The history of proprotein convertase subtilisin kexin-9 inhibitors and their role in the treatment of cardiovascular disease

Eun Ji Kim and Anthony S. Wierzbicki (ID)

Abstract: A consensus has formed based on epidemiological studies and clinical trials that intervention to reduce low density lipoprotein cholesterol (LDL-C) will reduce cardiovascular disease (CVD) events. This has progressively reduced the thresholds for intervention and targets for treatment. Whist statins are sufficient for many people in primary prevention, they only partially achieve the newer targets of secondary prevention for established CVD. Increasing use of statins has highlighted that 1–2% cannot tolerate these drugs. Other cholesterol-lowering drugs such as ezetimibe add to the benefits of statins but have limited efficacy. The discovery of activating mutations in proprotein convertase subtilisin kexin-9 (PCSK9) as a cause of familial hypercholesterolaemia while inactivating mutations lower LDL-C led to the idea to develop PCSK9 inhibitors as drugs. This article reviews the history of lipid-lowering therapies, the discovery of PCSK9 and the development of PCSK9 inhibitors. It reviews the key trials of the current antibody-based drugs and how these have influenced new guidelines. It also reviews the controversy caused by their cost and the increasing application of health economics to determine the optimum strategy for implementation of novel therapeutic pathways and surveys other options for targeting PCSK9 as well as other LDL-C lowering compounds in late development.

Keywords: cardiovascular disease, cardiovascular risk, cholesterol, ezetimibe, LDL-C, proprotein convertase subtilisin kexin-9 (PCSK9), statins

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Introduction

The history of intervention on low-density lipoprotein cholesterol

The role of lipids in cardiovascular disease (CVD) was established in epidemiological studies such as Framingham or the Munster Heart Study about 50 years ago, but its occurrence had been described in histological specimens of atheromatous plaque about 100 years ago by Anitschow.[1–3] The role of lipids in atherosclerosis has been widely recognised and so the trials of lipid-lowering drugs in patients with moderate elevations of low-density lipoprotein cholesterol (LDL-C) have a long history. In hyperlipidaemia (as opposed to hypertension) genetic causes of elevated lipids are frequent. Familial hypercholesterolaemia (FH) caused by defects affecting the function of the LDL receptor pathway and presenting consequently with elevated LDL-C and premature CVD has an incidence of 1 in 350.[4] Unfortunately, there have been no CVD outcomes studies in FH with any lipid-lowering therapy despite its frequency. The contrast with trials in type 1 diabetes is notable.

Initial attempts at intervention in the 1970s with drugs such as niacin, bile acid sequestrants and clofibrate in patients with moderate hypercholesterolaemia (with or without CVD) were disappointing.[5] Though these early outcome studies often showed reductions in CVD events, no effect on mortality could be demonstrated. This, allied with the adverse effects seen, particularly with clofibrate, led to considerable scepticism about the benefits of lipid-lowering

Correspondence to:
Anthony S. Wierzbicki
Department of Chemical Pathology, St Thomas' Hospital, Westminster Bridge Road, London, SE1 7EH, UK
Anthony.Wierzbicki@kcl. ac.uk

Eun Ji Kim
Department of Metabolic Medicine/Chemical Pathology, Guy's & St Thomas' Hospitals, London, UK

for CVD. The echo of this controversy continues in the popular media to this day. The new statin class of LDL-C lowering therapy with double the efficacy of previous compounds was launched in 1990.[6] The groundbreaking study that resolved the questions about the efficacy of LDL-C lowering in CVD was the Scandinavian Simvastatin Survival Study in 1994.[7] In this study of 4444 patients with chronic coronary heart disease and LDL-C > 5 mmol/l who were treated with simvastatin 20–40 mg to a target LDL-C of 3 mmol/l, statin therapy resulted in a 30% decrease in mortality, and a 30% decrease in CVD mortality, CVD events and interventions as well as hospitalisation. The intervention was also cost-saving to health services.[8] Multiple further trials with statins followed, extending the results to primary prevention [e.g. West of Scotland Coronary Outcomes Prevention Study (WOSCOPS)[9]], patients with type 2 diabetes (e.g. Collaborative Atorvastatin Diabetes Study; CARDS[10]) and progressively lower levels of initial LDL-C in each of these sub-groups. All these studies have now been subjected to pooled individual patient meta-analysis, confirming the benefits in all subgroups and establishing a relationship of 21% reduction in CVD events per 1 mmol/l reduction in LDL-C.[11,12] All these studies pushed the baseline and post-intervention levels for LDL-C progressively lower, but, as a residual risk of CVD events still existed in treated cohorts, the question were still asked about 'whether still lower is better'? The only areas where questions remain about the efficacy of LDL-C lowering with statins are in advanced chronic kidney disease (>CKD 4) or established chronic cardiac failure, though subgroups analysis show benefits in less severely affected population with these conditions.[13]

Non-statin therapies and CVD outcomes
Though these studies conclusively identified the benefits of statin therapy, controversy has raged about other interventions.[14] Surrogate outcome studies confused the story with ezetimibe, although eventually an outcomes study in acute coronary syndromes (ACS) with this drug added to statin therapy did finally confirm its utility. In the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT), ezetimibe therapy allowed patients to reach an average LDL-C of 1.4 mmol/l (*versus* 1.8 mmol/l

in statin-alone treated patients) and reduced CVD events by 8% in line with the regression relationship predicted for the degree of LDL-C change from statins.[15] Studies with other drugs such as anacetrapib, which incidentally reduce LDL-C, also followed the same relationship.[16] Currently, the consensus is that any drug intervention that lowers LDL-C is likely to lower CVD events unless it has off-target side-effects.[3]

Proprotein convertase subtilisin kexin-9
The search for causes of the genetic defect in FH identified mutations in two genes – the LDL receptor and apolipoprotein B – as causing the majority of cases. However, the search continued for other causes, and mutations in proprotein convertase subtilisin kexin-9 [PCSK9; Neural apoptosis-regulated convertase-1 (NARC-1)] were identified.[17] Further work clarified that these mutations activated the protein, causing functional inactivation (enhanced intracellular degradation) of LDL receptors, whereas other inactivating mutations increasing LDL receptor function were associated with lower LDL-C.[18,19] In the Dallas Heart Study, 2.6% of 3363 black patients who had nonsense mutation of PCSK9 leading to a reduction of LDL-C by 28% with better coronary heart disease (CHD) outcomes.[20] A few clinically asymptomatic cases of homozygous PCSK9 deficiency associated with hypolipoproteinaemia have also been described.[21,22] These studies laid the theoretical basis for considering intervention to lower LDL-C by targeting PCSK9 (Figure 1).

Therapies targeting PCSK-9
Once the role of PCSK9 in controlling plasma LDL-C had been established and there were reasons for suspecting that this intervention would be safe, a systematic search began for compounds that would target this pathway.[23–25] PCSK9 exists as a dimer and auto-activates through mutual cleavage of furin-sensitive catalytic domains. The classical approach of small molecule inhibition has proved difficult due to the hydrophobic nature of the compounds required to reach those binding sites,[26] whereas other approaches remain exploratory.[27] Many of these hydrophobic molecules have poor bioavailability as oral compounds need to be water-soluble and for food (fat) effects to be limited to allow licensing.[27] Though no cases of autoimmune-based
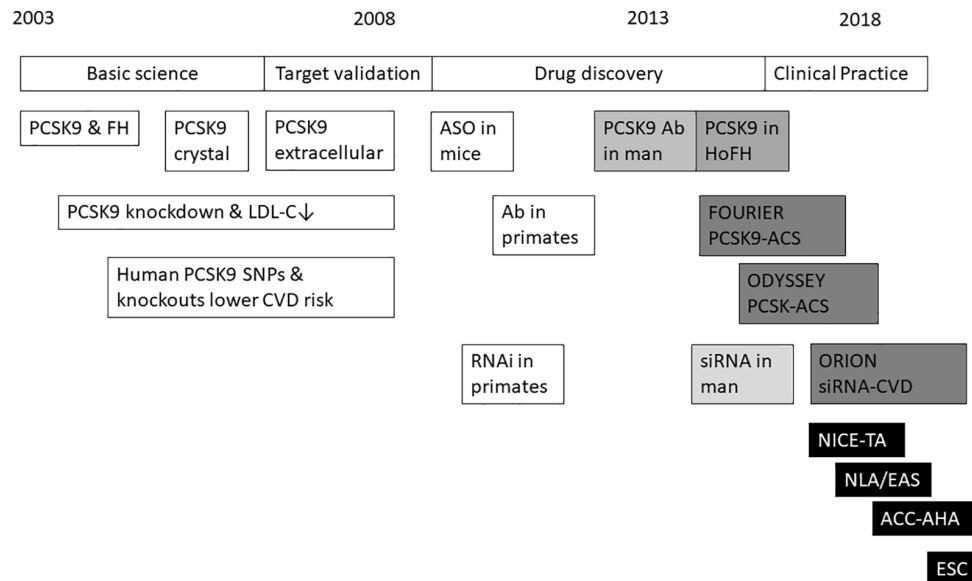
Figure 1. Timeline from PCSK9 discovery to use in clinical practice.
Phase I–II trials in light grey; phase III trials medium grey; CVD outcome studies dark grey; clinical guidelines in black boxes. Ab, antibody; ACS, acute coronary syndrome; ASO, antisense oligonucleotide; CVD, cardiovascular disease; EAS, European Atherosclerosis Society; FH, familial hypercholesterolaemia; HoFH, homozygous FH; NICE, National Institute for Health and Clinical Excellence; NLA, National Lipid Association USA; PCSK9, proprotein convertase subtilisin kexin-9; siRNA, short interfering RNA.

hyper- or hypolipoproteinaemia due to anti-PCSK9 antibodies have been described, animal studies showed that human PCSK9 was antigenic and this allowed the development of a series of antibody-based therapies based on humanised (-zumab) or human (-cumab) antibodies.

Alirocumab and Evolocumab are fully human anti-PCSK9 antibodies and are licensed for clinical practice as they reduce LDL-C by 54% when given fortnightly.[28] As with all antibody therapies, their adverse effects tend to be related to the structure of the antibody, hence causing increases in injection site reaction [1.51 *versus* 0.83 per 100 patient-years; relative risk (RR) 1.41, 95% confidence interval (CI) 1.21–1.65); $p < 0.001$], but also in practice symptoms of flu-like myalgia post-injection (up to 10%).[25,28,29] No compound-specific adverse events have yet been described. High expression of PCSK9 in the cerebellum and gut exists,[30] and it has been suggested that these drugs may be associated with neurological adverse effects, especially neurocognitive deterioration, though again nothing has been seen in the clinical trials to date [0.57 *versus* 0.55 per 100 patient-years; RR 1.01 (0.84–1.21); $p = 0.91$].[28,31]

PCSK9 inhibitors and new-onset diabetes

It took 15 years for the effect of statins in increasing blood glucose by approximately 0.3 mmol/l and hence rates of new diabetes to be noticed.[32] Mendelian randomisation studies that validated the association of variants in the 2-hydroxy-methyl-glutaryl-CoA (HMG-CoA) reductase gene with increased rates of diabetes, possibly related to an increase in body weight,[33] have also suggested that PCSK9 inhibition may cause similar problems.[34] A meta-analysis of 68,123 patients from 20 trials with median follow up of 78 weeks suggested that PCSK9 inhibitors increased fasting blood glucose (1.88 mg/dl; 0.1 mmol/l; $p = 0.001$) and HbA$_{1c}$ by 0.03% ($p < 0.001$) when compared with placebo but, over a 2-year time-scale, this was not sufficient to increase incidence of diabetes [RR 1.04 (0.96–1.13); $p = 0.43$]. An exploratory meta-regression analysis suggested an increased risk of diabetes with the potency ($p = 0.03$) and duration ($p = 0.03$) of PCSK9 inhibitor therapy.[35] However, in a meta-analysis of 39 trials with a weighted follow-up time of 2.3 years comprising 150,617 patient-years, the rate of new-onset of diabetes mellitus was unchanged [1.92 *versus* 1.93 per 100 patient-years

(RR 1.00 (0.93–1.07); $p = 0.97$].[28] Similar results were seen in the long-term clinical CVD outcome studies of these drugs.[28,35] In a pre-specified analysis of the ODSSEY trial by glycaemic status, alirocumab resulted in similar relative reductions in the incidence of the primary endpoint in each glycaemic category. Among patients without diabetes at baseline, no difference was seen in new onset diabetes [10.1 *versus* 9.6%; (HR 1.00 (0.89–1.11].[36] In the FOURIER study, 11,031 patients (40%) had diabetes, 10,344 had pre-diabetes and 6189 were normoglycaemic. No increase was seen in new-onset diabetes in patients without diabetes at baseline (HR 1.05 (0.94–1.17), including in patients with pre-diabetes (HR 1.00 (0.89–1.13). Levels of glucose and HbA_{1c} were similar between evolocumab and placebo over time in all groups.[37]

Antibodies to PCSK-9

However, the humanised antibody, Bococizumab, which contains murine sequence, although initially delivering a 55% reduction in LDL-C like other antibodies, has not been licensed following longer-term studies. This was caused by the attenuation of its effect secondary to development of anti-drug antibodies in the outcome trials (SPIRE-1 and SPIRE-2) with a frequency of 48% after 3 months resulting in titre-dependent attenuation of drug effect.[38,39] Though anti-drug antibodies have been described for human PCSK9 antibodies, none have been associated with loss of efficacy, yet there are growing anecdotal reports of patients who do not respond to PCSK9 therapy.[40] Whereas many of these non-responders have disturbances in lipid metabolism associated with increased synthesis of very-low-density lipoprotein (VLDL), and hence LDL-C, and thus logically might not respond to a drug that increases recycling of cholesterol back to the liver through the LDL receptor and so require additional treatment to correct this problem,[40] some seem to be primary non-responders. No relationship with anti-drug antibodies has been shown in the few cases investigated though potentially enhanced cell-mediated reticulo-endothelial clearance of PCSK9 antibodies through Fc receptors has not been investigated.

PCSK-9 development trials

All PCSK-9 inhibitors have followed the standard phase I–III development programme used for lipid-lowering therapies.[41] After individual dose, multiple dose and dose ranging studies, phase III trials have been conducted in patients with/without CHD, familial hypercholesterolaemia (FH), and type 2 diabetes either compared with placebo, added to baseline statins or in comparison with ezetimibe.[28] Data are still awaited on populations with chronic renal failure and human immunodeficiency virus (HIV). The most interesting studies were in homozygous FH (HoFH) and statin intolerance. Statins, in contrast to ezetimibe, show significantly reduced efficacy in HoFH and do not work in patients with null mutations. Initial studies with PCSK9 inhibition showed substantially preserved efficacy in patents with HoFH due to point mutations in the LDL receptor, reduced efficacy in patients with a single null allele and no response in dual null allele patients.[42] These findings were replicated in larger studies.[43,44] Up to 25% of patients with HoFH can discontinue apheresis,[45] though in most cases apheresis has been continued and LDL-C targets have been adjusted to those used in routine practice.

Statin intolerance is a substantial problem, with a claimed prevalence of 10% (although in reality 1% in re-challenge studies).[46] It is defined as inability to continue to take a statin despite trying three drugs at different doses.[47,48] Two studies have been conducted with PCSK9 inhibitors in this population. In the GAUSS-2 trial, 307 statin-intolerant patients with LDL-C 4.97 mmol/l were randomised to evolucumab or ezetimibe.[49] Evolocumab reduced LDL-C by 53–56%, compared with 14–16% for ezetimibe. Muscle adverse events occurred in 12% of evolocumab-treated patients and 23% of ezetimibe-treated patients. The larger GAUSS-3 study in 511 patients reproduced these effects.[50] In the ODYSSEY-alternative study, 361 statin-intolerant patients received an injection and oral placebo for 4 weeks during placebo run-in.[51] Patients (13%) reporting muscle-related symptoms during the run-in were to be withdrawn. Continuing patients were randomised (2:2:1) to alirocumab 75 mg fortnightly (with option to increase), ezetimibe 10 mg, or atorvastatin 20 mg/day with appropriate placebos for 24 weeks and then open follow up. The baseline LDL-C was 5.0 mmol/l. Alirocumab reduced LDL-C by 52% *versus* 17% with ezetimibe. Treatment was discontinued by 18% with alirocumab and 25% for ezetimibe or statin therapy. Skeletal muscle-related events were seen in 16%

with alirocumab compared with 20% with ezetimibe and 22% with atorvastatin. Open label statin was well tolerated by those patients who were unblinded at study completion.

PCSK-9 cardiovascular outcomes trials

There are increasing challenges in designing outcome trials in CVD. Progressive lowering of acceptable LDL-C levels for recruitment into studies as a result of guidelines and ethical considerations leads to lower event rates and thus potentially larger trial populations. It is now increasingly common to concentrate on high-risk sub-groups such as patients with ACS and to require the presence of additional CVD risk factors such as increased age, smoking, or type 2 diabetes. All of these changes make the trials possible in terms of timescales, logistics, and finance, but cause difficulties for guideline groups that have to generalise the findings outside the original trial population. The recent PCSK9 trials follow these design precepts.

FOURIER trial: evolocumab and CVD outcomes

The FOURIER recruited 27,564 patients with a history of recent atherosclerotic vascular disease (cerebrovascular, cardiovascular and peripheral artery disease) but also an additional one major or two minor risk factors allied with a LDL-C > 1.8 mmol/l (70 mg/dl) or nonHDL-C > 2.6 mmol/l (100 mg/dl).[52] Additional risk factors included hypertension (80%), type 2 diabetes (37%) and smoking (29%). These patients had to be receiving statin therapy of at least 20 mg of atorvastatin with or without ezetimibe, but only 70% were on high-intensity statin. In reality, given questions about the efficacy of ezetimibe at the time, 5% were on this treatment. Patients were randomised to evolocumab injections (either 140 mg every 2 weeks or 420 mg every 4 weeks) or matching placebo. The primary endpoint was a combination of fatal and non-fatal myocardial infarction (MI) and stroke (cerebrovascular accident; CVA) including unstable angina (UAS) warranting hospital admission or coronary revascularisation. The secondary outcome excluded revascularisation episodes. LDL-C decreased by 59% from 2.2 mmol/l (92 mg/dl) to 0.8 mmol/l (30 mg/dl) in the evolocumab group, while non-HDL-C levels decreased by 52% and ApoB levels by 49%. Evolocumab reduced the five-point primary endpoint (9.8% *versus* 11.3%; HR 0.85; $p < 0.001$) and secondary endpoint (a composite of CVD death, MI, or CVA) (5.9% *versus* 7.4%; HR 0.80; $p < 0.001$) at a median follow up of 2.2 years. This was driven by reduction in the risk of non-fatal MI, CVA and coronary revascularisation. Few serious adverse events were found, with no difference in new-onset diabetes or neurocognitive events. However, injection-site reactions were more common with evolocumab (2.1% *versus* 1.6%).

Major CVD events declined progressively with decreasing LDL-C concentrations achieved, with adjusted HR in the group with LDL-C < 0.2 mmol/l (10 mg/dl) of 0.69 for the primary and 0.59 for secondary endpoints compared with those with LDL-C > 2.6 mmol/l (100 mg/dl).[53] High-risk subgroups such as those with differing extent of coronary artery disease,[54] type 2 diabetes mellitus[37] or peripheral arterial disease[55] had higher event rates and consequently greater absolute benefits with evolucumab therapy.

ODYSSEY-outcomes: alirocumab and CVD outcomes

The ODYSSEY Outcomes trial randomised 18,924 patients with history of an ACS within the previous 12 months to either alirocumab 75 mg or placebo every 2 weeks. Inclusion criteria included either LDL-C > 1.8 mmol/l (70 mg/dl), nonHDL-C > 2.6 mmol/l (100 mg/dl) or ApoB > 80 mg/dl after maximal statin therapy (atorvastatin 40–80 mg or rosuvastatin 20–40 mg).[56] The primary composite end-point included death from CAD or CVD, myocardial infarction, ischemic stroke or angina requiring hospital admission. Most patients (93%) were enrolled due to LDL-C > 1.8 mmol/l (70 mg/dl). The on-treatment LDL-C target was 0.6–1.3 mmol/l (25–50 mg/dl). Alirocumab was up-titrated from 75 mg to 150 mg every 2 weeks in patients who had LDL > 1.3 mmol/l (50 mg/dl). Conversely, patients who had LDL-C levels consistently below 0.2 mmol/l (15 mg/dl) were switched to placebo. After a median follow up of 2.8 years, LDL-C levels had decreased by 55% to 1.3 mmol (53 mg/dl) from 2.4 mmol/l (101 mg/dl). The primary end-point was significantly lower in the alirocumab group compared with the placebo group (9.5% *versus* 11.1%, HR 0.85, $p = 0.0003$) after a median 2.8 years, with benefit seen only after 1 year of treatment as in statin trials. The reduction in MACE with alirocumab was primarily because of

reduction in ischaemic events: nonfatal MI was reduced by 14%, CVA by 27% and UAS by 39%. All-cause mortality was reduced by 15% (3.5% *versus* 4%, $p = 0.03$) but CAD (2.2% *versus* 2.3%) and CVD (2.5% *versus* 2.9%) deaths were similar. Minor injection site reactions were commoner with alirocumab (3.1 *versus* 2.1%). In patients with baseline LDL-C > 2.6 mmol/l (100 mg/dl) comprising 30% of total, CVD events were reduced by 24%, with all endpoints showing reductions, including CAD death and CVD death by 28% and 31%, respectively.

In a pre-specified analysis of ODYSSEY Outcomes study, the total number of nonfatal CVD events and deaths prevented with alirocumab was twice the number of first events prevented.[57] In this study, 3064 first and 5425 total events occurred in the whole cohort. In the alirocumab arm, there were 190 fewer first and 385 fewer total nonfatal CVD events or deaths. Alirocumab thus decreased the occurrence of first, but also subsequent, CVD events.

An overview of PCSK9 outcome trials
Both trials reduced primary composite CVD end points. The Kaplan–Meier curves diverged after 12 months and the effects accumulated as the length of treatment extended. The incidence of primary composite end points in the FOURIER trial decreased by 15 (12–27)%, similar to the 12 (7–16)% achieved during the first year of treatment with statins in the Cholesterol Treatment Trialists' (CTT) meta-analysis.[52] Similar results were obtained in ODYSSEY outcomes, indicating that the CVD event, i.e. change in LDL-C relationship (21% relative risk reduction per 1 mmol/LDL-C reduction), held for these drugs as well as statins and ezetimibe.

As usual for lipid trials, the greatest effects with PCSK9 inhibitors were seen on coronary intervention and non-fatal outcomes.[58] In a meta-analysis of 83,321 patients, PCSK9 inhibitor therapy did not reduce all-cause mortality [RR 0.94 (0.81–1.09); $p = 0.41$], even after excluding the bococizumab studies.[59] Predictably, this risk reduction varied with baseline LDL-C, in showing a significant reduction only in patients with LDL-C > 100 mg/dl (2.4 mmol/l) [RR 0.39 (0.20–0.76); $p = 0.01$]. PCSK9 inhibitor therapy showed no significant effect on CVD mortality, but, after

regrouping ODYSSEY OUTCOME estimates, again an effect restricted to patients with LDL-C > 100 mg/dl (2.4 mmol/l) [RR 0.67 (0.51–0.87); $p = 0.006$] was detected.[59]

Concerns have been expressed about the association of low LDL-C with excess CVD risk, especially for haemorrhagic stroke.[60] The PCSK-9 endpoint trials allowed this to be explored, though the ODYSSEY trials did have a compulsory dose reduction if achieved LDL-C was 0.5 mmol/l (20 mg/dl). In the FOURIER study, 8003 (31%) patients achieved concentrations of 0.5–1.3 mmol/l.[53] The relationship between lower LDL-C and lower risk of CVD endpoints continued and extended to the bottom first percentile (LDL-C < 0.2 mmol/l. No excess adverse events, including haemorrhagic strokes, were seen. This confirms the safety of achieving ultra-low LDL-C in patients with ACS.

Insights of PCSK-9 trials into optimal targets
Imaging-based studies have a long history in CVD studies. Originally studies were preformed using quantitative coronary angiography, and these showed the benefits of statin, fibrate, niacin and bile acid sequestrant treatment as well as strict LDL-C-lowering diets. The technology evolved and later trials were conducted using intravascular ultrasound (IVUS) in patients with one 50% coronary artery stenosis over a 2-year time period. The first trial using this methodology was the Reversal of Atherosclerosis With Lipitor (REVERSAL) trial comparing Pravastatin 40 mg with Atorvastatin 80 mg.[61] This data was later analysed by achieved LDL-C, and regression began to be seen with LDL-C < 1.8 mmol/l.[62,63]

Other groups performed similar studies with ezetimibe added to statin therapy. The largest is the plaque regression with cholesterol absorption inhibitor or synthesis inhibitor evaluated by intravascular ultrasound (PRECISE-IVUS) trial in 202 patients with previous CAD intervention.[64] Statin therapy was optimised to deliver LDL-C < 1.8 mol/l and patients were randomised to ezetimibe or placebo for 1 year and followed by IVUS imaging. The achieved LDL-C was 1.6 mmol/l (63 mg/dl) compared with 1.9 mmol/l (73 mg/dl) resulting in percent atheroma volume (PAV) change of 1.54 (0.003–3.08)%, which did

not exceed the pre-defined noninferiority margin of 3%. The absolute change with statin-ezetimibe in PAV was −1.4% *versus* −0.3% with statin alone ($p = 0.001$) and 78% *versus* 58% ($p = 0.004$) patients showed evidence of regression.

A similar study has been conducted with a PCSK-9 inhibitor. The global assessment of plaque regression with a PCSK9 antibody as measured by intravascular ultrasound (GLAGOV) trial randomised 968 patients aged 60 years with angiographic CAD to 420 mg monthly or placebo on top of statin therapy for 76 weeks.[65] The evolucumab group achieved a LDL-C of 0.9 mmol/l (37 mg/dl) compared with 2.2 mmol/l (93 mg/dl) in the placebo group. Evolocumab treatment reduced PAV by 1.0% overall, inducing 0.95% regression compared with a 0.05% increase in the placebo group. In 144 patients with LDL-C levels < 1.8 mmol/l (70 mg/dl) the change in PAV was more marked (−1.97% *versus* −0.35%; $p < 0.001$), with 81% of patients on evolocumab showing plaque regression. The study confirmed that evolocumab added to statin therapy induced significant plaque regression.

Health economics of PCSK-9 therapy

The main problem with PCSK-9 therapies has been their substantial price differential compared with other agents used in CVD medicine.[66] Though priced similarly to other antibody-based therapies, this has provoked resistance from payers and caused controversy within guidelines groups. Health economic analyses show that off-patent drug therapies are highly cost-effective in primary prevention, whereas in secondary prevention on-patent therapies costing about $100/month are cost effective in ACS but not chronic CVD if a low threshold of $30,000 ($30k) per quality adjusted life year (QALY) is used as in the United Kingdom (UK).[67] Using thresholds more typical of the United States (US) at $50–100k/QALY, these drugs may be cost effective in higher-risk secondary prevention but are limited in primary prevention.[68,69] Analyses using the initial process of PCSK-9 inhibitors suggested poor cost-effectiveness at a $150k threshold unless optimistic assumptions were made.[70,71] Value-based pricing analyses in the US suggested that a price in the region of $5–6k was reasonable given their benefits.[70] Prices for PCSK-9 inhibitors have since been reduced in the US from $15–16k

per year to a new global price in the region of $6k, rendering these drugs more acceptable to payers. European payers have negotiated further discounts to improve the acceptability of these drugs in social insurance/government-funded health systems, but concerns about price still limit access to these medications in many countries.[69,72]

The outcome of these concerns about the affordability of PCSK-9 inhibitors has been a tendency towards underuse of these drugs compared with specialist society guideline recommendations at 1–2% of the population concentrated on the highest risk groups.[73–75] However, significant differences are likely to arise between payer-derived guidelines [e.g. UK National Institute for Health and Clinical Excellence (NICE)] and specialist societies that do not need to consider reimbursement.[76] The gap between clinical desires and financial affordability opens the way to alternative methods of lowering LDL-C.

Alternative approaches to lowering LDL-C

Data from imaging and CVD outcomes studies suggest that most therapies that reduce LDL-C will also reduce CVD events. This has encouraged the development of further compounds that reduce LDL-C.

Reduction of LDL-C with small interfering RNA therapy: inclisiran

An alternative approach to inhibiting PCSK9 in plasma with antibodies is to inhibit translation of the protein in the cytoplasm by interfering with the messenger RNA (mRNA).[77] Theoretically, this has the advantage that this will inhibit the intracellular activities of PCSK9, whereas antibodies only affect the extracellular compartment. Two approaches exist to inhibit the mRNA. The longer established strategy in lipids is to inhibit mRNA translation through the use of subcutaneously administered antisense oligonucleotides (ASOs). The alternative is to use short interfering RNA (siRNA). The siRNA blocks translation of PCSK9 mRNA and promotes RNA degradation through RNA-induced silencing complex (RISC), in contrast with ASOs, which use a RNAse H1-based degradation pathway.[77,78] Further targeting specificity can be added through a n-acetyl-glucosamine (NAG) moiety that targets the drug to hepatocytes, enabling siRNA doses to be reduced

compared with untargeted ASOs. Unfortunately, ASO approaches to PCSK9 have proved unsuccessful due to high rates of injection-site reactions and renal tubular toxicity.[79]

Inclisiran is a long-acting siRNA molecule targeting PCSK9 mRNA. A dose-ranging study of several doses of inclisiran in adult healthy volunteers with an LDL-C > 2.6 mmol/l (100 mg/dl) showed that multiple-dose regimens of inclisiran reduced levels of PCSK9 and LDL-C by 84% and 60% from baseline at day 84.[80] Doses ≥300 mg reduced PCSK9 and LDL-C levels for at least 6 months. No serious adverse events were seen with inclisiran, and the most common adverse events in the trial were cough, musculoskeletal pain, nasopharyngitis, headache, back pain and diarrhea. This study prompted further development of inclisiran. In the ORION-1 study of 501 patients, inclisiran produced dose-dependent reductions in PCSK9 levels and LDL-C.[81] The greatest reduction in LDL-C was seen with two-doses of 300 mg inclisiran regimen (first dose at day 1 and second dose at day 90), resulting in 48% of patients achieving LDL-C < 1.2 mmol/l (50 mg/dl) at 6 months to 1 year.[82] Various phase III studies with inclisiran are ongoing. The ORION-4 study is randomising 15,000 patients aged >55 years with pre-existing CVD to inclisiran or placebo.[83] They will be followed up for 5 years for safety and efficacy in preventing major adverse CVD events. More than 1550 patient-years of safety data have been accumulated in the ORION phase III program with no significant adverse safety signals.

PCSK9 vaccines
As PCSK9 is antigenic and can be used to produce human antibodies, then it is feasible to attempt a long-term vaccine strategy to accomplish similar effects on a permanent basis. Initial experimental results in animals vaccinated with bacteriophage virus-like particle (VLP) conjugated with PCSK9 epitopes adjacent to the LDLR binding domain have been encouraging.[84] Immunisation against PCSK9 in various mouse models has been shown to decrease cholesterol and LDL-C levels, reduce development of CVD, and decrease systemic and vascular inflammation.[85] However, no human studies have yet been performed.

PCSK9 gene therapy
Individuals homozygous for PCSK9 deficiency have been identified and have clinical phenotypes of being healthy though profoundly hypocholesterolaemic.[21] Thus, clinical intervention to knock out PCSK9 using gene therapy technology such as Clustered regularly interspaced short palindromic repeat-(CRISPR)-associated protein -9 (CRISPR-Cas9) may be feasible to deliver a cure for conditions such as FH.[86,87] Again, no studies in man have yet been performed.

Bempedoic acid
As there is considerable confidence in the LDL-C hypothesis, and as statins target the cholesterol synthesis pathway, it may be possible to further inhibit this pathway.[88] Originally, studies concentrated on squalene synthase inhibition later in the pathway, but these drugs (e.g. zaragozic acid, lapaquistat) proved to have limited efficacy and potentially high toxicity.[89]

The newest development is to target the step before HMG-coA reductase in the liver: ATP-citrate lyase. Bempedoic acid is an oral inhibitor of hepatic ATP citrate lyase, which decreases LDL-C levels by 17% in patients receiving maximally tolerated statin therapy at 12 weeks without obvious serious adverse events.[90] In statin-intolerant patients, bempedoic acid added to other lipid lowering therapy, including ezetimibe, was well tolerated and resulted in 24% lower LDL-C compared with placebo or when added to ezetimibe therapy again after 12 weeks.[91] Longer-term safety has been demonstrated in 2230 patients.[92] This drug is now being evaluated in a CVD outcomes study.

Implications of PCSK-9 trials for guidelines
Lipid guidelines have generally followed the 50th centile of achieved LDL-C from the CVD outcome trials as a target. Thus, targets fell rapidly from 3 mmol/l in secondary prevention (4S) to 1.8–2.0 mmol/l (CARE and LIPID studies).[76] The high-dose compared with low-dose statin studies in secondary prevention reinforced the 1.8–2.0 mmol/l target especially after the CTT meta-analysis was published, but did not reduce the targets further despite the analysis showing continuing linear relative risk reduction down to 1.5 mmol/l. The results of recent studies, such as IMPROVE-IT or the

PCSK-9 trials, in ACS are just beginning to be implemented in the guidelines.[93,94] Almost all new guidelines have agreed that ezetimibe should be added to statin therapy in ACS as it is moderately effective and off-patent, and thus cheap.[93,94] UK NICE is the only exception as its appraisal is dated.

Incorporation of PCSK-9 trials into guidelines has proved problematic, due primarily to cost considerations. Specialist societies later followed by guidelines have based their recommendations on the trial populations by creating the concept of high-risk groups of patients with CVD distinguished by excess vascular risk (ACS or multivascular bed disease) hyperlipidaemia and additional risk factors.[95–97] The American and European society guidelines,[93,94] like their specialist lipid societies, introduced the concept of ultra-high-risk groups based on the secondary prevention populations recruited to trials, whereas others have suggested the use of number needed to treat (NNT) thresholds, which are a proxy for CVD risk.[98] Patients with FH or recurrent ACS, LDL-C > 2.5 mmol/l and additional risk factors qualify for treatment. European guidelines have gone further by extending the concept to ultra-high primary prevention. UK NICE is conservative in preserving the concept of one category of established CVD and suggesting prescription in FH with LDL-C > 5 mmol/l or patients with CVD and LDL-C > 4 mmol/l for monovascular or > 3.5 mmol/l in multi-vascular bed or recurrent disease in patients despite maximally tolerated statin and ezetimibe.

These guidelines might be thought to substantially increase the population eligible for PCSK-9 therapy. Currently, the UK NICE guidelines suggest 2% of patients with CVD may be eligible for PCSK9 inhibitors despite 29% not achieving LDL-C targets of <2 mmol/l,[99] whereas a Canadian analysis suggests up to 52% may require PCSK-9 therapy.[72] However, in both FOURIER and ODYSSEY, lipid-lowering therapy was not maximised prior to randomisation and ezetimibe usage was low. In IMPROVE-IT, baseline lipid levels were similar to the PCSK-9 trials at a LDL-C of 2.43 mmol/l (94/dl), but optimisation of statin therapy (simvastatin only) reduced this to 1.81 mmol/l (67 mg/dl), whereas addition of ezetimibe allowed a mean LDL of 1.38 mmol/l (53 mg/dl) to be achieved, in line with suggested targets from both major guidelines. The only major difference between these guidelines is in the new European guidelines, which suggests a lower target of 1 mmol/l for LDL-C in patients with recurrent ACS within 1 year of initial intervention based on the PCSK9 trials.

All the guidelines and some advisory strategies concentrate on lipid-lowering as part of their brief, in effect focusing on NNT and, hence, underlying CVD risk.[98] These approaches neglect the substantial benefits seen on CVD mortality and events seen in some sub-groups from non-lipid-lowering-based therapies. Sodium-glucose lithium transporter-2 (SGLT-2) inhibitors reduce mortality, heart failure and CVD events in patients with diabetes,[100] whereas glucagon-like peptide-1 (GLP-1) agonists reduce CVD events.[101] These patients may encompass 25–30% of eligible patients based on PCSK-9 trial recruitment. Furthermore, other interventions such as high-dose omega-3 fatty acids (reduction of cardiovascular events with EPA-intervention trial; REDUCE-IT) in patients with moderate hypertriglyceridaemia (which overlap with PCSK-9 trial recruitment criteria) also reduce CVD events.[102] Other data suggest that more aggressive anti-thrombotic therapy or even colchicine therapy may further reduce the eligible population by reducing CVD events from other causes without needing to prescribe expensive drugs.[103] Thus, the eligible population for PCSK9 inhibitors is, in reality, patients with hypercholesterolaemia who are statin-intolerant or those with FH.

Conclusion

PCSK-9 inhibitors are a major innovation in lipid management and are undoubtedly effective in reducing both CVD events and LDL-C by 50% with acceptable rates of discontinuation. Uptake of these drugs has been slow, driven by concerns about affordability from payers, but increasing flexibility on pricing is helping to resolve this in many countries. New PCSK-9 interventions are in development, as are alternative oral methods of lowering LDL-C. Though PCSK-9 inhibitors will undoubtedly have a place in lipid management, it is unclear as to whether they will be universally used or just prescribed to selected high-risk groups.

ORCID iD
Anthony S. Wierzbicki iD https://orcid.org/0000-0003-2756-372X

References

1. Steinberg D. Thematic review series: the pathogenesis of atherosclerosis. An interpretive history of the cholesterol controversy: part II: the early evidence linking hypercholesterolemia to coronary disease in humans. *J Lipid Res* 2005; 46: 179–190.

2. Steinberg D. Thematic review series: the pathogenesis of atherosclerosis: an interpretive history of the cholesterol controversy, part III: mechanistically defining the role of hyperlipidemia. *J Lipid Res* 2005; 46: 2037–2051.

3. Ference BA, Ginsberg HN, Graham I, *et al.* Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic and clinical studies. A consensus statement from the European atherosclerosis society consensus panel. *Eur Heart J* 2017; 38: 2459–2472.

4. Defesche JC, Gidding SS, Harada-Shiba M, *et al.* Familial hypercholesterolaemia. *Nat Rev Dis Primers* 2017; 3: 17093.

5. Steinberg D. The pathogenesis of atherosclerosis. An interpretive history of the cholesterol controversy, part IV: the 1984 coronary primary prevention trial ends it–almost. *J Lipid Res* 2006; 47: 1–14.

6. Steinberg D. Thematic review series: the pathogenesis of atherosclerosis. An interpretive history of the cholesterol controversy, part V: the discovery of the statins and the end of the controversy. *J Lipid Res* 2006; 47: 1339–1351.

7. The Scandinavian Simvastatin Survival Study (4S) investigators. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian simvastatin survival study (4S). *Lancet* 1994; 344: 1383–1389.

8. Johannesson M, Jonsson B, Kjekshus J, *et al.* Cost effectiveness of simvastatin treatment to lower cholesterol levels in patients with coronary heart disease. Scandinavian simvastatin survival study group. *N Engl J Med* 1997; 336: 332–336.

9. Shepherd J, Cobbe SM, Ford I, *et al.* Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland coronary prevention study group. *N Engl J Med* 1995; 333: 1301–1307.

10. Colhoun HM, Betteridge DJ, Durrington PN, *et al.* Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. *Lancet* 2004; 364: 685–696.

11. Baigent C, Keech A, Kearney PM, *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* 2005; 366: 1267–1278.

12. Baigent C, Blackwell L, Emberson J, *et al.* Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet* 2010; 376: 1670–1681.

13. Collins R, Reith C, Emberson J, *et al.* Interpretation of the evidence for the efficacy and safety of statin therapy. *Lancet* 2016; 388: 2532–2561.

14. Lloyd-Jones DM, Morris PB, Ballantyne CM, *et al.* 2017 focused update of the 2016 ACC expert consensus decision pathway on the role of non-statin therapies for LDL-cholesterol lowering in the management of atherosclerotic cardiovascular disease risk: a report of the American college of cardiology task force on expert consensus decision pathways. *J Am Coll Cardiol* 2017; 70: 1785–1822.

15. Cannon CP, Blazing MA, Giugliano RP, *et al.* Ezetimibe added to statin therapy after acute coronary syndromes. *N Engl J Med* 2015; 372: 2387–2397.

16. HPS3/TIMI55–REVEAL Collaborative Group, Bowman L, Hopewell JC, *et al.* Effects of anacetrapib in patients with atherosclerotic vascular disease. *N Engl J Med* 2017; 377: 1217–1227.

17. Abifadel M, Varret M, Rabes JP, *et al.* Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet* 2003; 34: 154–156.

18. Cariou B, Ouguerram K, Zair Y, *et al.* PCSK9 dominant negative mutant results in increased LDL catabolic rate and familial hypobetalipoproteinemia. *Arterioscler Thromb Vasc Biol* 2009; 29: 2191–2197.

19. Cariou B, Le MC and Costet P. Clinical aspects of PCSK. *Atherosclerosis* 2011; 216: 258–265.

20. Cohen JC, Boerwinkle E, Mosley TH Jr, *et al.* Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med* 2006; 354: 1264–1272.

21. Zhao Z, Tuakli-Wosornu Y, Lagace TA, *et al.* Molecular characterization of loss-of-function mutations in PCSK9 and identification of a compound heterozygote. *Am J Hum Genet* 2006; 79: 514–523.

22. Hooper AJ, Marais AD, Tanyanyiwa DM, *et al.* The C679X mutation in PCSK9 is present and lowers blood cholesterol in a Southern African population. *Atherosclerosis* 2007; 193: 445–448.

23. Warden BA, Fazio S and Shapiro MD. The PCSK9 revolution: current status, controversies, and future directions. *Trends Cardiovasc Med* 2019; 30: 179–185.

24. Blanchard V, Khantalin I, Ramin-Mangata S, *et al.* PCSK9: from biology to clinical applications. *Pathology* 2019; 51: 177–183.

25. Norata GD, Tibolla G and Catapano AL. Targeting PCSK9 for hypercholesterolemia. *Annu Rev Pharmacol Toxicol* 2014; 54: 273–293.

26. Min DK, Lee HS, Lee N, *et al.* In silico screening of chemical libraries to develop inhibitors that hamper the interaction of PCSK9 with the LDL receptor. *Yonsei Med J* 2015; 56: 1251–1257.

27. Petersen DN, Hawkins J, Ruangsiriluk W, *et al.* A small-molecule anti-secretagogue of PCSK9 targets the 80S ribosome to inhibit PCSK9 protein translation. *Cell Chem Biol* 2016; 23: 1362–1371.

28. Guedeney P, Giustino G, Sorrentino S, *et al.* Efficacy and safety of alirocumab and evolocumab: a systematic review and meta-analysis of randomized controlled trials. *Eur Heart J.* Epub ahead of print 3 July 2019. DOI: 10.1093/eurheartj/ehz430.

29. Rosenson RS, Hegele RA, Fazio S, *et al.* The evolving future of PCSK9 inhibitors. *J Am Coll Cardiol* 2018; 72: 314–329.

30. Stoekenbroek RM, Lambert G, Cariou B, *et al.* Inhibiting PCSK9 - biology beyond LDL control. *Nat Rev Endocrinol* 2018; 15: 52–62.

31. Giugliano RP, Mach F, Zavitz K, *et al.* Cognitive function in a randomized trial of evolocumab. *N Engl J Med* 2017; 377: 633–643.

32. Sattar N, Preiss D, Murray HM, *et al.* Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. *Lancet* 2010; 375: 735–742.

33. Swerdlow DI, Preiss D, Kuchenbaecker KB, *et al.* HMG-coenzyme a reductase inhibition, type 2 diabetes, and bodyweight: evidence from genetic analysis and randomised trials. *Lancet* 2015; 385: 351–361.

34. Schmidt AF, Swerdlow DI, Holmes MV, *et al.* PCSK9 genetic variants and risk of type 2 diabetes: a mendelian randomisation study. *Lancet Diabetes Endocrinol* 2017; 5: 97–105.

35. de Carvalho LSF, Campos AM and Sposito AC. Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors and incident type 2 diabetes: a systematic review and meta-analysis with over 96,000 patient-years. *Diabetes Care* 2018; 41: 364–367.

36. Ray KK, Colhoun HM, Szarek M, *et al.* Effects of alirocumab on cardiovascular and metabolic outcomes after acute coronary syndrome in patients with or without diabetes: a prespecified analysis of the ODYSSEY OUTCOMES randomised controlled trial. *Lancet Diabetes Endocrinol* 2019; 7: 618–628.

37. Sabatine MS, Leiter LA, Wiviott SD, *et al.* Cardiovascular safety and efficacy of the PCSK9 inhibitor evolocumab in patients with and without diabetes and the effect of evolocumab on glycaemia and risk of new-onset diabetes: a prespecified analysis of the FOURIER randomised controlled trial. *Lancet Diabetes Endocrinol* 2017; 5: 941–950.

38. Ridker PM, Revkin J, Amarenco P, *et al.* Cardiovascular efficacy and safety of bococizumab in high-risk patients. *N Engl J Med* 2017; 376: 1527–1539.

39. Ridker PM, Tardif JC, Amarenco P, *et al.* Lipid-reduction variability and antidrug-antibody formation with bococizumab. *N Engl J Med* 2017; 376: 1517–1526.

40. Kohli M, Patel K, MacMahon Z, *et al.* Pro-protein subtilisin kexin-9 (PCSK9) inhibition in practice: lipid clinic experience in 2 contrasting UK centres. *Int J Clin Pract* 2017; 71: e13032.

41. Schmidt AF, Pearce LS, Wilkins JT, *et al.* PCSK9 monoclonal antibodies for the primary and secondary prevention of cardiovascular

disease. *Cochrane Database Syst Rev* 2017; 4: CD011748.

42. Stein EA, Honarpour N, Wasserman SM, *et al.* Effect of the proprotein convertase subtilisin/kexin 9 monoclonal antibody, AMG 145, in homozygous familial hypercholesterolemia. *Circulation* 2013; 128: 2113–2120.

43. Raal FJ, Honarpour N, Blom DJ, *et al.* Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolaemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial. *Lancet* 2015; 385: 341–350.

44. Hartgers ML, Defesche JC, Langslet G, *et al.* Alirocumab efficacy in patients with double heterozygous, compound heterozygous, or homozygous familial hypercholesterolemia. *J Clin Lipidol* 2018; 12: 390–396.e8.

45. Tselmin S, Julius U, Weinert N, *et al.* Experience with proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i) in patients undergoing lipoprotein apheresis. *Atheroscler Suppl* 2019; 40: 38–43.

46. Zhang H, Plutzky J, Skentzos S, *et al.* Discontinuation of statins in routine care settings: a cohort study. *Ann Intern Med* 2013; 158: 526–534.

47. Stroes ES, Thompson PD, Corsini A, *et al.* Statin-associated muscle symptoms: impact on statin therapy-European atherosclerosis society consensus panel statement on assessment, aetiology and management. *Eur Heart J* 2015; 36: 1012–1022.

48. Newman CB, Preiss D, Tobert JA, *et al.* Statin safety and associated adverse events: a scientific statement from the American heart association. *Arterioscler Thromb Vasc Biol* 2019; 39: e38–e81.

49. Stroes E, Colquhoun D, Sullivan D, *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* 2014; 63: 2541–2548.

50. Nissen SE, Stroes E, Dent-Acosta RE, *et al.* Efficacy and tolerability of evolocumab vs ezetimibe in patients with muscle-related statin intolerance: the GAUSS-3 randomized clinical trial. *JAMA* 2016; 315: 1580–1590.

51. Moriarty PM, Thompson PD, Cannon CP, *et al.* Efficacy and safety of alirocumab vs ezetimibe in statin-intolerant patients, with a statin rechallenge arm: the ODYSSEY ALTERNATIVE randomized trial. *J Clin Lipidol* 2015; 9: 758–769.

52. Sabatine MS, Giugliano RP, Keech AC, *et al.* Evolocumab and clinical outcomes in patients with cardiovascular disease. *N Engl J Med* 2017; 376: 1713–1722.

53. Giugliano RP, Pedersen TR, Park JG, *et al.* Clinical efficacy and safety of achieving very low LDL-cholesterol concentrations with the PCSK9 inhibitor evolocumab: a prespecified secondary analysis of the FOURIER trial. *Lancet* 2017; 390: 1962–1971.

54. Sabatine MS, De Ferrari GM, Giugliano RP, *et al.* Clinical benefit of evolocumab by severity and extent of coronary artery disease. *Circulation* 2018; 138: 756–766.

55. Bonaca MP, Nault P, Giugliano RP, *et al.* Low-density lipoprotein cholesterol lowering with evolocumab and outcomes in patients with peripheral artery disease: insights from the FOURIER trial (further cardiovascular outcomes research with PCSK9 inhibition in subjects with elevated risk). *Circulation* 2018; 137: 338–350.

56. Schwartz GG, Steg PG, Szarek M, *et al.* Alirocumab and cardiovascular outcomes after acute coronary syndrome. *N Engl J Med* 2018; 379: 2097–2107.

57. Szarek M, White HD, Schwartz GG, *et al.* Alirocumab reduces total nonfatal cardiovascular and fatal events: the ODYSSEY OUTCOMES trial. *J Am Coll Cardiol* 2019; 73: 387–396.

58. Mihaylova B, Emberson J, Blackwell L, *et al.* The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. *Lancet* 2012; 380: 581–590.

59. Khan SU, Riaz H, Rahman H, *et al.* Association of baseline LDL-C with total and cardiovascular mortality in patients using proprotein convertase subtilisin-kexin type 9 inhibitors: a systematic review and meta-analysis. *J Clin Lipidol* 2019; 13: 538–549.

60. O'Regan C, Wu P, Arora P, *et al.* Statin therapy in stroke prevention: a meta-analysis involving 121,000 patients. *Am J Med* 2008; 121: 24–33.

61. Nissen SE, Tuzcu EM, Schoenhagen P, *et al.* Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: a randomized controlled trial. *JAMA* 2004; 291: 1071–1080.

62. Nicholls SJ, Hsu A, Wolski K, *et al*. Intravascular ultrasound-derived measures of coronary atherosclerotic plaque burden and clinical outcome. *J Am Coll Cardiol* 2010; 55: 2399–2407.

63. Nicholls SJ, Tuzcu EM, Crowe T, *et al*. Relationship between cardiovascular risk factors and atherosclerotic disease burden measured by intravascular ultrasound. *J Am Coll Cardiol* 2006; 47: 1967–1975.

64. Tsujita K, Sugiyama S, Sumida H, *et al*. Impact of dual lipid-lowering strategy with ezetimibe and atorvastatin on coronary plaque regression in patients with percutaneous coronary intervention: the multicenter randomized controlled PRECISE-IVUS trial. *J Am Coll Cardiol* 2015; 66: 495–507.

65. Nicholls SJ, Puri R, Anderson T, *et al*. Effect of evolocumab on progression of coronary disease in statin-treated patients: the GLAGOV randomized clinical trial. *JAMA* 2016; 316: 2373–2384.

66. Wierzbicki AS. The challenges of proprotein convertase subtilisin-kexin-9 inhibitors for lipid guidelines and models of care. *Curr Opin Lipidol* 2017; 28: 470–476.

67. Ara R, Pandor A, Stevens J, *et al*. Prescribing high-dose lipid-lowering therapy early to avoid subsequent cardiovascular events: is this a cost-effective strategy? *Eur J PrevCardiol* 2012; 19: 474–483.

68. Hlatky MA and Kazi DS. PCSK9 Inhibitors: economics and policy. *J Am Coll Cardiol* 2017; 70: 2677–2687.

69. Korman M and Wisloff T. Modelling the cost-effectiveness of PCSK9 inhibitors vs. ezetimibe through LDL-C reductions in a Norwegian setting. *Eur Heart J Cardiovasc Pharmacother* 2018; 4: 15–22.

70. Kazi DS, Moran AE, Coxson PG, *et al*. Cost-effectiveness of PCSK9 inhibitor therapy in patients with heterozygous familial hypercholesterolemia or atherosclerotic cardiovascular disease. *JAMA* 2016; 316: 743–753.

71. Toth PP, Danese M, Villa G, *et al*. Estimated burden of cardiovascular disease and value-based price range for evolocumab in a high-risk, secondary-prevention population in the US payer context. *J Med Econ* 2017; 20: 555–564.

72. Ko DT, Khan AM, Kotrri G, *et al*. Eligibility, clinical outcomes, and budget impact of PCSK9 inhibitor adoption: the CANHEART PCSK9 study. *J Am Heart Assoc* 2018; 7: e010007.

73. Piccinni C, Antonazzo IC, Maggioni AP, *et al*. PCSK9 inhibitors' new users: analysis of prescription patterns and patients' characteristics from an Italian real-world study. *Clin Drug Investig* 2020; 40: 173–181.

74. Chamberlain AM, Gong Y, Shaw KM, *et al*. PCSK9 inhibitor use in the real world: data from the national patient-centered research network. *J Am Heart Assoc* 2019; 8: e011246.

75. Tai MH, Shepherd J, Bailey H, *et al*. Real-world treatment patterns of PCSK9 inhibitors among patients with dyslipidemia in Germany, Spain, and the United Kingdom. *Curr Med Res Opin* 2019; 35: 829–835.

76. Kim EJ and Wierzbicki AS. Cardiovascular prevention: frontiers in lipid guidelines. *Clin Med* 2020; 20: 36–42.

77. Macchi C, Sirtori CR, Corsini A, *et al*. A new dawn for managing dyslipidemias: the era of RNA-based therapies. *Pharmacol Res* 2019; 150: 104413.

78. Wierzbicki AS and Viljoen A. Anti-sense oligonucleotide therapies for the treatment of hyperlipidaemia. *Expert Opin Biol Ther* 2016; 16: 1125–1134.

79. van Poelgeest EP, Hodges MR, Moerland M, *et al*. Antisense-mediated reduction of proprotein convertase subtilisin/kexin type 9 (PCSK9): a first-in-human randomized, placebo-controlled trial. *Br J Clin Pharmacol* 2015; 80: 1350–1361.

80. Fitzgerald K, White S, Borodovsky A, *et al*. A highly durable RNAi therapeutic inhibitor of PCSK9. *N Engl J Med* 2017; 376: 41–51.

81. Leiter LA, Teoh H, Kallend D, *et al*. Inclisiran lowers LDL-C and PCSK9 irrespective of diabetes status: the ORION-1 randomized clinical trial. *Diabetes Care* 2019; 42: 173–176.

82. Ray KK, Stoekenbroek RM, Kallend D, *et al*. Effect of 1 or 2 doses of inclisiran on low-density lipoprotein cholesterol levels: one-year follow-up of the ORION-1 randomized clinical trial. *JAMA Cardiol* 2019; 4: 1067–1075.

83. Stoekenbroek RM, Kallend D, Wijngaard PL, *et al*. Inclisiran for the treatment of cardiovascular disease: the ORION clinical development program. *Future Cardiol* 2018; 14: 433–442.

84. Crossey E, , Amar MJA, Sampson M, *et al*. A cholesterol-lowering VLP vaccine that targets PCSK9. *Vaccine* 2015; 33: 5747–5755.

85. Landlinger C, Pouwer MG, Juno C, *et al*. The AT04A vaccine against proprotein convertase subtilisin/kexin type 9 reduces total cholesterol, vascular inflammation, and atherosclerosis in APOE★3Leiden.CETP mice. *Eur Heart J* 2017; 38: 2499–2507.

86. Wang X, Raghavan A, Chen T, *et al*. CRISPR-Cas9 targeting of PCSK9 in human hepatocytes in vivo-brief report. *Arterioscler Thromb Vasc Biol* 2016; 36: 783–786.

87. Zhang L, Wang L, Xie Y, *et al*. Triple-targeting delivery of CRISPR/Cas9 to reduce the risk of cardiovascular diseases. *Angew Chem Int Ed Engl* 2019; 58: 12404–12408.

88. Wierzbicki AS, Reynolds TM and Viljoen A. An update on trials of novel lipid-lowering drugs. *Curr Opin Cardiol* 2018; 33: 416–422.

89. Stein EA, Bays H, O'Brien D, *et al*. Lapaquistat acetate: development of a squalene synthase inhibitor for the treatment of hypercholesterolemia. *Circulation* 2011; 123: 1974–1985.

90. Ballantyne CM, McKenney JM, MacDougall DE, *et al*. Effect of ETC-1002 on serum low-density lipoprotein cholesterol in hypercholesterolemic patients receiving statin therapy. *Am J Cardiol* 2016; 117: 1928–1933.

91. Ballantyne CM, Laufs U, Ray KK, *et al*. Bempedoic acid plus ezetimibe fixed-dose combination in patients with hypercholesterolemia and high CVD risk treated with maximally tolerated statin therapy. *Eur J Prev Cardiol*. Epub ahead of print 31 July 2019. DOI: 10.1177/2047487319864671.

92. Ray KK, Bays HE, Catapano AL, *et al*. Safety and efficacy of bempedoic acid to reduce LDL cholesterol. *N Engl J Med* 2019; 380: 1022–1032.

93. Mach F, Baigent C, Catapano AL, *et al*. 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *Eur Heart J* 2019; 41: 111–188.

94. Grundy SM, Stone NJ, Bailey AL, *et al*. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a report of the American college of cardiology/ American heart association task force on clinical practice guidelines. *Circulation* 2019; 139: e1082–e1143.

95. Orringer CE, Jacobson TA, Saseen JJ, *et al*. Update on the use of PCSK9 inhibitors in adults: recommendations from an expert panel of the national lipid association. *J Clin Lipidol* 2017; 11: 880–890.

96. Jellinger PS, Handelsman Y, Rosenblit PD, *et al*. American association of clinical endocrinologists and American college of endocrinology guidelines for management of dyslipidemia and prevention of cardiovascular disease. *Endocr Pract* 2017; 23: 1–87.

97. Landmesser U, Chapman MJ, Stock JK, *et al*. 2017 update of ESC/EAS task force on practical clinical guidance for proprotein convertase subtilisin/kexin type 9 inhibition in patients with atherosclerotic cardiovascular disease or in familial hypercholesterolaemia. *Eur Heart J* 2018; 39: 1131–1143.

98. Annemans L, Packard CJ, Briggs A, *et al*. 'Highest risk-highest benefit' strategy: a pragmatic, cost-effective approach to targeting use of PCSK9 inhibitor therapies. *Eur Heart J* 2018; 39: 2546–2550.

99. Elamin AFM, Grafton-Clarke C, Wen Chen K, *et al*. Potential use of PCSK9 inhibitors as a secondary preventative measure for cardiovascular disease following acute coronary syndrome: a UK real-world study. *Postgrad Med J* 2019; 95: 61–66.

100. Zelniker TA, Wiviott SD, Raz I, *et al*. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet* 2019; 393: 31–39.

101. Bethel MA, Patel RA, Merrill P, *et al*. Cardiovascular outcomes with glucagon-like peptide-1 receptor agonists in patients with type 2 diabetes: a meta-analysis. *Lancet Diabetes Endocrinol* 2018; 6: 105–113.

102. Bhatt DL, Steg PG, Miller M, *et al*. Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. *N Engl J Med* 2019; 380: 11–22.

103. Tardif JC, Kouz S, Waters DD, *et al*. Efficacy and safety of low-dose colchicine after myocardial infarction. *N Engl J Med* 2019; 381: 2497–2505.