *PCSK9* mutations

In 2003, a mutation in *PCSK9* was discovered in French families, and this gene became the third to be implicated in autosomal-dominant familial hypercholesterolaemia, in addition to *LDLR* and *APOB*.[11] *PCSK9* cDNA has 3,617 bp that encode the 692–amino-acid PCSK9 protein.[11,12] Autosomal-dominant familial hypercholesterolaemia can be caused by gain-of-function mutations in *PCSK9*, resulting in a low level of LDL receptors that, in turn, causes a high level of LDL-C.[12–14] Autosomal-dominant

Key points

- Although statins are the primary drug therapy for high LDL-cholesterol (LDL-C) level, statins are inadequate for many individuals because of limitations in tolerability or efficacy
- Proprotein convertase subtilisin/kexin type 9 (PCSK9), an important enzyme in lipid metabolism, is a promising therapeutic target to lower the LDL-C level
- Phase II trials of inhibition with anti-PCSK9 antibodies have shown promising lipid-lowering effects and short-term tolerability
- Phase III trials of PCSK9 inhibitors are providing evidence on the benefit of additional LDL-C lowering in high-risk patients whose LDL-C level remains elevated despite statin therapy

familial hypercholesterolaemia has been associated with an increased risk of premature CVD.[15,16]

Of particular interest is that loss-of-function mutations in *PCSK9* are characterized by very low plasma levels of LDL-C and apoB.[17,18] The association between variants causing loss of function of *PCSK9* and incident CVD events was evaluated in several large epidemiological studies. In the ARIC study,[19] loss-of-function *PCSK9* mutations resulted in reductions in the LDL-C level in African-American (by 28% in heterozygous carriers) and white (by 15% in heterozygous carriers) individuals. These mutations were also associated with a significant reduction in coronary events (HR 0.11, 95% CI 0.02–0.81, $P = 0.03$ for African-American individuals; HR 0.50, 95% CI 0.32–0.79, $P = 0.003$ for white individuals).[19] The most-important finding in this study was that the loss of function in *PCSK9* that resulted in a 37 mg/dl reduction in LDL-C level was also associated with an 88% reduction in incident coronary heart disease in African-American individuals.[19] In white individuals with a loss-of-function *PCSK9* mutation, a 21 mg/dl reduction in LDL-C level was associated with a 47% reduction in incident coronary heart disease.[19]

These results were confirmed by studies on other cohorts, such as the Copenhagen Heart Study,[20] which showed that loss of function of *PCSK9* was associated with an 11–15% reduction in the LDL-C level, which resulted in a 6–46% risk reduction in coronary heart disease events. In another study, a 27% reduction in LDL-C level was described in black women from Zimbabwe, in whom the frequency of *PCSK9* mutation was found to be 3.7%.[21] In contrast to the African-American population, the Y142X variant was not present in the Zimbabwean population.[21] In a case report, an individual who was a compound heterozygote for two inactivating mutations in *PCSK9* had an LDL-C level of 14 mg/dl.[22] The reduction in the risk of coronary heart disease observed with *PCSK9* mutations is much higher than that associated with reductions in cholesterol levels achieved in trials with statins.[23] This disparity might be explained by the sustained, lifelong reduction in LDL-C level as a consequence of the hereditary genetic variation,[24] compared with lowering the LDL-C level later in life with medications. Other epidemiological studies also suggest that the nonsense mutation of *PCSK9* is associated with significantly reduced peripheral artery disease,[25] and subclinical atherosclerosis measured by carotid intima–media thickness.[26] Therefore, genetic variation in human *PCSK9* led to interest in PCSK9 as a possible target for the treatment of hypercholesterolaemia and prevention of CVD.[27–29]

PCSK9 metabolism and statins

PCSK9 is a member of the proprotein convertase family that is secreted in the liver as an inactive enzyme precursor that contains a triad of residues required for catalytic activity.[30,31] Diurnal variations (nadir between 1500 h and 2100 h, peak at 0400 h), fasting state (reduction in PCSK level), and sex (higher in women than in men) are all factors that influence the level of PCSK9 in the blood.[32,33] The PCSK9 precursor undergoes intramolecular autocatalytic cleavage of its N-terminal prosegment in the endoplasmic reticulum.[31] After PCSK9 is secreted, the cleaved prodomain remains associated with the catalytic domain, permitting the mature PCSK9 protein to move out of the endoplasmic reticulum and enter the secretory pathway.[30,34] PCSK9 circulates in the plasma as a phosphoprotein and has no known substrate other than itself.[35] After secretion from the cell, PCSK9 can immediately bind to the surrounding LDL receptors and be endocytosed together with the receptor, or the protein can enter the circulation.[36,37] After reaching the bloodstream, PCSK9 can modulate LDL-receptor recycling in organs such as the liver, intestines, kidneys, lungs, pancreas, and adipose tissue.[38,39] PCSK9 binds to an LDL receptor on the surface of cells at the first epidermal growth factor-like (EGF-A) domain. The PCSK9–LDL-receptor complex is internalized into endosomal or lysosomal compartments and undergoes degradation, leading to a decreased number of LDL receptors on the surface of the cell (Figure 1a).[40,41] This physiological function of PCSK9 leads to an inverse relationship between the level of PCSK9 in the blood and the number of LDL receptors, which has been demonstrated in several studies.[42–44]

Several researchers have investigated the relationship between statins and PCSK9 metabolism and secretion in animals and humans, and have shown that statins increase the concentration of PCSK9 by 14–47%, which is dose-dependent and proportional to the duration of treatment.[44–46] Statins act as competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, which results in reduced endogenous cholesterol synthesis that, in turn, causes upregulation of LDL receptors through the sterol regulatory element-binding protein pathway.[47–49] Therefore, silencing of *PCSK9* was hypothesized to result in additional LDL-C lowering beyond that achieved with statin therapy. This theory was first confirmed by Berge and colleagues, who showed that a missense mutation in *PCSK9* might increase the response to statins.[50] These findings suggest that silencing *PCSK9* with treatment might enhance the response to statin therapy and increase LDL-C lowering. Several treatment options to block PCSK9 have now been developed and tested.[51]

Preclinical studies

Antisense oligonucleotides

One of the initial approaches to inhibit PCSK9 secretion was to target its mRNA, which can be achieved using antisense oligonucleotides (ASOs)—short sequences of

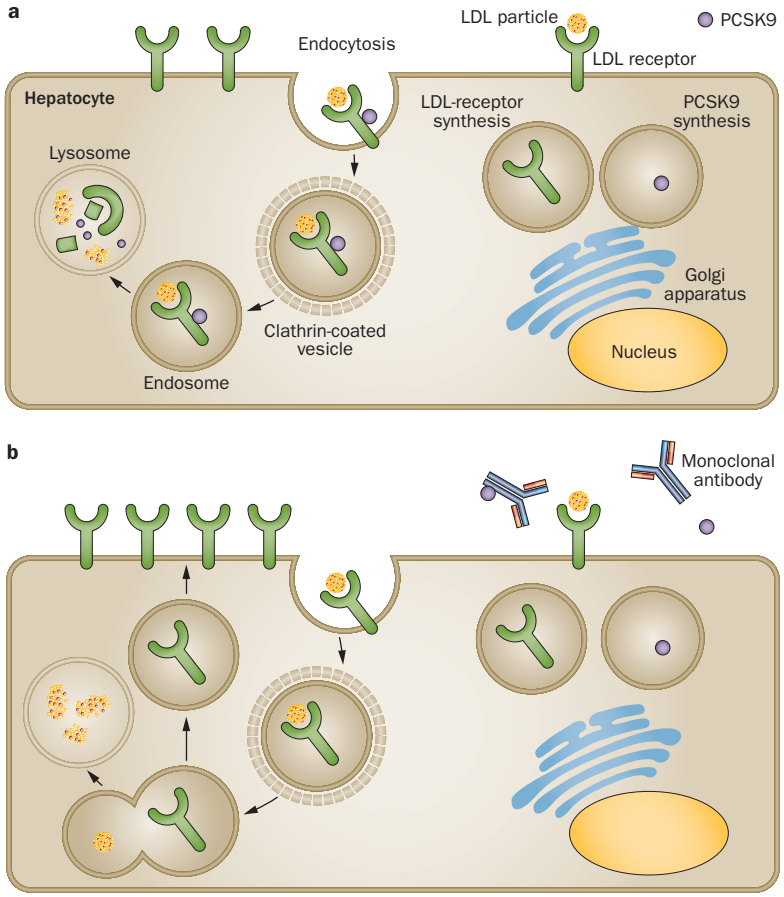

Figure 1 | LDL-cholesterol metabolism in the presence or absence of PCSK9. **a** | PCSK9 is synthesized in the liver as an inactive enzyme precursor that contains a triad of residues required for catalytic activity. PCSK9 circulates in the plasma as a phosphoprotein and, after having been secreted, can immediately bind to, and be endocytosed with, surrounding LDL receptors. The complex of the PCSK9 molecule and the LDL receptor is internalized and undergoes degradation in endosomal and lysosomal compartments, with few receptors recycled to the cell surface. This leads to a decreased number of LDL receptors on the surface of cells. **b** | Human monoclonal antibodies can bind to PCSK9 adjacent to the region that is required for interaction with LDL receptors. PCSK9 is, therefore, prevented from binding to LDL receptors. After endocytosis, the LDL receptor is recycled back to the surface of the cell, with few receptors degraded in the lysosome. Abbreviation: PCSK9, proprotein convertase subtilisin/kexin type 9.

nucleotides that bind to the mRNA and inhibit its translation to protein.[52] The first second-generation ASO for PCSK9 safely reduced murine hepatic *Pcsk9* mRNA secretion by 92% and the LDL-C level by 32%.[53] This ASO also produced an increase in the expression of *Apobec1* mRNA, a threefold decrease in the level of apoB-48, and a 50% decrease in the level of apoB-100.[53] Furthermore, after injection of ASOs into monkeys, the serum level of PCSK9 was reduced by 85%, and the LDL-C level was decreased by 50%.[54] In this study, no adverse effects from the ASOs were reported.[51] Currently, no trials of ASOs targeting PCSK9 are ongoing; a phase I trial was prematurely terminated in 2011 for undisclosed reasons.[55,56]

Small interfering RNA

Another method of silencing mRNA is to use single-stranded RNA, or small interfering RNA (siRNA), which can be administered intravenously in the form of small lipoid nanoparticles. In rats, liver-specific siRNA targeting PCSK9 achieved 50–60% maximal mRNA silencing and resulted in a 30% lowering of the plasma LDL-C level.[57] In nonhuman primates, single-dose administration of 5 mg of the drug resulted in a 56–70% lowering of the LDL-C level after 72 h, which was sustained up to 3 weeks.[57]

Monoclonal antibodies

The most studied and clinically advanced approach to PCSK9 inhibition is the use of monoclonal antibodies (mAbs; Figure 1b).[58] Chan and colleagues discovered the first neutralizing anti-PCSK9 mAb in 2009.[59] They showed that mAb1, a human mAb that binds to PCSK9 adjacent to the region that is required for LDL-receptor interaction, prevents the interaction between PCSK9 and LDL receptors *in vitro*.[59] *In vivo*, mAb1 increased the expression of hepatic LDL receptors and lowered the LDL-C level by approximately 30% in mice and nonhuman primates.[59] mAb1 also lowered the LDL-C level in mice expressing human PCSK9, suggesting that this antibody might effectively lower the LDL-C level in humans.[59] Several other antibodies with similar properties have been developed, and were tested in monkeys either as monotherapy or in combination with statins. The results of these studies were similar to those from Chan colleagues, showing that administration of antibodies against PCSK9 significantly increases the antibody-bound level of PCSK9 in the plasma, and significantly lowers the LDL-C level by 20–50% compared with baseline.[60–62] PCSK9 mAbs combined with statins were more effective in lowering the LDL-C level than either therapy alone.[61] No substantial adverse effects were reported in mice or primates, and the reduction in the LDL-C level was sustained for >2 weeks.[60–62] After successful experimentation with primates, several mAbs have undergone further testing in various phase I and II trials.

Clinical studies

Phase I trials

Small interfering RNA

In a phase I trial, Fitzgerald and colleagues evaluated the siRNA oligonucleotide ALN-PCS02 (Alnylam, USA; Table 1).[63] Healthy volunteers with an LDL-C level >116 mg/dl received a single intravenous infusion of the siRNA at escalating doses, and the effect was compared with placebo. The drug produced a rapid, dose-dependent reduction in the levels of PCSK9 (mean 68%) and LDL-C (mean 41%).[63] The largest reductions were observed with the highest dose of ALN-PCS02. The drug seemed to be safe, with no major adverse effects.[63]

Monoclonal antibodies

Several mAbs have been tested in phase I trials (Table 1). In both phase I studies whose results have been published, a single intravenous dose was first compared with escalating subcutaneous doses, then the effect of escalating doses of the compound on blood lipids was tested. No significant difference in the incidence of adverse effects between the placebo and treatment groups was observed for either compound.[64,65]

Table 1 | Phase I clinical trials of PCSK9 inhibitors

Type of agent	Name	Company	Duration (days)	Change from baseline (%)				
				LDL-C	Lp(a)	apoB	HDL-C	Tg
Small interfering RNA[63]	ALN-PCS	Alnylam (USA)	NA	–41	NA	NA	None	NA
Antibody[64]	Alirocumab	Sanofi (USA) and Regeneron Pharmaceuticals (USA)	64	–61	–27	–48	+18	–16
Antibody[65]	Evolocumab	Amgen (USA)	60	–81	–50	–59	None	None
Antibody[66–70]	Bococizumab	Pfizer (USA)	NR*	NR*	NR*	NR*	NR*	NR*
Antibody[71–73]	LY3015014	Lilly (USA)	NR	NR	NR	NR	NR	NR

*Results reported in abstract form.[60,74] Abbreviations: apoB, apolipoprotein B; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; Lp(a), lipoprotein(a); NA, not available; NR, trial completed, but data not yet published in a peer-reviewed journal; Tg, triglyceride.

Alirocumab (previously known as SAR236553/REGN727; Sanofi, USA and Regeneron Pharmaceuticals, USA) was tested in healthy volunteers and patients with familial or nonfamilial hypercholesterolaemia.[64] In this trial, the patients with hypercholesterolaemia were divided into those treated with statins and those treated by diet modification alone. Escalating doses of alirocumab administered subcutaneously reduced the LDL-C level in the statin-treated population by up to 61% compared with baseline (P<0.001).[64] The magnitude of the response was dose-dependent. The decrease in the LDL-C level was similar between patients with familial or nonfamilial hypercholesterolaemia. The decrease in the LDL-C level was also similar in patients treated with a statin and those previously treated by diet modification alone. A significant reduction in the apoB level of up to 48% compared with baseline was also observed in both groups.[64] The HDL-C level was increased by up to 18% in the statin-treated patients, and a nonsignificant maximal decrease of 16% in the level of triglycerides was reported. The lipoprotein(a) [Lp(a)] level was decreased by up to 27% compared with baseline, but the difference was not significant at all doses.[64]

Evolocumab (previously known as AMG 145; Amgen, USA) was tested in 56 healthy volunteers and 57 individuals receiving statins.[65] After a single dose in healthy volunteers, evolocumab reduced the LDL-C level by up to 64% compared with placebo (P<0.0001).[65] In statin-treated patients, multiple doses of the compound reduced the LDL-C level by up to 81% compared with placebo (P<0.001), and by 75% at the end of treatment (P<0.001).[65] Compared with placebo, evolocumab also reduced the apoB level by up to 55% in healthy volunteers (P<0.0001), and by up to 59% in statin-treated patients (P<0.001).[65] In the same study, evolocumab significantly reduced the Lp(a) level by 27–50% between baseline and the end of the dosing interval (6 weeks).[65] The decrease in the LDL-C level associated with the reduction in free PCSK9 was rapid and consistent between cohorts. The magnitude and duration of LDL-C lowering observed in the cohort receiving the highest doses of statins were similar to those in individuals receiving a lower dose of a statin.[65]

Several phase I trials of other PCSK9 mAbs, including bococizumab (previously known as RN316/PF-04950615; Pfizer, USA)[66–70] and LY3015014 (Eli Lilly, USA),[71–73] have been completed and presented in abstract form.[60,74] However, full reports have not yet been published in peer-reviewed journals.

Phase II trials

Several PCSK9 mAbs have been tested in phase II clinical trials (Table 2). Alirocumab, bococizumab, or evolocumab administered subcutaneously at various time intervals had impressive results on blood lipids and were well tolerated, with no difference in the incidence of adverse effects between the placebo and active-treatment groups. Phase II studies of LY3015014 are ongoing.[75]

Alirocumab was studied in doses of 50 mg, 100 mg, or 150 mg administered every 2 weeks, or 200 mg or 300 mg administered every 4 weeks.[76] This compound was tested in addition to atorvastatin (10 mg, 20 mg, or 40 mg daily) therapy in patients with hypercholesterolaemia and an LDL-C level >100 mg/dl. The reduction in the LDL-C level with 2-weekly administration measured at week 12 was dose-dependent and varied between 40% and 72%.[76] With the 200 mg and 300 mg doses given every 4 weeks, the reductions in the LDL-C level at week 12 were 43% and 48%, respectively.[76] The LDL-C reduction did not differ significantly according to atorvastatin dose. Alirocumab also reduced the apoB level to a similar extent as the LDL-C level, and reduced the Lp(a) level by 8–30% compared with placebo.[76] Changes in the triglyceride and HDL-C levels with alirocumab were small and variable, but greater than with placebo. In this trial, 89–100% of patients receiving alirocumab achieved a target LDL-C level of <100 mg/dl.[76] The compound was well tolerated, with no substantial increase in adverse effects reported.[76]

Another trial was conducted in patients with hypercholesterolaemia and an LDL-C level >100 mg/dl who were receiving atorvastatin 10 mg.[77] These patients were randomly assigned to receive either atorvastatin 80 mg only, atorvastatin 80 mg plus alirocumab 150 mg every 2 weeks, or atorvastatin 10 mg plus alirocumab 150 mg every 2 weeks. The largest reduction in LDL-C level was reported in the group receiving alirocumab plus atorvastatin 80 mg (least-squares mean percent reduction 73.5 ± 23.5), followed by those receiving alirocumab plus atorvastatin 10 mg (least-squares mean percent reduction 66.2 ± 3.5).[77] The group that received only atorvastatin 80 mg had a significantly lower reduction in the LDL-C

Table 2 | Phase II clinical trials of monoclonal antibodies against PCSK9

Study	PCSK9 inhibitor	Population; *n*	Treatment groups	Change versus placebo (LSM%)*			
				LDL-C	Lp(a)	HDL-C	Tg
McKenney *et al.*[76]	Alirocumab	LDL-C level ≥100 mg/dl with stable-dose atorvastatin; 183	50 mg, 100 mg, or 150 mg every 2 weeks	–34.5 to –67.3	–13.3 to –28.6	+5.1 to +7.7	–15.2 to –28.6
			200 mg or 300 mg every 4 weeks	–38.1 to –42.6	–7.9 to –16.7	+7.3 to +9.5	–18.1 to –20.5
Roth *et al.*[77]	Alirocumab	LDL-C level ≥100 mg/dl with atorvastatin; 92	150 mg every 2 weeks plus atorvastatin 10 mg daily	–48.9	–32.0‡	+6.2	+7.9‡
			150 mg every 2 weeks plus atorvastatin 80 mg daily	–55.9	–28.2‡	+9.4	–12.8‡
Stein *et al.*[78,79]	Alirocumab	LDL-C level ≥100 mg/dl with a statin, with or without ezetimibe; 77	150 mg every 2 weeks	–57.2	–19.5‡	+10.1	–5.7‡
			150 mg, 200 mg, or 300 mg every 4 weeks	–18.2 to –31.5	–3.5 to –11.4‡	+4.3 to +7.8	–6.2 to +5.6‡
LAPLACE–TIMI 57 trial[80]	Evolocumab	LDL-C level ≥85 mg/dl with a statin, with or without ezetimibe; 631	70 mg, 105 mg, or 140 mg every 2 weeks	–41.8 to –66.1	NA	+6.6 to +8.1	–18.1 to –33.7
			280 mg, 350 mg, or 420 mg every 4 weeks	–41.8 to –50.3	NA	+1.6 to +5.5	–13.4 to –19.4
MENDEL trial[81]	Evolocumab	LDL-C level ≥100 mg/dl and ≤190 mg/dl without lipid-lowering therapy; 406	70 mg, 105 mg, or 140 mg every 2 weeks	–37.3 to –47.2	–11.1 to –29.3	+4.2 to +10.2	–7.4 to –12.0
			280 mg, 350 mg, or 420 mg every 4 weeks	–43.6 to –52.5	–21.6 to –29.2	+3.3 to +5.8	–1.7 to –5.3
RUTHERFORD trial[82]	Evolocumab	Heterozygous FH, LDL-C level ≥100 mg/dl with a statin, with or without ezetimibe; 167	350 mg every 4 weeks	–43.8	–23.1	+7.8	–15.0
			420 mg every 4 weeks	–56.4	–31.5	+6.8	–19.9
GAUSS trial[83]	Evolocumab	Statin intolerant (no statin therapy), LDL-C level >ATP III target; 236	280 mg, 350 mg, or 420 mg every 4 weeks	–26.0 to –35.9	–12.4 to –18.0	+6.6 to +8.5	–8.7 to –13.8
			420 mg every 4 weeks plus ezetimibe 10 mg daily	–47.3	–21.2	+13.1	–4.0

Phase II studies of bococizumab have been completed,[86–88] and presented in abstract form,[85] but full reports have not yet been published in peer-reviewed journals. Phase II studies of LY3015014 are ongoing. *Change versus atorvastatin 80 mg monotherapy in Roth *et al.*; change versus ezetimibe 10 mg monotherapy in GAUSS trial. ‡Median. Abbreviations: ATP III, US National Cholesterol Education Program Adult Treatment Panel III; FH, familial hypercholesterolemia; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; Lp(a), lipoprotein(a); LSM, least-squares mean; NA, not available; Tg, triglyceride.

level (least-squares mean percent reduction 17.3 ± 3.5) than the other groups.[77] Significant reductions in the levels of Lp(a) and apoB were also observed in both combination-therapy groups. All the patients in the two groups assigned to receive alirocumab, compared with only 52% of the group assigned to atorvastatin 80 mg plus placebo, achieved the target LDL-C level of <100 mg/dl.[77] The effect on blood lipids did not differ significantly between the two groups receiving alirocumab.[77]

In a third trial, alirocumab was tested in 77 patients with heterozygous familial hypercholesterolaemia who were at target LDL-C level with stable lipid-lowering therapy involving statins with or without ezetimibe.[78] Administration of alirocumab resulted in a least-squares mean reduction of 29–68% in the LDL-C concentration between baseline and week 12.[78] Reductions in the apoB level were also consistent with those reported for LDL-C. Median percent change from baseline to week 12 in the Lp(a) level showed a nonsignificant trend towards a reduction compared with placebo. In this trial, 80% of patients treated with alirocumab achieved the target LDL-C level of <70 mg/dl.[78] In a 1-year open-label extension of this study, 150 mg alirocumab given every 2 weeks in addition to previous, randomly assigned, baseline lipid therapy in 57 patients with familial hypercholesterolaemia led to LDL-C level reductions of 57–66%.[79] Treatment was well tolerated, with >90% compliance.[79] No significant elevations in levels of muscle or liver enzymes were observed at 1-year follow-up, and the most-frequent adverse effect was injection-site reaction.[79]

The efficacy and safety of evolocumab was tested in the LAPLACE–TIMI 57 study[80] at doses of 70 mg, 105 mg, or 140 mg every 2 weeks, and doses of 280 mg, 350 mg, or 420 mg every 4 weeks in 631 patients who were taking a stable dose of a statin with or without ezetimibe. At 12 weeks, evolocumab administered every 2 weeks produced a significant dose-dependent reduction in the LDL-C level of 42–66% compared with placebo.[80] For evolocumab administered every 4 weeks, the LDL-C reduction was 42–50% compared with placebo.[80] The LDL-C lowering achieved between dosing intervals was much greater than that at the end of the dosing interval (85% and 70% reductions measured at 1 week after the first dose given every 2 weeks or 4 weeks, respectively).[80] The apoB level was lowered to a similar extent as the LDL-C level. Evolocumab also significantly lowered the level of triglycerides. No significant difference in adverse effects was reported between the evolocumab and placebo groups.[80]

In the MENDEL study,[81] evolocumab was compared with ezetimibe or placebo in 406 patients with hypercholesterolaemia who were not receiving concurrent lipid-lowering treatment. The LDL-C-lowering effect of evolocumab compared with placebo in this study

was similar in magnitude to that in the LAPLACE–TIMI 57 trial,[80] with a difference in least-square means of 37–53%.[81] The MENDEL study[81] also showed significant superiority of evolocumab over ezetimibe for LDL-C lowering, with a difference between the two drugs of 25–37%. Evolocumab also significantly reduced the Lp(a) level by 11–29% compared with placebo, but no significant reduction in the level of triglycerides was observed.[81]

In the RUTHERFORD trial,[82] the effect of evolocumab was assessed in 167 patients with heterozygous familial hypercholesterolaemia who were treated with lipid-lowering therapy (a statin with or without ezetimibe), but who were not achieving a target LDL-C level. At week 12, treatment with evolocumab at a dose of 350 mg or 420 mg every 4 weeks resulted in 70% and 89% of patients, respectively, reaching an LDL-C level of <100 mg/dl, and 44% and 65% of patients, respectively, achieving an LDL-C level of <70 mg/dl.[82]

In the GAUSS study,[83] the efficacy and tolerability of evolocumab was tested at doses of 280 mg, 350 mg, or 420 mg every 4 weeks, evolocumab 420 mg plus ezetimibe 10 mg, and ezetimibe 10 mg only in 160 patients who were statin intolerant. In this study, myalgia was the most-common adverse effect of treatment, occurring in seven patients (7.4%) taking evolocumab only (five patients taking 280 mg, one patient taking 350 mg, and one patient taking 420 mg), six patients (20.0%) taking evolocumab plus ezetimibe, and one patient (3.1%) taking ezetimibe plus placebo.[83] Treatment with evolocumab resulted in a reduction in the LDL-C level of 41–51%,[83] similar to that in the other three studies.[80–82] The largest reduction in the LDL-C level was in the group receiving evolocumab 420 mg plus ezetimibe 10 mg.[83] All four studies involving evolocumab[80–83] suggest that the compound is safe and effectively lowers the levels of LDL-C, apoB, and Lp(a) when used as monotherapy or in combination with other LDL-C-lowering drugs. Furthermore, a small ($n = 33$) phase Ib study showed that evolocumab reduces the small LDL particle number and increases mean LDL particle size.[84]

Bococizumab has been evaluated in several phase II studies. The results of two studies have been presented in abstract form,[85,86] and the other studies have been completed, but full results have not been published.[87,88] In one study that has been presented, 135 patients with a mean baseline LDL-C level of 123 mg/dl who were already taking high-dose statins were randomly allocated to one of four doses of bococizumab given by intravenous infusion every 4 weeks or placebo.[85] The largest reductions in the LDL-C level (46% and 56%) were achieved with doses of 3 mg/kg and 6 mg/kg, respectively.[85] Patients who received all three administrations of the agent at a dose of 3 mg/kg or 6 mg/kg had an increased baseline LDL-C level, which was reduced by 75%.[85] A small number of patients (5%) had antidrug antibodies, but their presence did not seem to affect drug efficacy.[85] In the other trial that has been presented, bococizumab significantly reduced the LDL-C level by up to 53 mg/dl at 12 weeks.[86]

All these phase II studies indicate that PCSK9 antibodies provide substantial LDL-C lowering when used as monotherapy or in conjunction with a statin. The efficacy of LDL-C lowering is dose-dependent and does not differ between patients treated with statins and patients receiving no other lipid-lowering therapy. The greatest reduction in the LDL-C level is achieved between the dosing intervals, and more-constant LDL-C concentrations seem to be achieved with dosing every 2 weeks than with 4-week intervals. However, no data are available from these trials to indicate an effect on CVD outcomes. In addition to substantial LDL-C lowering, PCSK9 inhibitors also lowered the apoB level to a similar extent as the LDL-C level. Most of the studies showed a significant Lp(a)-lowering capacity of the drugs; however, they were not designed to investigate the effect of PCSK9 on Lp(a), and the precise mechanism by which PCSK9 antibodies decrease the Lp(a) level is not known. Nevertheless, Lp(a) lowering seems to be a class effect of PCSK9 inhibitors, given that similar reductions (18–33%) are observed with alirocumab[76] and evolcumab.[89] Whether Lp(a) lowering translates into a reduction in clinical outcomes needs to be tested in future trials. The effect on the levels of triglyceride and HDL-C levels was small and not consistent between studies. Although PCSK9 inhibitors allowed patients with familial or nonfamilial hypercholesterolaemia to achieve the target LDL-C level, the effect of these drugs on cardiovascular outcomes remains to be established. No significant adverse effects were recorded in the phase II trials, and the therapy seems to be well tolerated by patients who are statin intolerant.

Phase III trials

Phase III trials (summarized in Figure 2) of alirocumab (Table 3),[90–101] evolocumab (Table 4),[102–112] and bococizumab (Table 5)[113–117] are being conducted. Results from six of these trials were presented at the ACC Scientific Sessions in 2014. The ODYSSEY MONO trial[90] showed a 47% reduction in the LDL-C level with alirocumab at 24 weeks, which was significantly more than with ezetimibe. The other five trials were designed to evaluate the long-term tolerability and adverse effects of evolocumab in various patient populations.

In the MENDEL-2 trial,[102] evolocumab reduced the LDL-C level by 56% (140 mg every 2 weeks) and 57% (420 mg every 4 weeks) after 12 weeks in statin-naive patients. Treatment given every 2 weeks or every 4 weeks was similarly effective in lowering the LDL-C level. Similar results were observed in the GAUSS-2 trial,[103] in which the same doses of evolocumab produced an equally sustainable effect in patients intolerant to statins. In both the MENDEL-2[102] and GAUSS-2[103] trials, evolocumab monotherapy was superior to ezetimibe monotherapy.

The DESCARTES-2 trial,[104] which had the longest follow-up (52 weeks), involved 901 patients with or without coronary heart disease who had an LDL-C level >75 mg/dl with maximal lipid-lowering therapy. Individuals were randomized to receive evolocumab 420 mg every 4 weeks or placebo, in addition to background therapy consisting of diet modification alone, atorvastatin 10 mg plus diet modification, atorvastatin 80 mg, or atorvastatin 80 mg plus ezetimibe 10 mg. The

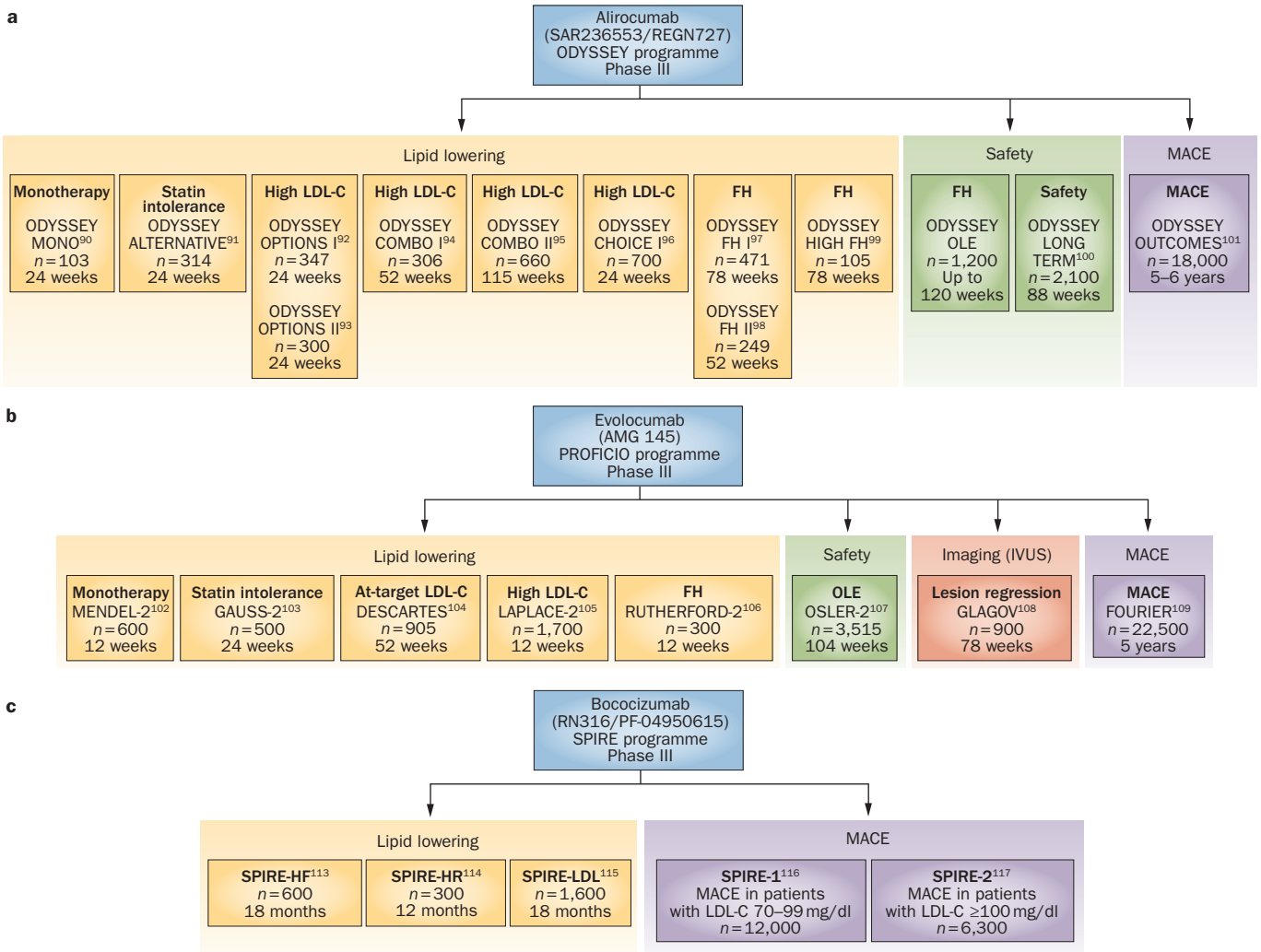

Figure 2 | Ongoing phase III clinical trials of PCSK9 inhibitors. Phase III trials designed to evaluate the long-term safety and tolerability of **a** | alirocumab, **b** | evolocumab, and **c** | bococizumab, and their efficacy in reducing the rate of cardiovascular events in various populations of patients. Abbreviations: FH, familial hypercholesterolaemia; IVUS, intravascular ultrasonography; MACE, major adverse cardiac events; OLE, open-label extension; PCSK9, proprotein convertase subtilisin/kexin type 9.

reduction in LDL-C level from baseline to 52 weeks in this wide spectrum of patients (which varied from those at low cardiovascular risk to those who already had coronary heart disease) was 57% greater with evolocumab than with placebo.[104] This reduction is similar to that observed at 12 weeks in the phase II studies reviewed above.

In the LAPLACE-2 trial,[105] the efficacy and tolerability of evolocumab were evaluated in addition to either moderate-intensity or high-intensity statin therapy. Evolocumab in doses of 120 mg every 2 weeks or 420 mg every 4 weeks proved to be efficacious and safe.[105] Evolocumab was also found to lower Lp(a) and apoB levels significantly, and to increase the HDL-C level. In the RUTHERFORD-2 trial,[106] evolocumab combination therapy proved safe and efficacious in lowering the LDL-C level in patients with heterozygous familial hypercholesterolaemia. In these patients, all of whom were already taking a statin and 60% of whom were taking ezetimibe, evolocumab reduced the LDL-C level by 56–63% after 12 weeks.[106]

Statins remain the mainstay of treatment for lowering the LDL-C level. However, a substantial number of individuals cannot tolerate high doses of a statin, some cannot tolerate any dose of a statin, and many individuals with hereditary lipid disorders or a previous coronary heart disease event have elevated levels of LDL-C, apoB, and Lp(a) despite maximal tolerated therapy. Phase II trials have shown promising lipid-lowering effects and short-term tolerability of PCSK9 antibodies in these particular subgroups of patients, who might benefit from additional LDL-C lowering. The new ACC/AHA guidelines[118] on treatment of blood cholesterol do not define LDL-C target levels and make no recommendation on using LDL-C-lowering therapy in addition to statins, because of a lack of evidence from randomized clinical trials. Data from the phase III trials reviewed above suggest that evolocumab might be a tolerable and efficacious option for these patients. These and other ongoing phase III trials of PCSK9 inhibitors will

Table 3 | Phase III clinical trials of alirocumab

Study	Population (background statin therapy)	Purpose	Estimated completion
ODYSSEY MONO[90]	Hypercholesterolaemia (with or without statin therapy)	To demonstrate the reduction in LDL-C level by alirocumab versus ezetimibe, after 24 weeks of treatment	Completed
ODYSSEY ALTERNATIVE[91]	Statin intolerance; primary hypercholesterolaemia (heterozygous FH or non-FH); and moderate, high, or very high CVD risk (no statin therapy)	To evaluate the efficacy and safety of alirocumab versus ezetimibe and versus atorvastatin, after 24 weeks of treatment	May 2014
ODYSSEY OPTIONS I[92]	Hypercholesterolaemia (heterozygous FH or non-FH) not adequately controlled (atorvastatin with or without other lipid-modifying therapy), and high CVD risk	To evaluate the reduction in LDL-C level by alirocumab as an add-on therapy to atorvastatin, versus ezetimibe as an add-on therapy to atorvastatin, versus doubling the atorvastatin dose, and versus switching from atorvastatin to rosuvastatin, after 24 weeks of treatment	May 2014
ODYSSEY OPTIONS II[93]	Hypercholesterolaemia not adequately controlled (rosuvastatin with or without other lipid-modifying therapy), and high CVD risk	To evaluate the reduction in LDL-C level by alirocumab as an add-on therapy to rosuvastatin, versus ezetimibe as an add-on therapy to rosuvastatin, and versus doubling the rosuvastatin dose, after 24 weeks of treatment	April 2014
ODYSSEY COMBO I[94]	Hypercholesterolaemia not adequately controlled (with maximum dose of a statin with or without other lipid-modifying therapy), and high CVD risk	To demonstrate the reduction in LDL-C level by alirocumab versus placebo as an add-on therapy to stable, maximally tolerated daily statin therapy with or without other lipid-modifying therapy, after 24 weeks of treatment	April 2014
ODYSSEY COMBO II[95]	Hypercholesterolaemia not adequately controlled (maximum dose of a statin with or without other lipid-modifying therapy), and high CVD risk	To demonstrate the reduction in LDL-C level by alirocumab versus ezetimibe as an add-on therapy to stable, maximally tolerated daily statin therapy in comparison with ezetimibe, after 24 weeks of treatment	July 2015
ODYSSEY CHOICE 1[96]	Hypercholesterolaemia	To evaluate the efficacy and safety of alirocumab every 4 weeks versus placebo with or without statin therapy, after 24 weeks of treatment	May 2014
ODYSSEY FH I[97]	Heterozygous FH not adequately controlled with current lipid-modifying therapy (no specification regarding statin therapy)	To evaluate the effect of alirocumab versus placebo on LDL-C level after 24 weeks of treatment (including background statin or other lipid-modifying therapy)	December 2014
ODYSSEY FH II[98]	Heterozygous FH not adequately controlled (with maximally tolerated statin with or without other lipid-modifying therapy)	To demonstrate the reduction in LDL-C level by alirocumab versus placebo as an add-on therapy to stable, maximally tolerated daily statin (atorvastatin, rosuvastatin, or simvastatin) therapy, with or without other lipid-modifying therapy, after 24 weeks of treatment	December 2014
ODYSSEY HIGH FH[99]	Heterozygous FH not adequately controlled with current lipid-modifying therapy (no specification regarding statin therapy)	To evaluate the effect of alirocumab versus placebo on LDL-C level after 24 weeks of treatment (including background statin or other lipid-modifying therapy)	January 2015
ODYSSEY LONG TERM[100]	Hypercholesterolaemia not adequately controlled with current lipid-modifying therapy, and high CVD risk (no specification regarding statin therapy)	To evaluate the long-term safety and tolerability of alirocumab versus placebo, after 78 weeks of treatment	October 2014
ODYSSEY OUTCOMES[101]	Recent (in the past 4–16 weeks) acute coronary syndrome event requiring hospitalization	To compare the effect of alirocumab versus placebo on CVD events (cardiovascular death, nonfatal myocardial infarction, fatal and nonfatal ischaemic stroke, unstable angina requiring hospitalization), for up to 64 months	March 2018

Abbreviations: CVD, cardiovascular disease; FH, familial hypercholesterolaemia; LDL-C, LDL cholesterol.

definitively answer the question of whether additional LDL-C lowering is beneficial in high-risk patients who are taking statin therapy, but continue to have an elevated LDL-C level.

Adverse effects of PCSK9 inhibitors

Clinical trial data

No differences in the rate of adverse effects between treatment and placebo groups were reported in the phase II clinical trials with alirocumab or evolocumab. None of the patients who received these drugs had severe or life-threatening adverse reactions that were associated with the active medication. The most-common adverse effects reported in the active-treatment groups were injection-site reaction, either pain or localized rash (2–9%); upper respiratory tract infection (6–10%); nasopharyngitis (4–15%); and mild gastrointestinal complications, such as diarrhoea (4%) or nausea (4–6%).[76–83] No antibodies against evolocumab were detected at the end of treatment in any trials. One case of allergic reaction was reported with alirocumab, which resolved with antihistamine.[77] Only one case of leukocytoclastic vasculitis

Table 4 | Phase III clinical trials of evolocumab

Study	Population (background statin therapy)	Purpose	Estimated completion
MENDEL-2[102]	Framingham Risk Score ≤10% and LDL-C level ≥100 mg/dl (no specification regarding statin therapy)	To evaluate the safety and efficacy of evolocumab every 2 or 4 weeks versus ezetimibe and versus placebo, at 10 and 12 weeks	Completed
GAUSS-2[103]	Statin intolerance; hypercholesterolaemia (no statin or low-dose statin)	To evaluate the safety and efficacy of evolocumab every 2 or 4 weeks versus ezetimibe, at 10 and 12 weeks	Completed
DESCARTES[104]	LDL-C level ≥85 mg/dl and either at ATP III target with background lipid therapy or taking maximum background lipid therapy (no specification regarding statin therapy)	To evaluate the efficacy, safety, and tolerability of evolocumab every 4 weeks versus placebo, at 52 weeks, when added to assigned background lipid-lowering therapy	Completed
LAPLACE-2[105]	Primary hypercholesterolaemia or mixed dyslipidaemia (taking statin therapy with or without ezetimibe)	To evaluate the safety, tolerability, and efficacy of evolocumab every 2 or 4 weeks plus a statin versus a statin plus ezetimibe, at 10 and 12 weeks	Completed
RUTHERFORD-2[106]	Heterozygous FH and LDL-C level ≥100 mg/dl with statin therapy (no specification regarding statin therapy)	To evaluate the safety, tolerability, and efficacy of evolocumab every 2 or 4 weeks versus placebo, at 10 and 12 weeks	Completed
OSLER-2[107]	Hypercholesterolaemia or mixed dyslipidaemia; completion of previous evolocumab study (no specification regarding statin therapy)	To evaluate the long-term safety, tolerability, and efficacy of evolocumab versus usual care, at 104 weeks	January 2017
GLAGOV[108]	Coronary heart disease; clinical indication for coronary catheterization; and LDL-C level ≥80 mg/dl or, with additional risk factors, ≥60 mg/dl and <80 mg/dl (no specification regarding statin therapy)	To determine the effects of evolocumab every 4 weeks on atherosclerotic disease burden (percent atheroma volume measured by intravascular ultrasonography), at 72 weeks	July 2015
FOURIER[109]	Clinical CVD, high risk of recurrent CVD event, and LDL-C level ≥70 mg/dl or non-HDL-C ≥100 mg/dl (no specification regarding statin therapy)	To assess the effect of evolocumab every 2 or 4 weeks plus a statin versus placebo plus a statin on major CVD events (CVD death, nonfatal myocardial infarction, unstable angina requiring hospitalization, stroke, or coronary revascularization), at 5 years	February 2018
TESLA[110]	Homozygous FH and LDL-C level >130 mg/dl with stable lipid therapy (no specification regarding statin therapy)	To determine the safety, tolerability, and efficacy of evolocumab in patients with homozygous FH, at 12 weeks	February 2014
TAUSSIG[111]	Homozygous FH or *PCSK9* mutations; LDL-C level above ATP III target or receiving apheresis; and completion of previous evolocumab study (no specification regarding statin therapy)	To assess the long-term safety and efficacy of evolocumab every 2 or 4 weeks on LDL-C level in patients with severe FH, at 5 years	January 2020
Study of AMG 145 in high-risk Japanese patients[112]	Japanese, high CVD risk, and hypercholesterolaemia or mixed dyslipidaemia (receiving statin therapy)	To evaluate the efficacy, safety, and tolerability of evolocumab every 2 or 4 weeks plus low-dose or high-dose statin versus placebo plus low-dose or high-dose statin, at 10 and 12 weeks	August 2014

Abbreviations: ATP III, US National Cholesterol Education Program Adult Treatment Panel III; CVD, cardiovascular disease; FH, familial hypercholesterolaemia; LDL-C, LDL cholesterol; non-HDL-C, non-HDL cholesterol.

was reported with alirocumab,[76] and no instances of vasculitis were reported with evolocumab. The incidence of these adverse effects in the active-treatment groups was not significantly different from that in the placebo groups. However, because of the short duration and fairly small numbers of participants in these phase II trials, larger phase III trials are required to assess the safety of PCSK9 inhibition with mAbs, particularly given that adverse effects on the liver or in muscle have been observed with statins and other lipid-lowering drug therapies.[119]

Information regarding the safety and tolerability of alirocumab and evolocumab in phase III clinical trials was presented at the ACC Scientific Sessions 2014. In the ODYSSEY MONO trial[90] of alirocumab, injection-site reactions were uncommon (<4%), and the incidence of muscle-related symptoms was similar in each group (3.8% with alirocumab and 3.9% with ezetimibe). In the DESCARTES-2 trial[104] of evolocumab, no significant difference existed in the incidence of adverse effects with evolocumab treatment or placebo. The most-common adverse effects were nasopharingytis, upper respiratory tract infections, influenza, and back pain.[104] An elevated creatine kinase level (more than fivefold the upper limit of normal) occurred in 1.2% of patients treated with evolocumab compared with 0.3% of those who received placebo; myalgia was reported in 4.0% and 3.0% of each group, respectively.[104] The low incidence of muscle-related adverse effects gives hope that this drug could be used as alternative therapy in statin-intolerant patients. The results of the GAUSS-2 trial,[103] in which

Table 5 | Phase III clinical trials of bococizumab

Study	Population (background statin therapy)	Purpose	Estimated completion
SPIRE-HF[113]	Heterozygous FH; high or very high CVD risk; LDL-C level >70 mg/dl and Tg level ≤400 mg/dl (with statin therapy)	To compare the effect of bococizumab and a statin versus placebo and a statin on LDL-C level in patients with heterozygous FH, at 12 weeks	January 2016
SPIRE-HR[114]	High or very high CVD risk; LDL-C level >70 mg/dl and Tg level ≤400 mg/dl (with statin therapy)	To compare the effect of bococizumab and a statin versusxplacebo and a statin on LDL-C level, at 12 weeks	January 2016
SPIRE-LDL[115]	High or very high CVD risk; LDL-C level >70 mg/dl and Tg level ≤400 mg/dl (with statin therapy)	To compare the effect of bococizumab and a statin versusxplacebo and a statin on LDL-C level, at 12 weeks	December 2015
SPIRE-1[116]	High CVD risk; LDL-C level ≥70 mg/dl and <100 mg/dl, or non-HDL-C level ≥100 mg/dl and <130 mg/dl, with lipid-lowering therapy (no specification regarding statin therapy)	To compare the effect of bococizumab versus placebo on reducing the occurrence of major cardiovascular events, including cardiovascular death, myocardial infarction, stroke, and unstable angina requiring urgent revascularization, at 5 years	August 2017
SPIRE-2[117]	High CVD risk; LDL-C level ≥100 mg/dl or non-HDL-C level ≥130 mg/dl, with lipid-lowering therapy (no specification regarding statin therapy)	To compare the effect of bococizumab versus placebo on reducing the occurrence of major cardiovascular events, including cardiovascular death, myocardial infarction, stroke, and unstable angina requiring urgent revascularization, at 5 years	August 2017

Abbreviations: CVD, cardiovascular disease; FH, familial hypercholesterolaemia; LDL-C, LDL cholesterol; non-HDL-C, non-HDL cholesterol; Tg, triglyceride.

all patients had muscle-related pain before inclusion, support this possibility. In this study, muscle-related adverse effects occurred in 12% of patients treated with evolocumab (13% in those treated every 2 weeks, and 12% in those treated every 4 weeks), compared with 23% of patients treated with ezetimibe.[103] However, the trial lacked a blinded statin challenge, which will be included in future studies.

Very low LDL-C level

The magnitude of the LDL-C lowering achieved with PCSK9 mAbs might mean that the safety of a very low level of LDL-C is a cause for concern.[120] The data obtained from the phase II trials show that PCSK9 antibodies can reduce the level of LDL-C to as low as 18 mg/dl. This level is much lower than the average observed in statin trials, such as the JUPITER study,[121] in which patients achieved a mean LDL-C concentration of 44 mg/dl with rosuvastatin treatment. Among potential risks thought to be associated with a very low level of LDL-C are haemorrhagic stroke, neurocognitive impairment, haemolytic anaemia, and hormonal and vitamin deficiency.[120] Another concern is associated with the accuracy of the Friedewald equation in measuring very low LDL-C levels. The Friedewald equation is routinely used to calculate LDL-C cholesterol level; however, several reports have shown that this equation underestimates LDL-C when the level is low.[122–124] This problem needs to be taken into consideration, and direct measurement of LDL-C concentration might be required as a confirmatory measure in patients with a low LDL-C level.

Meta-analyses of statin trials have not shown a significant association between statin treatment and intracerebral haemorrhage,[125] but some studies showed a possible association between intracerebral haemorrhage and cholesterol levels,[126] and an increased rate of intracerebral bleeding with intensive statin treatment in patients with stroke.[127] Neither direct action of PCSK9 on brain cells nor penetration by PCSK9 of the blood barrier has been reported. mAbs do not readily cross the blood–brain barrier because of their large size[128] and, therefore, are unlikely to reach pharmacologically or clinically relevant concentrations in the brain.

Cholesterol is an important component of the neurons and, additionally, data from cell-culture systems show that PCSK9 is involved in cortical neuron regeneration.[41] Therefore, PCSK9 inhibition in the central nervous system might, theoretically, cause neurological concerns because of low levels of both PCSK9 and cholesterol. However, mAbs are not thought to cross the blood–brain barrier readily into the central nervous system. Several isolated reports of patients treated with statins have noted neurological complaints, but this association has not been proven in clinical trials.[129] Furthermore, a Cochrane review on statins showed that a mean 22% reduction in the LDL-C level was not associated with either improvement or worsening in cognitive function.[130]

Another potential complication from a low cholesterol level that might occur with long-term administration of PCSK9 inhibitors is hormonal insufficiency. Studies have shown that adrenal cells have a reduced secretion of hormones in the presence of a very low level of cholesterol.[131,132] Whether decreasing cholesterol with PCSK9 inhibitors adversely affects functioning of the adrenal glands will be examined in phase III–IV trials.

Systemic effects

Given that PCSK9 is also expressed in organs other than the liver, such as the intestines, pancreas, and nervous system, the concern of adverse effects associated with PCSK9 and LDL metabolism has been raised. Studies on *Pcsk9*$^{-/-}$ mice have suggested that PCSK9 inhibition might result in increased visceral adiposity,[133] decreased glucose tolerance,[134] increased susceptibility to hepatic viruses,[135]

and altered expression of other genes.[136,137] However, none of these effects has been confirmed in humans.

Conclusions

In conclusion, in <1 decade since the observation that rare *PCSK9* mutations alter LDL metabolism, PCSK9 inhibition has emerged as a promising therapeutic strategy that might be used in addition to the currently available LDL-C-lowering therapies, with phase III trials already in progress. PCSK9 inhibitors produce a 40–72% reduction in the LDL-C level when combined with a statin or when administered to patients not taking other LDL-C-lowering drugs. This powerful LDL-C-lowering effect might enable patients who are statin intolerant or whose LDL-C level remains high despite maximal statin therapy to achieve a target LDL-C level. To date, no major adverse effects have been reported in humans, and ongoing phase III trials will provide needed information on the long-term safety and efficacy of PCSK9 inhibition with mAbs in reducing CVD events.

Review criteria

The PubMed and MEDLINE databases, ClinicalTrials.gov, and press-release statements were searched in March 2013 for research articles and clinical trials published in English, using the following key terms: "PCSK9", "PCSK9 metabolism", "PCSK9 inhibitor", "PCSK9 and statins", "PCSK9 antibody", "AMG 145", "SAR236553/REGN727", "ALN-PCS", "PF-04950615", "LY3015014", "PCSK9 genetic variants", and "PCSK9 gene and cardiovascular disease". Another search of ClinicalTrials.gov was performed in February 2014.

1. Agarwal, S. K. *et al.* Sources of variability in measurements of cardiac troponin T in a community-based sample: the atherosclerosis risk in communities study. *Clin. Chem.* **57**, 891–897 (2011).
2. Boekholdt, S. M. *et al.* Association of LDL cholesterol, non-HDL cholesterol, and apolipoprotein B levels with risk of cardiovascular events among patients treated with statins: a meta-analysis. *JAMA* **307**, 1302–1309 (2012).
3. Sniderman, A. D. *et al.* A meta-analysis of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B as markers of cardiovascular risk. *Circ. Cardiovasc. Qual. Outcomes* **4**, 337–345 (2011).
4. Genser, B. & Marz, W. Low density lipoprotein cholesterol, statins and cardiovascular events: a meta-analysis. *Clin. Res. Cardiol.* **95**, 393–404 (2006).
5. Baigent, C. *et al.* Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* **366**, 1267–1278 (2005).
6. Cannon, C. P. *et al.* Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N. Engl. J. Med.* **350**, 1495–1504 (2004).
7. Avis, H. J. *et al.* Efficacy and safety of rosuvastatin therapy for children with familial hypercholesterolemia. *J. Am. Coll. Cardiol.* **55**, 1121–1126 (2010).
8. Toth, P. P., Harper, C. R. & Jacobson, T. A. Clinical characterization and molecular mechanisms of statin myopathy. *Expert Rev. Cardiovasc. Ther.* **6**, 955–969 (2008).
9. Preiss, D. *et al.* Risk of incident diabetes with intensive-dose compared with moderate-dose statin therapy: a meta-analysis. *JAMA* **305**, 2556–2564 (2011).
10. Preiss, D. & Sattar, N. Statins and the risk of new-onset diabetes: a review of recent evidence. *Curr. Opin. Lipidol.* **22**, 460–466 (2011).
11. Abifadel, M. *et al.* Mutations in *PCSK9* cause autosomal dominant hypercholesterolemia. *Nat. Genet.* **34**, 154–156 (2003).
12. Abifadel, M. *et al.* Mutations and polymorphisms in the proprotein convertase subtilisin kexin 9 (*PCSK9*) gene in cholesterol metabolism and disease. *Hum. Mutat.* **30**, 520–529 (2009).
13. Maxwell, K. N. & Breslow, J. L. Proprotein convertase subtilisin kexin 9: the third locus implicated in autosomal dominant hypercholesterolemia. *Curr. Opin. Lipidol.* **16**, 167–172 (2005).
14. Jensen, H. K. The molecular genetic basis and diagnosis of familial hypercholesterolemia in Denmark. *Dan. Med. Bull.* **49**, 318–345 (2002).
15. Humphries, S. E. *et al.* Genetic causes of familial hypercholesterolaemia in patients in the UK: relation to plasma lipid levels and coronary heart disease risk. *J. Med. Genet.* **43**, 943–949 (2006).
16. Humphries, S. E. *et al.* Mutational analysis in UK patients with a clinical diagnosis of familial hypercholesterolaemia: relationship with plasma lipid traits, heart disease risk and utility in relative tracing. *J. Mol. Med. (Berl.)* **84**, 203–214 (2006).
17. Cariou, B. *et al.* PCSK9 dominant negative mutant results in increased LDL catabolic rate and familial hypobetalipoproteinemia. *Arterioscler. Thromb. Vasc. Biol.* **29**, 2191–2197 (2009).
18. Fasano, T. *et al.* A novel loss of function mutation of *PCSK9* gene in white subjects with low-plasma low-density lipoprotein cholesterol. *Arterioscler. Thromb. Vasc. Biol.* **27**, 677–681 (2007).
19. Cohen, J. C., Boerwinkle, E., Mosley, T. H. Jr & Hobbs, H. H. Sequence variations in *PCSK9*, low LDL, and protection against coronary heart disease. *N. Engl. J. Med.* **354**, 1264–1272 (2006).
20. Benn, M., Nordestgaard, B. G., Grande, P., Schnohr, P. & Tybjaerg-Hansen, A. *PCSK9* R46L, low-density lipoprotein cholesterol levels, and risk of ischemic heart disease: 3 independent studies and meta-analyses. *J. Am. Coll. Cardiol.* **55**, 2833–2842 (2010).
21. Hooper, A. J., Marais, A. D., Tanyanyiwa, D. M. & Burnett, J. R. The C679X mutation in *PCSK9* is present and lowers blood cholesterol in a Southern African population. *Atherosclerosis* **193**, 445–448 (2007).
22. Zhao, Z. *et al.* Molecular characterization of loss-of-function mutations in *PCSK9* and identification of a compound heterozygote. *Am. J. Hum. Genet.* **79**, 514–523 (2006).
23. Anand, S. S. Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. *Vasc. Med.* **8**, 289–290 (2003).
24. Brown, M. S. & Goldstein, J. L. Biomedicine: lowering LDL—not only how low, but how long? *Science* **311**, 1721–1723 (2006).
25. Folsom, A. R., Peacock, J. M. & Boerwinkle, E. Variation in *PCSK9*, low LDL cholesterol, and risk of peripheral arterial disease. *Atherosclerosis* **202**, 211–215 (2009).
26. Huang, C. C. *et al.* Longitudinal association of PCSK9 sequence variations with low-density lipoprotein cholesterol levels: the Coronary Artery Risk Development in Young Adults Study. *Circ. Cardiovasc. Genet.* **2**, 354–361 (2009).
27. Horton, J. D., Cohen, J. C. & Hobbs, H. H. Molecular biology of PCSK9: its role in LDL metabolism. *Trends Biochem. Sci.* **32**, 71–77 (2007).
28. Chen, S. N. *et al.* A common *PCSK9* haplotype, encompassing the E670G coding single nucleotide polymorphism, is a novel genetic marker for plasma low-density lipoprotein cholesterol levels and severity of coronary atherosclerosis. *J. Am. Coll. Cardiol.* **45**, 1611–1619 (2005).
29. Brown, W. V., Breslow, J. & Ballantyne, C. Clinical use of genetic typing in human lipid disorders. *J. Clin. Lipidol.* **6**, 199–207 (2012).
30. Benjannet, S. *et al.* NARC-1/PCSK9 and its natural mutants: zymogen cleavage and effects on the low density lipoprotein (LDL) receptor and LDL cholesterol. *J. Biol. Chem.* **279**, 48865–48875 (2004).
31. Zaid, A. *et al.* Proprotein convertase subtilisin/kexin type 9 (PCSK9): hepatocyte-specific low-density lipoprotein receptor degradation and critical role in mouse liver regeneration. *Hepatology* **48**, 646–654 (2008).
32. Persson, L. *et al.* Circulating proprotein convertase subtilisin kexin type 9 has a diurnal rhythm synchronous with cholesterol synthesis and is reduced by fasting in humans. *Arterioscler. Thromb. Vasc. Biol.* **30**, 2666–2672 (2010).
33. Cui, Q. *et al.* Serum PCSK9 is associated with multiple metabolic factors in a large Han Chinese population. *Atherosclerosis* **213**, 632–636 (2010).
34. Seidah, N. G. *et al.* The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. *Proc. Natl Acad. Sci. USA* **100**, 928–933 (2003).
35. Cunningham, D. *et al.* Structural and biophysical studies of PCSK9 and its mutants linked to familial hypercholesterolemia. *Nat. Struct. Mol. Biol.* **14**, 413–419 (2007).
36. Cariou, B., Le May, C. & Costet, P. Clinical aspects of PCSK9. *Atherosclerosis* **216**, 258–265 (2011).
37. Tibolla, G., Norata, G. D., Artali, R., Meneghetti, F. & Catapano, A. L. Proprotein convertase subtilisin/kexin type 9 (PCSK9): from structure-function relation to therapeutic inhibition. *Nutr. Metab. Cardiovasc. Dis.* **21**, 835–843 (2011).

38. Lagace, T. A. *et al.* Secreted PCSK9 decreases the number of LDL receptors in hepatocytes and in livers of parabiotic mice. *J. Clin. Invest.* **116**, 2995–3005 (2006).
39. Schmidt, R. J. *et al.* Secreted proprotein convertase subtilisin/kexin type 9 reduces both hepatic and extrahepatic low-density lipoprotein receptors *in vivo*. *Biochem. Biophys. Res. Commun.* **370**, 634–640 (2008).
40. Zhang, D. W. *et al.* Binding of proprotein convertase subtilisin/kexin type 9 to epidermal growth factor-like repeat A of low density lipoprotein receptor decreases receptor recycling and increases degradation. *J. Biol. Chem.* **282**, 18602–18612 (2007).
41. Poirier, S. *et al.* The proprotein convertase PCSK9 induces the degradation of low density lipoprotein receptor (LDLR) and its closest family members VLDLR and ApoER2. *J. Biol. Chem.* **283**, 2363–2372 (2008).
42. Maxwell, K. N., Fisher, E. A. & Breslow, J. L. Overexpression of PCSK9 accelerates the degradation of the LDLR in a post-endoplasmic reticulum compartment. *Proc. Natl Acad. Sci. USA* **102**, 2069–2074 (2005).
43. Rashid, S. *et al.* Decreased plasma cholesterol and hypersensitivity to statins in mice lacking *Pcsk9*. *Proc. Natl Acad. Sci. USA* **102**, 5374–5379 (2005).
44. Alborn, W. E. *et al.* Serum proprotein convertase subtilisin kexin type 9 is correlated directly with serum LDL cholesterol. *Clin. Chem.* **53**, 1814–1819 (2007).
45. Careskey, H. E. *et al.* Atorvastatin increases human serum levels of proprotein convertase subtilisin/kexin type 9. *J. Lipid Res.* **49**, 394–398 (2008).
46. Welder, G. *et al.* High-dose atorvastatin causes a rapid sustained increase in human serum PCSK9 and disrupts its correlation with LDL cholesterol. *J. Lipid Res.* **51**, 2714–2721 (2010).
47. Brown, M. S. & Goldstein, J. L. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. *Proc. Natl Acad. Sci. USA* **96**, 11041–11048 (1999).
48. Dubuc, G. *et al.* Statins upregulate *PCSK9*, the gene encoding the proprotein convertase neural apoptosis-regulated convertase-1 implicated in familial hypercholesterolemia. *Arterioscler. Thromb. Vasc. Biol.* **24**, 1454–1459 (2004).
49. Nohturfft, A., DeBose-Boyd, R. A., Scheek, S., Goldstein, J. L. & Brown, M. S. Sterols regulate cycling of SREBP cleavage-activating protein (SCAP) between endoplasmic reticulum and Golgi. *Proc. Natl Acad. Sci. USA* **96**, 11235–11240 (1999).
50. Berge, K. E., Ose, L. & Leren, T. P. Missense mutations in the *PCSK9* gene are associated with hypocholesterolemia and possibly increased response to statin therapy. *Arterioscler. Thromb. Vasc. Biol.* **26**, 1094–1100 (2006).
51. Brautbar, A. & Ballantyne, C. M. Pharmacological strategies for lowering LDL cholesterol: statins and beyond. *Nat. Rev. Cardiol.* **8**, 253–265 (2011).
52. Gupta, N. *et al.* A locked nucleic acid antisense oligonucleotide (LNA) silences PCSK9 and enhances LDLR expression *in vitro* and *in vivo*. *PLoS ONE* **5**, e10682 (2010).
53. Graham, M. J. *et al.* Antisense inhibition of proprotein convertase subtilisin/kexin type 9 reduces serum LDL in hyperlipidemic mice. *J. Lipid Res.* **48**, 763–767 (2007).
54. Lindholm, M. W. *et al.* PCSK9 LNA antisense oligonucleotides induce sustained reduction of LDL cholesterol in nonhuman primates. *Mol. Ther.* **20**, 376–381 (2012).
55. Do, R. Q., Vogel, R. A. & Schwartz, G. G. PCSK9 inhibitors: potential in cardiovascular therapeutics. *Curr. Cardiol. Rep.* **15**, 345 (2013).
56. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01350960 (2011).
57. Frank-Kamenetsky, M. *et al.* Therapeutic RNAi targeting PCSK9 acutely lowers plasma cholesterol in rodents and LDL cholesterol in nonhuman primates. *Proc. Natl Acad. Sci. USA* **105**, 11915–11920 (2008).
58. Stein, E. A. & Swergold, G. D. Potential of proprotein convertase subtilisin/kexin type 9 based therapeutics. *Curr. Atheroscler. Rep.* **15**, 310 (2013).
59. Chan, J. C. *et al.* A proprotein convertase subtilisin/kexin type 9 neutralizing antibody reduces serum cholesterol in mice and nonhuman primates. *Proc. Natl Acad. Sci. USA* **106**, 9820–9825 (2009).
60. Gumbiner, B. *et al.* The effects of multiple dose administration of RN316 (PF-04950615), a humanized IgG2a monoclonal antibody binding proprotein convertase subtilisin kexin type 9, in hypercholesterolemic subjects [abstract 13524]. *Circulation* **126**, 2776–2799 (2012).
61. Zhang, L. *et al.* An anti-PCSK9 antibody reduces LDL-cholesterol on top of a statin and suppresses hepatocyte SREBP-regulated genes. *Int. J. Biol. Sci.* **8**, 310–327 (2012).
62. Ni, Y. G. *et al.* A PCSK9-binding antibody that structurally mimics the EGF(A) domain of LDL-receptor reduces LDL cholesterol *in vivo*. *J. Lipid Res.* **52**, 78–86 (2011).
63. Fitzgerald, K. *et al.* Effect of an RNA interference drug on the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: a randomised, single-blind, placebo-controlled, phase 1 trial. *Lancet* **383**, 60–68 (2014).
64. Stein, E. A. *et al.* Effect of a monoclonal antibody to PCSK9 on LDL cholesterol. *N. Engl. J. Med.* **366**, 1108–1118 (2012).
65. Dias, C. S. *et al.* Effects of AMG 145 on low-density lipoprotein cholesterol levels: results from 2 randomized, double-blind, placebo-controlled, ascending-dose phase 1 studies in healthy volunteers and hypercholesterolemic subjects on statins. *J. Am. Coll. Cardiol.* **60**, 1888–1898 (2012).
66. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT00991159 (2012).
67. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01163838 (2010).
68. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01163851 (2011).
69. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01243151 (2012).
70. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01435382 (2012).
71. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01618916 (2013).
72. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01671085 (2013).
73. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01426412 (2013).
74. Gumbiner, B. *et al.* The effects of single dose administration of RN316 (PF-04950615), a humanized IgG2a monoclonal antibody binding proprotein convertase subtilisin kexin type 9, in hypercholesterolemic subjects treated with and without atorvastatin [abstract]. *Circulation* **126**, A13322 (2012).
75. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01890967 (2014).
76. McKenney, J. M. *et al.* Safety and efficacy of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease, SAR236553/REGN727, in patients with primary hypercholesterolemia receiving ongoing stable atorvastatin therapy. *J. Am. Coll. Cardiol.* **59**, 2344–2353 (2012).
77. Roth, E. M., McKenney, J. M., Hanotin, C., Asset, G. & Stein, E. A. Atorvastatin with or without an antibody to PCSK9 in primary hypercholesterolemia. *N. Engl. J. Med.* **367**, 1891–1900 (2012).
78. Stein, E. A. *et al.* Effect of a monoclonal antibody to PCSK9, REGN727/SAR236553, to reduce low-density lipoprotein cholesterol in patients with heterozygous familial hypercholesterolaemia on stable statin dose with or without ezetimibe therapy: a phase 2 randomised controlled trial. *Lancet* **380**, 29–36 (2012).
79. Stein, E. A. *et al.* One year open-label treatment with alirocumab 150 mg every two weeks in heterozygous familial hypercholesterolemic patients [abstract 1183-134]. Presented at ACC Scientific Sessions (2014).
80. Giugliano, R. P. *et al.* Efficacy, safety, and tolerability of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 in combination with a statin in patients with hypercholesterolaemia (LAPLACE-TIMI 57): a randomised, placebo-controlled, dose-ranging, phase 2 study. *Lancet* **380**, 2007–2017 (2012).
81. Koren, M. J. *et al.* Efficacy, safety, and tolerability of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 as monotherapy in patients with hypercholesterolaemia (MENDEL): a randomised, double-blind, placebo-controlled, phase 2 study. *Lancet* **380**, 1995–2006 (2012).
82. Raal, F. *et al.* Low-density lipoprotein cholesterol-lowering effects of AMG 145, a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease in patients with heterozygous familial hypercholesterolemia: the Reduction of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder (RUTHERFORD) randomized trial. *Circulation* **126**, 2408–2417 (2012).
83. Sullivan, D. *et al.* Effect of a monoclonal antibody to PCSK9 on low-density lipoprotein cholesterol levels in statin-intolerant patients: the GAUSS randomized trial. *JAMA* **308**, 2497–2506 (2012).
84. Xu, R. *et al.* Effects of evolocumab on lipoprotein particles and subclasses in hypercholesterolemic and heterozygous familial hypercholesterolemia subjects on statin therapy [abstract 1183-134]. Presented at ACC Scientific Sessions (2014).
85. Gumbiner, B. Effects of 12 weeks of treatment with RN316 (PF-04950615), a humanized IgG2a monoclonal antibody binding proprotein convertase subtilisin kexin type 9, in hypercholesterolemic subjects on high and maximal dose statins. Presented at AHA Scientific Sessions 2012.
86. Ballantyne, C. M. *et al*. Efficacy and safety of bococizumab (RN316/PF-04950615), a monoclonal antibody against proprotein convertase subtilisin/kexin type 9 in statin-treated hypercholesterolemic subjects: results from a randomized, placebo-controlled,

dose-ranging study (NCT: 01592240) [abstract 1183-129]. Presented at ACC Scientific Sessions 2014.
87. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01350141 (2013).
88. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01342211 (2013).
89. Desai, N. R. *et al.* AMG145, a monoclonal antibody against proprotein convertase subtilisin kexin type 9, significantly reduces lipoprotein(a) in hypercholesterolemic patients receiving statin therapy: an analysis from the LDL-C Assessment with Proprotein Convertase Subtilisin Kexin Type 9 Monoclonal Antibody Inhibition Combined with Statin Therapy (LAPLACE)-Thrombolysis in Myocardial Infarction (TIMI) 57 trial. *Circulation* **128**, 962–969 (2013).
90. Roth, E. M. *et al*. A 24-week study of alirocumab as monotherapy versus ezetimibe: the first phase 3 data of a proprotein convertase subtilisin/kexin type 9 inhibitor [abstract 1183-125]. Presented at ACC Scientific Sessions (2014).
91. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01709513 (2013).
92. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01730040 (2013).
93. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01730053 (2013).
94. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01644175 (2013).
95. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01644188 (2014).
96. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01926782 (2014).
97. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01623115 (2013).
98. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01709500 (2013).
99. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01617655 (2013).
100. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01507831 (2013).
101. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01663402 (2014).
102. Koren, M. J. *et al.* Anti-PCSK9 monotherapy for hypercholesterolemia—the MENDEL-2 randomized, controlled phase 3 clinical trial of evolocumab. *J. Am. Coll. Cardiol.* http://dx.doi.org/10.1016/j.jacc.2014.03.018.
103. Stroes, E. *et al.* Anti-PCSK9 antibody effectively lowers cholesterol in patients with statin intolerance: the GAUSS-2 randomized, placebo-controlled phase 3 clinical trial of evolocumab. *J. Am. Coll. Cardiol.* http://dx.doi.org/10.1016/j.jacc.2014.03.019.
104. Blom, D. J. *et al.* A 52-week placebo-controlled trial of evolocumab in hyperlipidemia. *N. Engl. J. Med.* **370**, 1809–1819 (2014).
105. Robinson, J. G. *et al*. The LAPLACE-2 trial: a phase 3, double-blind, randomized, placebo and ezetimibe controlled, multicenter study to evaluate safety, tolerability and efficacy of evolocumab (AMG 145) in combination with statin therapy in subjects with primary hypercholesterolemia and mixed dyslipidemia. Presented at ACC Scientific Sessions (2014).
106. Raal, F. *et al.* The addition of evolocumab (AMG 145) allows the majority of heterozygous familial hypercholesterolemic patients to achieve low-density lipoprotein cholesterol goals—results from the phase 3 randomized, double-blind, placebo-controlled study [abstract 400-005]. Presented at ACC Scientific Sessions (2014).
107. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01854918 (2014).
108. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01813422 (2014).
109. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01764633 (2014).
110. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01588496 (2014).
111. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01624142 (2014).
112. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01953328 (2014).
113. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01968980 (2014).
114. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01968954 (2014).
115. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01968967 (2014).
116. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01975376 (2014).
117. US National Library of Medicine. *ClinicalTrials.gov* [online], http://clinicaltrials.gov/ct2/show/NCT01975389 (2014).
118. Stone, N. J. *et al.* 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J. Am. Coll. Cardiol.* http://dx.doi.org/10.1016/j.jacc.2013.11.002.
119. Pasternak, R. C. *et al.* ACC/AHA/NHLBI clinical advisory on the use and safety of statins. *J. Am. Coll. Cardiol*. **40**, 567–572 (2002).
120. Larosa, J. C., Pedersen, T. R., Somaratne, R. & Wasserman, S. M. Safety and effect of very low levels of low-density lipoprotein cholesterol on cardiovascular events. *Am. J. Cardiol.* **111**, 1221–1229 (2013).
121. Hsia, J., MacFadyen, J. G., Monyak, J. & Ridker, P. M. Cardiovascular event reduction and adverse events among subjects attaining low-density lipoprotein cholesterol <50 mg/dl with rosuvastatin. The JUPITER trial (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin). *J. Am. Coll. Cardiol.* **57**, 1666–1675 (2011).
122. Martin, S. S. *et al.* Friedewald-estimated versus directly measured low-density lipoprotein cholesterol and treatment implications. *J. Am. Coll. Cardiol.* **62**, 732–739 (2013).
123. Sibal, L., Neely, R. D., Jones, A. & Home, P. D. Friedewald equation underestimates low-density lipoprotein cholesterol at low concentrations in young people with and without type 1 diabetes. *Diabet. Med.* **27**, 37–45 (2010).
124. Scharnagl, H., Nauck, M., Wieland, H. & Marz, W. The Friedewald formula underestimates LDL cholesterol at low concentrations. *Clin. Chem. Lab. Med.* **39**, 426–431 (2001).
125. McKinney, J. S. & Kostis, W. J. Statin therapy and the risk of intracerebral hemorrhage: a meta-analysis of 31 randomized controlled trials. *Stroke* **43**, 2149–2156 (2012).
126. Lewington, S. *et al.* Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet* **370**, 1829–1839 (2007).
127. Amarenco, P. *et al.* High-dose atorvastatin after stroke or transient ischemic attack. *N. Engl. J. Med.* **355**, 549–559 (2006).
128. Jones, A. R. & Shusta, E. V. in *Therapeutic Monoclonal Antibodies: From Bench to Clinic* (ed. An, Z.) 483–502 (John Wiley & Sons, 2009).
129. McGuinness, B. *et al.* Statins for the treatment of dementia. *Cochrane Database of Systematic Reviews*, Issue 8, Art. No.: CD007514. http://dx.doi.org/10.1002/14651858.CD007514.pub2.
130. McGuinness, B. *et al.* Cochrane review on 'Statins for the treatment of dementia'. *Int. J. Geriatr. Psychiatry* **28**, 119–126 (2013).
131. Heikkila, P., Kahri, A. I., Ehnholm, C. & Kovanen, P. T. The effect of low- and high-density lipoprotein cholesterol on steroid hormone production and ACTH-induced differentiation of rat adrenocortical cells in primary culture. *Cell Tissue Res.* **256**, 487–494 (1989).
132. Heikkila, P., Kahri, A. I., Kovanen, P. T. & Ehnholm, C. Effects of mevinolin, an inhibitor of cholesterol synthesis, on the morphology and function of differentiating and differentiated rat adrenocortical cells in primary culture. *Cell Tissue Res.* **261**, 125–132 (1990).
133. Roubtsova, A. *et al.* Circulating proprotein convertase subtilisin/kexin 9 (PCSK9) regulates VLDLR protein and triglyceride accumulation in visceral adipose tissue. *Arterioscler. Thromb. Vasc. Biol.* **31**, 785–791 (2011).
134. Mbikay, M. *et al.* PCSK9-deficient mice exhibit impaired glucose tolerance and pancreatic islet abnormalities. *FEBS Lett.* **584**, 701–706 (2010).
135. Labonte, P. *et al.* PCSK9 impedes hepatitis C virus infection *in vitro* and modulates liver CD81 expression. *Hepatology* **50**, 17–24 (2009).
136. Ranheim, T. *et al.* Genome-wide expression analysis of cells expressing gain of function mutant D374Y-PCSK9. *J. Cell. Physiol.* **217**, 459–467 (2008).
137. Lan, H. *et al.* Proprotein convertase subtilisin/kexin type 9 (PCSK9) affects gene expression pathways beyond cholesterol metabolism in liver cells. *J. Cell. Physiol.* **224**, 273–281 (2010).

Author contributions

Both authors researched data for the article, discussed its content, wrote the manuscript, and reviewed/edited the article before submission.