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The pharmacology of cholesterol-lowering drugs

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ABSTRACT

The causal role of low-density lipoprotein cholesterol LDL-C in atherosclerotic-related cardiovascular disease (ASCVD) has been undoubtedly established over the last decades, and lowering plasma LDL-C levels represents the main approach to reduce the risk of cardiovascular (CV) events. A large number of observations has definitely proven that the protective effect is independent of the drug used to lower LDL-C, with a continuous linear reduction of CV risk with further LDL-C reductions. Although high-intensity statin therapy may significantly reduce CV event incidence, frequently statins are insufficient to achieve the large reductions recommended by current guidelines for high and very high risk patients.

Several non-statin drugs, having mechanisms of action complementary to that of statins, are now available, and include ezetimibe, monoclonal antibodies targeting PCSK9, and, more recently, inclisiran, bempedoic acid, and evinacumab. Combining these drugs based on the recommendations by current and future guidelines should be considered for optimal risk reduction, although several gaps in clinical practice remain to be filled.

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Introduction

Since the discovery of statins, the landscape of cardiovascular disease (CVD) management has changed drastically, having shown unequivocally that reducing low-density lipoprotein cholesterol (LDL-C) levels results in a reduced incidence of CV events. The causality of LDL-C in the aetiology of atherosclerotic-related CVDs (ASCVDs) has been clearly established over the last decades (1, 2), with concordant observations from a variety of sources spanning from basic research, to genetic and clinical studies, further strengthening the evidence that the pharmacological control of plasma LDL-C levels is the major route to prevent CV outcomes, independently of the drug used to lower LDL-C (3, 4). Another major finding arising from clinical trials is that therapy intensification, either as statin dose/type or combination therapy, associates with significant reduction of CV event incidence in high and very high risk patients. Altogether these observations have led to intensify the research of new non-statin drugs having mechanisms of action that can "complement" the effect of statins; as a result, several alternative approaches for the treatment of hypercholesterolemia became available for therapy with unprecedented speed, thus enriching the tools for therapy to lower LDL-C.

In this context, statins still represent the cornerstone for the treatment of hypercholesterolemia, having shown approximately a 20% reduction in the risk of CV events per each mmol/L LDL-C reduction (5). Despite that, this approach might not be enough to reach the recommended goals in all individuals, especially when taking into consideration the lower LDL-C goals introduced by the most recent guidelines for the management of hypercholesterolemia (6). The need of additional approaches, together with the observation that, while there is no evidence of detrimental health effects associated with very low LDL-C-levels, there is a continuous linear reduction of CV risk (7), led to the development of other cholesterol-lowering drugs, including ezetimibe, monoclonal antibodies targeting PCSK9, and, more recently, inclisiran, bempedoic acid, and evinacumab.

The pharmacology of statins

Statins are competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase(HMG-CoAR), the rate-limiting enzyme of cholesterol synthesis pathway. The inhibition of this enzyme results in the reduction of intracellular cholesterol synthesis, which, in turn, upregulates the hepatic surface expression of low-density lipopro-

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tein receptor (LDLR), increases the uptake of LDL particle and reduces plasma LDL-C levels. A large number of randomized clinical trials have shown that statin-induced LDL-C lowering translates into a clinical benefit, with reduction of cardiovascular morbidity and mortality, in primary as well as secondary prevention (5, 8-13). More specifically, statin therapy reduces the risk of major atherosclerotic vascular events by ~20% per mmol/l (~39 mg/dL) absolute reduction in LDL-C (5), with the absolute benefit being determined by the individual CV risk. Compared with less intensive regimens, more intensive statin regimens were associated with a further 15% reduction in major cardiovascular events (MACE), the first demonstration that greater reductions in LDL-C produce further reductions in the incidence of MACE (**Figure 1**) (8).

Statin therapy has been shown to be effective in a wide range of patient categories. First of all, the proportional effects of statins on MACE is comparable in women and men having equivalent baseline risk of cardiovascular disease, as shown by a meta-analysis of data from 174,000 participants in 27 RCTs (14). This represents a relevant finding, as previous clinical trials and meta-analyses generated uncertainty about the effects of statin therapy in women, largely due to the lower number of women among participants in clinical trials. Statin therapy is effective among patients with diabetes, a condition conferring an increased CV risk: statin-treated diabetic patients show a significant 21% proportional reduction in MACE per mmol/l reduction in LDL-C (comparable to that observed in non-diabetic individuals) (15), but, being the absolute risk of CV events and death much higher compared to nondiabetic subjects, the same absolute reduction in LDL-C will result in a greater absolute CV risk reduction. In addition, the benefit of statin therapy applies both to high CV risk and low CV risk patients: the analysis of participants in 22 RCT of statins versus control, divided into categories of baseline 5-year risk of MACE, showed that the proportional reductions in MACE per 1 mmol/L LDL-C reduction in the two lowest risk categories (<5% and ≥5% to <10%) was at least as large as for higher risk participants (16). Again, people at highest risk have the highest absolute risk reduction per mmol/l LDL-C reduction, resulting in 61 MACE avoided per 1000 compared with 6 MACE avoided per 1000 in the lowest CV risk category over 5 years (16). Special consideration must be given to patients with chronic kidney disease (CKD): although statin therapy is effective in preventing coronary heart disease (CHD) and stroke in patients with mild-to-moderate CKD, in those with more advanced CKD or even on dialysis the relative reductions in MACE achieved with statin therapy became smaller as eGFR declined, with little evidence of benefit in patients on dialysis (17).

From these studies, a linear relationship between proportional reduction in the incidence of major cardiovascular events and mean absolute LDL-C reduction has been derived, indicating that the lower the LDL-C levels achieved, the greater the clinical benefit. There are, however, some challenges remain in clinical practice regarding the potential unfavourable effects related to the long-term daily use of

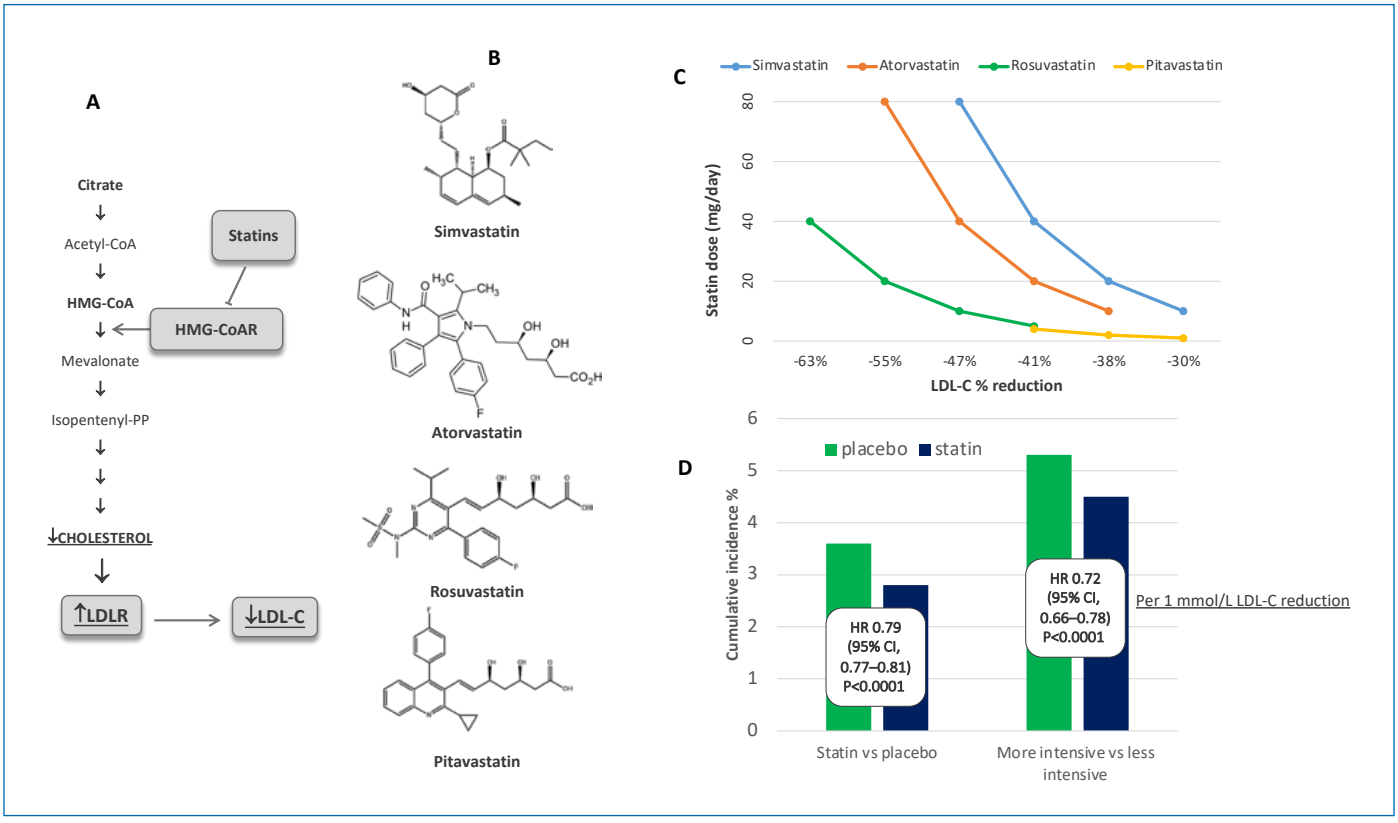

Figure 1 | Mechanism of action (A), structures (B), and LDL-C-lowering properties (C, D) of statins. (A) Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoAR), the rate-limiting enzyme of cholesterol synthesis pathway, thus reducing intracellular cholesterol synthesis and upregulating LDL-C levels. (B) Chemical structures of most commonly used statins. (C) LDL-C % reduction with different statins and doses. (D) Cardiovascular outcome incidence in patients treated with statins vs placebo or with more intensive vs less intensive statin regimens.
HMG-CoA, hydroxyl-methyl-glutaryl coenzyme A; HMG-CoAR, hydroxyl-methyl-glutaryl coenzyme A reductase; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol.

statins. Among these potentially negative effects, the most commonly studied is the occurrence of muscle-related adverse events and an increased incidence of new-onset diabetes. Statin-intolerance is referred as the inability to tolerate an effective dose of statin due to the occurrence of muscular symptoms while taking statin (18); such adverse events limit the effectiveness of statin therapy, and commonly lower the adherence to therapy or drug discontinuation, an effect that is more frequently observed in the everyday clinical practice rather than in clinical trials. Although a true statin intolerance condition is much rarer than reported, due to a "nocebo effect" (19), it represents a relevant issue as it places patients at high risk for CV events (20). Furthermore, a link between statin therapy (and in particular high intensity statin dose) and an increased risk in new-onset diabetes has been reported in several clinical trials and meta-analyses (21-25); such an increased risk, however, is modest and emerges mostly in patients with insulin resistance or prediabetes (26), and the clinical benefit in terms of CV event risk reduction largely exceeds this risk. This observation is supported by the results of a mendelian randomization analysis showing that variants in *HMGCR* (the gene encoding HMG-CoAR) associated with low LDL-C levels and a reduced risk of CV events also associate with an increased risk of diabetes (13% for each 10 mg/dL decrease in LDL-C) in patients with impaired fasting glucose (≥100 mg/dL), but not in those with normal fasting glucose (27).

The pharmacology of ezetimibe

Niemann-Pick C1L1 (NPC1L1) protein is a sterol transporter highly expressed in intestinal epithelial cells and involved in the intestinal absorption of cholesterol (**Figure 2A**), thus contributing to the regulation of cholesterol plasma levels.(28, 29) Subjects carrying inactivating mutations in *NPC1L1* have lower LDL-C levels compared with noncarriers, and a 53% reduction in the risk of CHD, suggesting this protein as a pharmacological target (30).

Ezetimibe, by interfering with the activity of NPC1L1, inhibits the absorption of biliary and dietary cholesterol. This drug exhibit a complementary mechanism of action as compared to statins, and their combination results in an LDL-C reduction greater than those observed using these two drugs in monotherapy, due to their mechanisms of action. In fact, statins, by inhibiting cholesterol synthesis pathway, produce the upregulation of hepatic LDLR and increase the uptake of LDL from the circulation. In turn, this causes a feedback mechanism resulting in an increased intestinal cholesterol absorption and a partially reduced efficacy of statin therapy. On the other hand, ezetimibe, by inhibiting intestinal cholesterol absorption, induces a compensatory mechanism increasing cholesterol synthesis in the intestine and the liver (31). When statins are combined with ezetimibe, both cholesterol synthesis and absorption are reduced, resulting in a further 15–20% LDL-C level decrease,(32-34) and adding ezetimibe to a statin is much more effective than doubling the dose of the statin, which only provides an additional 5-6% reduction in LDL-C (35, 36). The efficacy of this combination has been proved also in diabetic patients, who achieved greater LDL-C reductions compared with those observed in patients doubling the statin dose (37, 38), and in patients with familial hypercholesterolemia (FH) showing a residual LDLR activity (39-41).

The first demonstration that this combination has also a clinical benefit derived from the IMPROVE-IT trial, that compared the effect of a 6-year administration of ezetimibe+simvastatin or simvastatin alone in patients with a recent acute coronary syndrome (42). LDL-C level was further reduced by 24% with the combination therapy compared with simvastatin alone, translating into a significant 6.4% reduced risk of the primary composite endpoint (**Figure 2B, 2C**) (42). A secondary analysis of this trial showed an even higher benefit in specific subgroups of patients, such as women, aged people, and diabetic patients (43-45).

At present, the combination statin+ezetimibe represents a main approach for the treatment of hypercholesterolemia, and guidelines indicate that the combination will be used as a second step when patients cannot reach the recommended goals (or cannot tolerate an effective dose of statin). For a more detailed description of findings from clinical trials using ezetimibe, please see the paper by H. Bays in this issue.

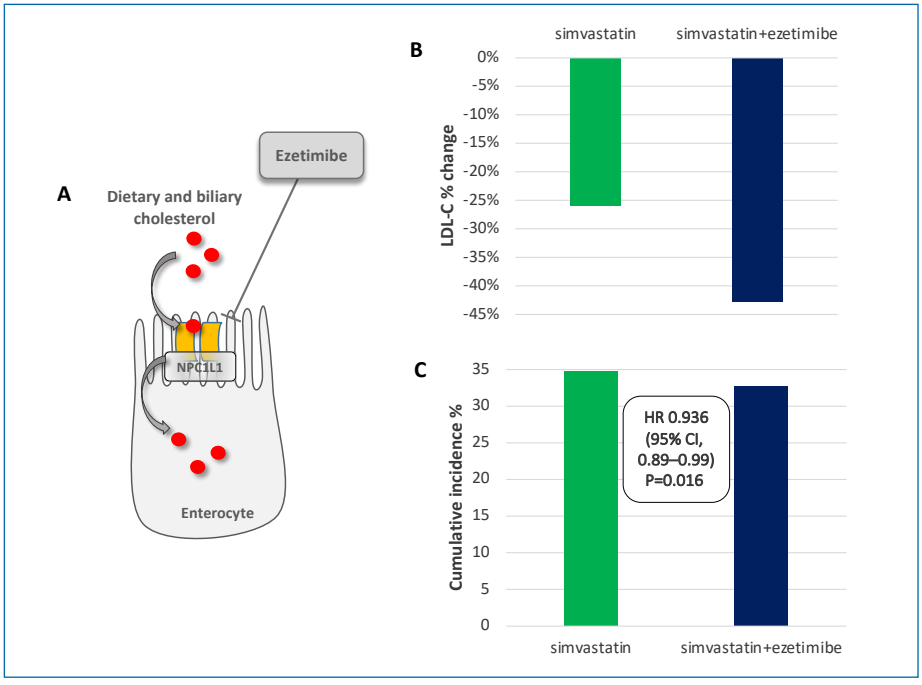

Figure 2 | Mechanism of action of ezetimibe (A) and results from the IMPROVE-IT (B, C). (A) NPC1L1 is localized on the brush border of the enterocytes and mediates the uptake of dietary and biliary cholesterol. Ezetimibe inhibits the activity of NPC1L1. (B) LDL-C percent change and incidence of cardiovascular events in patients receiving simvastatin monotherapy or the combination ezetimibe+simvastatin (IMPROVE-IT trial). NPC1L1, Niemann-Pick C1-Like 1; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol.

The pharmacology of PCSK9 inhibitors

Proprotein convertase subtilisin kexin 9 (PCSK9) is a serine protease highly expressed in the liver, intestine, kidney, and brain (46); it plays a crucial role in regulating the expression of hepatic LDLR by targeting it to degradation and, as a consequence, modulates plasma LDL-C levels (**Figure 3A**) (47-49). Individuals carrying loss-of-function mutations in *PCSK9* associated with lower levels of LDL-C also have a significantly reduced CV risk (50-54), whereas genetic gain of function variants associated with higher levels of LDL-C confer an increased risk of premature cardiovascular disease and are a cause of FH (55-57). These observations have suggested PCSK9 as a pharmacological target for the control of dyslipidaemia, and great research efforts have generated two monoclonal antibodies targeting circulating PCSK9 and, more recently, a gene silencing approach able to control more efficiently the production of PCSK9 only in the liver. In fact, despite PCSK9 is produced mainly by the liver, which contributes for circulating PCSK9 levels, other tissues express this protein, raising uncertainties on the potentially harmful effects of the pharmacological inhibition of PCSK9 in extrahepatic tissues.

Two monoclonal antibodies (evolocumab and alirocumab) have been developed and approved for the treatment of hypercholesterolemia, and are recommended by guidelines as an add-on to current lipid-lowering therapy when patients with high or very high CV risk cannot achieve the recommended goals with maximally tolerated dose of statin with or without ezetimibe; this recommendation stems on the results of randomized clinical trials having shown a substantial cholesterol-lowering efficacy (50%-60%) and a consequent clinical benefit. The development of an additional antibody (bococizumab) was halted due to the production of anti-drug antibodies that reduced the efficacy of the treatment.

Evolocumab. Evolocumab was evaluated in several phase 2 clinical trials, showing a cholesterol-lowering efficacy either as monotherapy or as add-on to ongoing lipid-lowering therapy (LLT) in different groups of patients (58-60). The evolocumab clinical trial program **PROFICIO** included phase 3 clinical trials that assessed the effectiveness of evolocumab in comparison with placebo or ezetimibe across a wide range of patient categories. Evolocumab alone was more effective than placebo or ezetimibe in reducing LDL-C levels (61), and adding evolocumab to the ongoing LLT resulted in a greater reduction in LDL-C (60%-65%) than adding ezetimibe (15%-20%) or placebo (62). Evolocumab was shown to be effective in statin-intolerant patients (63, 64), and in patients with heterozygous FH (65), whereas in HoFH patients the reduction was smaller (20-30%) and strictly related to the presence of a residual LDLR activity (as for all drugs acting by increasing LDLR expression) (60, 66, 67). The evaluation of the long-term effects of evolocumab showed a persistent hypocholesterolemic effect up to 5 years, and an overall safe profile, with no neutralizing antibodies detected (68).

The clinical benefit of PCSK9 inhibition has been addressed in the FOURIER trial, that evaluated the effect of evolocumab or placebo added to a background of statin therapy in patients with ASCVD and LDL-C ≥70 mg/dL (69). At week 48, LDL-C levels were reduced by 59% which translated into a 15% lower risk of the primary endpoint (a composite of cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, or coronary revascularization) (**Figure 3B, 3C**) and by 20% the secondary endpoint (a composite of cardiovascular death, myocardial infarction, or stroke) after

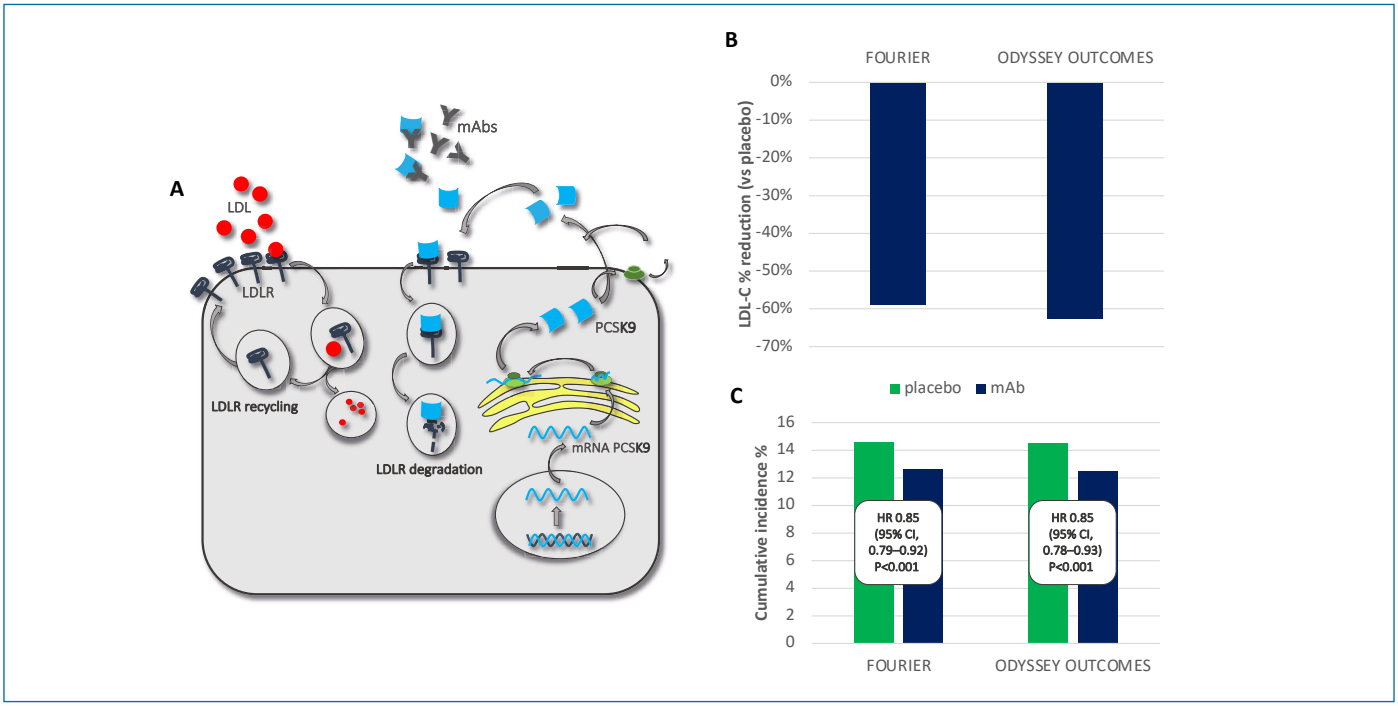

Figure 3 | Mechanism of action of mAbs to PCSK9 (A) and results from FOURIER and ODYSSEY OUTCOMES trials (B, C). (A) monoclonal antibodies to PCSK9 bind extracellular (secreted) PCSK9, thus preventing its binding to LDLR and the subsequent degradation of LDLR. LDL-C percent change (B) and incidence of cardiovascular events (C) with evolocumab (FOURIER) and alirocumab (ODYSSEY OUTCOMES).
LDL, low density lipoprotein; LDLR, low-density lipoprotein receptor; mAbs, monoclonal antibodies; PCSK9, proprotein convertase subtilisin kexin 9; HR, hazard ratio.

a median follow-up of 2.2 years (69). Relative risk reductions were comparable across patient categories, but larger absolute risk reductions were observed among patients at higher baseline risk, such as patients with diabetes (70), peripheral artery disease (71), advanced chronic kidney disease (72), recent MI (<2y), multiple prior MIs, or residual multivessel coronary artery disease (73), or elevated polygenic risk score (74). The use of monoclonal antibodies targeting PCSK9, for the first time, allowed patients to achieve very low LDL-C levels (<0.5 mmol/L (<~20 mg/dL), without specific safety concerns related to the low levels of LDL cholesterol achieved (75), and further supported the hypothesis of a linear relationship between LDL-C levels and CV outcomes even for very low LDL-C levels (1). Of note, no adverse cognitive effects were reported among patients treated with evolocumab over a median of 19 months (76), neither in those who achieved very low LDL-C levels (75).

Alirocumab. Based on the results obtained in phase 2 trials, suggesting substantial reductions in LDL-C levels in alirocumab-treated patients, ranging from 40% to 73% (77-80), the **ODYSSEY** program was started to assess the efficacy and safety of alirocumab alone or in combination with other LLT across different subgroups of hypercholesterolemic patients. The administration of alirocumab 75 mg Q2W or ezetimibe 10 mg/day showed that LDL-C levels were reduced in both groups compared with placebo, but the reduction observed among alirocumab-treated patients was higher than that observed among ezetimibe-treated patients (47.2% vs 15.6%) (81). The higher LDL-C-lowering efficacy of alirocumab has been shown also when given in combination with the ongoing therapy (maximum tolerated statin±other LLT) in high CV risk populations, when compared with either placebo (82, 83) or ezetimibe (84). Furthermore, adding alirocumab to atorvastatin or rosuvastatin was more effective than adding ezetimibe, or doubling the statin dose (85, 86). Finally, alirocumab can represent a valuable approach to reduce significantly hypercholesterolemia in specific groups of patients, such as statin-intolerant patients, in whom alirocumab reduced LDL-C levels substantially more than ezetimibe (45% and 14.6% at week 24, respectively) (87), and in FH (83, 88, 89).

The clinical benefit of alirocumab-based therapy was tested in an outcome trial (ODYSSEY OUTCOMES) that recruited patients with a recent acute coronary syndrome and LDL-C levels not at target despite high-intensity statin therapy (90). Alirocumab reduced LDL-C levels by 62.7% at 4 months and 54.7% at 48 months (90). After a median follow-up of 2.8 years, the risk of the primary endpoint (a composite of death from coronary heart disease, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, or unstable angina requiring hospitalization) was significantly reduced by 15% in alirocumab treated patients (**Figure 3B, 3C**); individuals with the highest baseline LDL-C levels (≥100 mg/dL) achieved the highest absolute risk reduction (90). Among participants in this study, those who did not receive background statin therapy had higher baseline LDL-C levels and were at higher risk of recurrent events, but also experienced a greater absolute LDL-C reduction and absolute MACE risk reduction (91). The beneficial effect of alirocumab was independent of patient age, but, because the higher absolute risk in older individuals, the absolute benefit deriving from alirocumab treatment increased with advancing age (92). An analysis of the ODYSSEY OUTCOMES trial using a polygenic risk score (PRS) for CAD showed that patients having a high PRS have a higher incidence of MACE than those with lower PRS, but also derive a larger absolute and relative risk reduction when treated with alirocumab (93), suggesting the potential of using PRS to stratify patients and identify those who may benefit more from a more intensive cholesterol-lowering approach.

Altogether, the results obtained in RCTs have substantiated PCSK9 inhibitors as an effective and safe approach to further reduce the CV risk in several groups of patients, thanks to a remarkable and sustained reduction of LDL-C levels beyond that obtained with statins±other LLT, with patients at increased CV risk having the greatest absolute benefit. As per the safety a major concern in statin therapy is the increased risk of new-onset diabetes. Although the mechanism by which anti-PCSK9 mAbs increase LDLR differs from that of statins, it is well established that LDLR plays a role in cholesterol metabolism in pancreatic beta cells (94) and indeed PCSK9 deficiency has been associated with an increased risk of new onset diabetes both in animal models and humans (27, 95). To date, results from an experimental model suggest that locally produced rather than circulating PCSK9 plays a role in the homeostasis of cholesterol in beta cells, and thus the inhibition of PCSK9 by mAbs should not affect this pathway; accordingly, evolocumab and alirocumab treatments do not appear to increase the risk of new-onset diabetes and do not worsen glycaemia (70, 96-100).

New cholesterol-lowering drugs

Inclisiran. Over the last few years, gene-based approaches targeting key players in the metabolism of lipids, and in particular LDL, led to the development and approval of inclisiran (101). Inclisiran is a small interfering RNA (siRNA) targeting PCSK9 mRNA thus inhibiting the intracellular production of PCSK9 (**Figure 4A**), in contrast with monoclonal antibodies against PCSK9 which bind and inhibit extracellular, circulating PCSK9.

Different experimental models have shown a rapid, durable, and reversible reduction in circulating PCSK9 and LDL-C levels with a single dose of a siRNA targeting PCSK9 (a precursor of inclisiran) (102); next, healthy volunteers who received a single intravenous dose of this siRNA showed a mean 70% reduction in circulating PCSK9 plasma levels and a 40% reduction in LDL-C levels (103). The N-acetylgalactosamine (GalNAc) modification of the double-stranded molecule, leveraging on the asialoglycoprotein receptor for its uptake, ensures a prompt and specific uptake by the liver, where this receptor is abundantly expressed (while only minimally expressed in extrahepatic tissues). The introduction of modifications that have led to the development of the GalNAc-siRNA conjugate (inclisiran) has largely improved the administration, increased the potency of the drug (allowing the use of lower doses), and reduced the potential for side effects. Following the demonstration of a dose-dependent reduction of plasma PCSK9 levels (up to 83.8%) and LDL-C levels (up to 59.7%) in healthy volunteers, inclisiran has been evaluated in the ORION clinical program that includes phase 2 and 3 clinical trials, some of which are still ongoing (**Figure 4A**). The phase 2 trial ORION-1 showed for the first time that inclisiran given as a single dose or two doses (at days 1 and 90) was effective in reducing LDL-C levels in hypercholesterolemic patients at high CV risk (104). Reduced levels of PCSK9 and LDL-C were maintained up to day 240 in inclisiran-treated patients (104), and one year after administration of either a single dose or two doses of inclisiran LDL-C were persistently low, with a 50% LDL-C reduction being maintained for at least 6 months after 2 doses of 300mg inclisiran (105). The rate of adverse events was similar in inclisiran and placebo groups, and injection-site reactions were rare and similar to those reported with monoclonal antibodies (104, 106). The ongoing open-label extension study of ORION-1 (ORION-3) is comparing the long-term effect inclisiran 300 mg administered on day 1 and every 180 day thereafter or evolocumab 140 mg every 2 weeks for up to 4 years (NCT03060577); the trial is expected to be completed in 2022. An interim analysis at ~22 months reported a 51% reduction

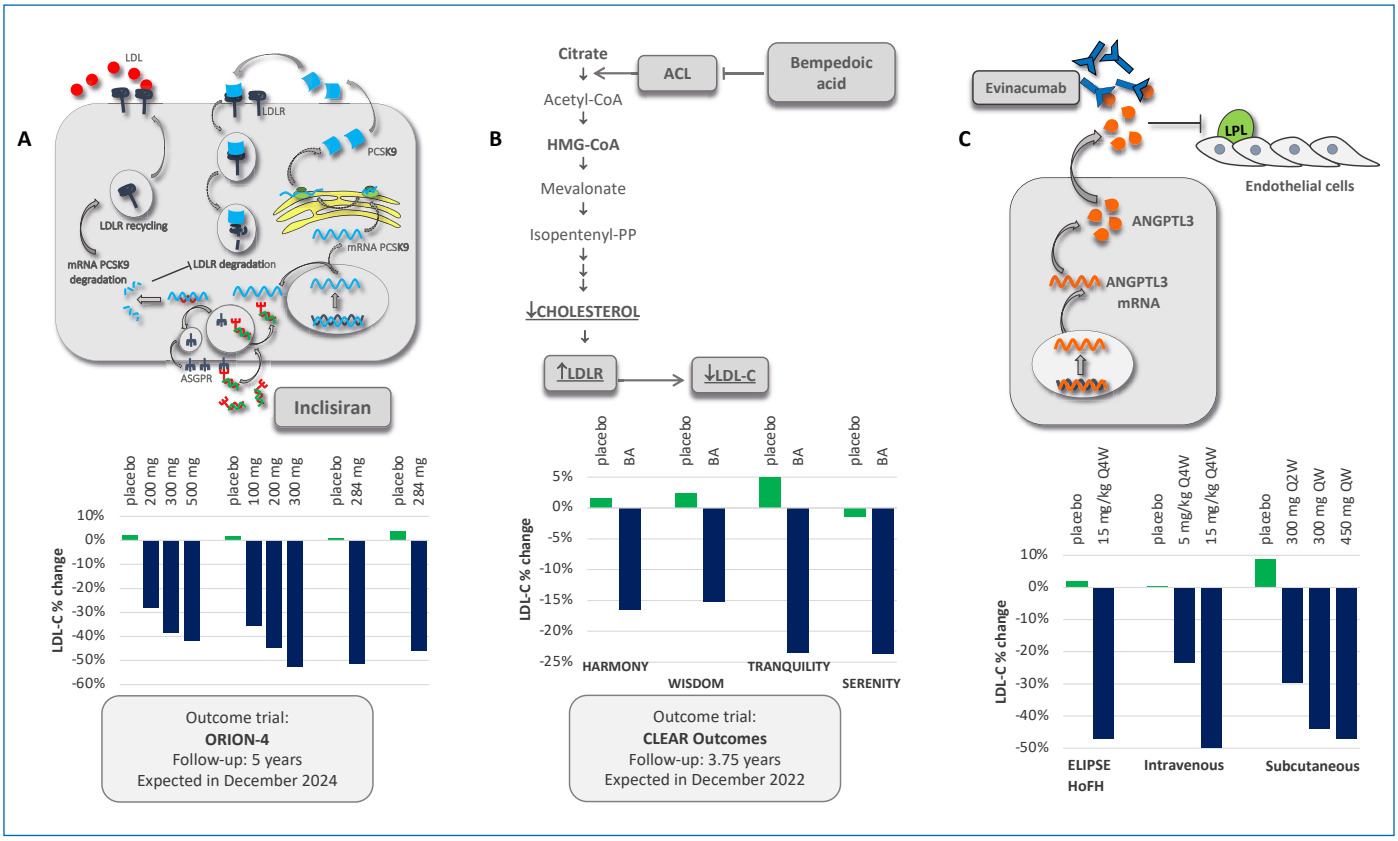

Figure 4 | New LDL-C-lowering drugs. Mechanism of action and LDL-C-lowering properties of inclisiran (A), bempedoic acid (B), evinacumab (C). LDL-C, low density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; PCSK9, proprotein convertase subtilisin kexin 9; ACL, adenosine triphosphate-citrate lyase; BA, bempedoic acid; ANGPTL3, angiopoietin-like 3; LPL, lipoprotein lipase; QW, once weekly; Q2W, once every 2 weeks; Q4W, once every 4 weeks.

in LDL-C levels in all patients, with a time-averaged lowering of ~60 mg/dL, and a good safety profile (107).

Three phase 3 trials have reported a significant efficacy of inclisiran in patients with HeFH (108), ASCVD, or an ASCVD risk equivalent taking a stable LLT (109). HeFH patients showed a ~40% reduction in LDL-C levels with inclisiran 300 mg injected on days 1, 90, 270, and 450 (compared with a 8.2% increase with placebo, with a between-group difference of -47.9%) (108). Similar reductions were achieved in the ORION-10 and -11 trials (52.3% and 53.8% between-group differences, respectively), independently of the gender, age, intensity of statin treatment, and underlying co-morbidities (109). Based on the observation that inclisiran significantly reduces PCSK9 levels also in 4 patients with HoFH in the ORION-2 pilot study, but lowers LDL-C levels at an extent related to the type of causative mutation (110), the ORION-5 trial (NCT03851705) has evaluated the effect of the administration of inclisiran or placebo in 45 HoFH patients in a 6-month double blind period, after which all patients have received inclisiran for an 18-month open-label follow-up period; the results of this study are now expected. The ongoing ORION-4 trial will establish whether inclisiran 300 mg may safely reduce the risk of major atherosclerotic cardiovascular events in ≥15,000 patients with pre-existing ASCVD during a median treatment duration of 5 years (NCT03705234). Estimated primary completion date is December 2024.

Bempedoic acid. Bempedoic acid is a recently developed lipid-lowering drug that inhibits adenosine triphosphate-citrate lyase (ACL), an enzyme involved in cholesterol biosynthesis (**Figure 4B**). The activity of this drug produces an upregulation of hepatic LDLR expression, leading to a reduction of circulating LDL-C levels (111). The potential clinical benefit of bempedoic acid therapy is suggested by the observation that genetic variants in *ACLY* (the gene encoding ACL) associated with lower LDL-C levels predict a reduced risk of cardiovascular disease (112). Being a pro-drug, bempedoic acid needs to be converted into the active form by very-long chain acyl-CoA synthetase (ACSVL1), an enzyme highly expressed in hepatocytes but not detectable in skeletal muscles. This represents an advantageous characteristic of bempedoic acid, as it should avoid any muscle-related adverse effects, which are instead frequently reported with statin therapy, conferring to this drug a potential role for use in patients who cannot tolerate an effective dose of statin.

Phase 2 clinical trials have shown that bempedoic acid significantly reduces LDL-C levels either in monotherapy or in combination with a statin or ezetimibe. When given alone, bempedoic acid dose-dependently reduced LDL-C levels (form 17.9% up to 26.6%) and improved lipid profile (113). Maximum LDL-C lowering was achieved after 2 weeks and was maintained for the course of the trial. CRP was significantly reduced among patients treated with bempedoic acid (~20% at all doses), but the reduction was more marked in individuals with higher CRP at baseline (≥2 mg/l), who reported reductions from 43% to 63.5% (compared to 7.0% reduction with placebo) (113). In patients with hypercholesterolemia and diabetes mellitus bempedoic acid determined an even greater reduction

in LDL-C levels (43% vs 4% reduction with placebo); CRP was reduced by 41% and no worsening of glycaemic control was observed (114). The addition of bempedoic acid to a background statin therapy resulted in greater LDL-C reductions compared with placebo (115, 116). The triple combination of bempedoic acid, ezetimibe, and atorvastatin has been evaluated in patients with hypercholesterolemia, showing a 63.6% reduction in LDL-C levels compared with a 3.1% reduction with placebo at week 6; 95% of patients had their LDL-C levels halved following the triple therapy, and 90% achieved levels <70 mg/dL (117). Also CRP was significantly lowered by 47.7% (vs 2.7% reduction with placebo) (117). Bempedoic acid was effective in reducing LDL-C also in patients with a history of statin intolerance (118, 119). In all these studies, a good safety profile of bempedoic acid was observed, without specific concerns. A modest, fully reversible increase in uric acid levels has been reported among patients treated with bempedoic acid, likely related to the drug-mediated inhibition of a specific transporter (organic anion transporter 2) (120).

The CLEAR (Cholesterol Lowering via Bempedoic acid, an ACL-Inhibiting Regimen) program of bempedoic acid includes 5 phase 3 studies, 4 of which have been completed (**Figure 4B**). Two of these studies have evaluated the effect of bempedoic acid or placebo in patients with ASCVD, HeFH, or both and with persistent hypercholesterolemia despite maximally tolerated LLT: LDL-C were reduced similarly in both studies (placebo corrected differences: -18.1% and -17.4%), with an overall improvement of lipid profile and significant reductions in CRP levels (placebo-corrected differences: -21.5% and -8.7%) (121, 122). The other two studies were performed in statin intolerant patients, in which bempedoic acid therapy was even more effective in reducing both LDL-C (placebo-corrected differences: -28.5% and -21.4%) and CRP (-32.5% and -24.3%) (123, 124). The ongoing CLEAR Outcomes study will evaluate the effect of bempedoic acid or placebo on cardiovascular outcomes in statin intolerant patients with, or at high risk for, cardiovascular disease.

Evinacumab. Angiopoietin-like 3 (ANGPTL3) is a physiological inhibitor of two enzymes crucially involved in lipoprotein metabolism, namely lipoprotein lipase (LPL) and endothelial lipase (EL) (125). Complete ANGPTL3 deficiency is associated with very low plasma lipid levels and no evidence of coronary atherosclerosis (126); heterozygous carriers of *ANGPTL3* LOF mutations had approximately 50% lower ANGPTL3 levels than noncarriers, lower levels of TG (-17%, -27%) and LDL-C (-12%, -9%), associated with a substantially reduced risk of CAD (-34%, -39%) (126, 127). Despite the reasons for the reduction in LDL-C are still not completely elucidated, these observations suggested ANGPTL3 as a potential target for the pharmacological control of hypercholesterolemia, and the evidence of LDLR-independent mechanism(s) advocated a potential suitability for patients with HoFH, particularly those carrying null *LDLR* mutations (128). A fully human monoclonal antibody targeting ANGPTL3, evinacumab (**Figure 4C**), was shown to reduce dose-dependently LDL-C (up to 23%) and TG (up to 76%) levels in healthy volunteers (127). When tested in an a single-group, open-label study involving nine HoFH patients, evinacumab added to their background lipid-lowering therapy (which included statins, ezetimibe, lomitapide, PCSK9 mAbs, or a portacaval shunt) reduced LDL-C level by a mean of 49%, but with a wide range of variability among patients; three patients with null/null mutations (2 homozygotes and 1 compound heterozygote) had significant, although different, responses to evinacumab (26%, 42%, and 44%, respectively) (129). A subsequent phase 3 trial in 65 HoFH patients (ELIPSE HoFH) reported similar results, with patients treated with evinacumab achieving a 47.1% reduction in LDL-C from baseline (compared with a 1.9% increase reported in the placebo group) (**Figure 4C**) (130). Evinacumab was effective both in patients with non-null mutations (having a residual LDLR activity) and patients with null/null variants (130), and recently it was shown to induce a profound plaque regression in two severely affected young FH patients (131). This represents a worthwhile observation, as HoFH patients with null/null variants have the highest CV risk and the lowest response to pharmacological approaches with either conventional or new cholesterol-lowering drugs acting through LDLR upregulation in the liver. It appears that inactivation of ANGPTL3 decreases the production rate of VLDL-apoB (132), suggesting the possibility that the reduction in LDL-C levels observed in HoFH patients treated with evinacumab could be the consequence of a reduced production of lipoproteins. A recent small study in 4 HoFH patients examined apoB (apolipoprotein B) containing lipoprotein kinetic parameters before and after treatment with evinacumab and observed that ANGPTL3 inhibition was associated with an increase in the fractional catabolic rate of IDL-apoB and LDL-apoB (133), suggesting that evinacumab lowers LDL-cholesterol predominantly by increasing apoB-containing lipoprotein clearance from the circulation.

Current gaps in therapy and evolving approaches to address gaps, improve adherence – real world data on current practice

Randomized clinical trials have unequivocally established the cholesterol-lowering effectiveness of newly developed drugs, although for some of them, including inclisiran, bempedoic acid, and evinacumab the clinical efficacy is currently under evaluation. Despite this, both inclisiran and bempedoic acid have been approved based on their LDL-C-lowering effect, that is expected to translate into a clinical benefit.

Nevertheless, the everyday clinical practice shows unmet needs and gaps that hinder the achievement of lipid goals related to the prevention of CV outcomes. Two major issues deserving a more in-depth discussion relate to 1) the relationship between the cost and efficacy of cholesterol-lowering drugs and 2) the adherence to cholesterol-lowering therapies.

Cost-effectiveness considerations. As already discussed above, current guidelines have introduced more and more stringent LDL-C goals for all risk categories; this calls for the use of more effective pharmacological approaches able to reduce LDL-C levels to <55 mg/dl in very high risk patients. Most of these patients cannot reach the recommended goal with statin monotherapy and in some instances also after ezetimibe; they would thus be eligible for the use of a PCSK9 inhibitor, as specified in the treatment algorithm, allowing a substantial percentage of patients to reach their LDL-C goals, but raising a question about the costs for the healthcare system. In fact, on one hand, PCSK9 inhibitors (but this applies also to inclisiran and other biologics) have a higher cost compared with conventional oral cholesterol-lowering agents; on the other hand, they have definitely a higher cholesterol-lowering efficacy. Starting from these considerations, which can be the role for the new oral bempedoic acid in this context? Patients can be not too far from their goal, but having LDL-C above the recommended level, they are virtually eligible for PCSK9 therapy; in these patients, the addition of bempedoic acid to the ongoing LLT might favour a further (although modest if compared with PCSK9 mAbs) LDL-C reduction, allowing to reach the goal without a PCSK9 mAb. Furthermore, statin-intolerant patients, who commonly show a poor adherence and, instead, a high discontinuation rate of statin therapy, might benefit from the use of bempedoic acid. A recent simulation study performed in a cohort

of patients with coronary heart disease showed that the introducing bempedoic acid in the algorithm will reduce substantially the percentage of patients requiring a PCSK9 inhibitor to reach their goal, thus lowering medical expenditure (134). It appears that patients with fully statin intolerance might have the greatest benefit in relation to cost (134).

Improving adherence to therapy. In spite of the clearly established clinical benefit of cholesterol-lowering therapies, the everyday clinical practice shows inadequacy in the pharmacological approach among patients with established ASCVD, with a poor attainment of LDL-C target in patients at high CV risk (135, 136). Furthermore, there is a low awareness of the danger of CV risk factors, and the occurrence of adverse events that are ascribed to the therapy easily translates into a time-decreasing adherence to therapy. This is even more evident among patients experiencing muscle-related adverse events (no matter if they are really imputable to therapy or not), or having mild-to-moderate response to the therapy (which is inevitably related to the individual response, but more likely to an inadequate approach), with an increasing percentage of patients discontinuing medications. Thus, improving adherence is crucial and every step must be taken to fill this gap. It is evident that the use of fixed-dose combination therapies, by combining in one pill two or more drugs, may make the patient more willing to take medications, with more chances to attain substantial reductions in LDL-C levels, which in turn may favour the adherence to an "effective" (from the patient point of view) treatment. It is also evident that biological cholesterol-lowering drugs (mAbs, siRNA), having administration regimens different from the oral agents that must be taken daily, together with a higher efficacy, may provide significant reductions in LDL-C with infrequent dosing (although at substantially higher costs).

Conclusions

In the last three decades since the approval of statin therapy, an extraordinary accumulation of evidence which has shown that reduction of LDL-C levels results in reduced incidence of CV events and that achieving lower levels of LDL-C leads to greater event reduction, which led to new guidelines for the treatment of high-risk and very-high-risk patients. With rapid progress in identification of treatment targets through genetic epidemiology and advances in both pharmacology and biotechnology, several options are now available in addition to statins that are highly effective in lowering LDL-C levels. However, there is currently a major gap between the evidence-based goals of treatment in the guidelines and clinical practice. Changes in approach, with earlier use of combination therapy including two agents in a single pill (137), as routinely used to treat hypertension successfully, and increased use of infrequently used therapies may provide great opportunities to improve guideline implementation in clinical practice.

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