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Review article

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Novel and future lipid-modulating therapies for the prevention of cardiovascular disease

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Abstract

Lowering the levels of LDL cholesterol in the plasma has been shown to reduce the risk of atherosclerotic cardiovascular disease (ASCVD). Several other lipoproteins, such as triglyceride-rich lipoproteins, HDL and lipoprotein(a) are associated with atherosclerosis and ASCVD, with strong evidence supporting causality for some. In this Review, we discuss novel and upcoming therapeutic strategies targeting different pathways in lipid metabolism to potentially attenuate the risk of cardiovascular events. Key proteins involved in lipoprotein metabolism, such as PCSK9, angiopoietin-related protein 3, cholesteryl ester transfer protein and apolipoprotein(a), have been identified as viable targets for therapeutic intervention through observational and genetic studies. These proteins can be targeted using a variety of approaches, such as protein inhibition or interference, inhibition of translation at the mRNA level (with the use of antisense oligonucleotides or small interfering RNA), and the introduction of loss-of-function mutations through base editing. These novel and upcoming strategies are complementary to and could work synergistically with existing therapies, or in some cases could potentially replace therapies, offering unprecedented opportunities to prevent ASCVD. Moreover, a major challenge in the prevention and treatment of non-communicable diseases is how to achieve safe, long-lasting reductions in causal exposures. This challenge might be overcome with approaches such as small interfering RNAs or genome editing, which shows how far the field has advanced from when the burden of achieving this goal was placed upon patients through rigorous adherence to daily small-molecule drug regimens.

Sections

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Key points

- LDL cholesterol is a causal and cumulative factor in the development of atherosclerosis; therefore, reducing plasma LDL cholesterol levels, irrespective of the approach used, decreases the risk of cardiovascular disease.
- Data from observational and genetic studies have revealed novel treatment targets beyond LDL cholesterol lowering.
- Novel treatment targets can be classified into those that increase the number of LDL receptors (LDLRs) in hepatocytes and those that interfere with the modification and composition of atherogenic lipoproteins.
- Besides statins and ezetimibe, treatments that enhance LDLR function (LDLR-dependent treatments) include bempedoic acid, which targets ATP citrate lyase, and PCSK9-targeting therapies, such as monoclonal antibodies or the small interfering RNA inclisiran.
- Emerging treatments that modify lipoprotein composition target apolipoprotein C-III, ANGPTL3, cholesteryl ester transfer protein and lecithin–cholesterol acyltransferase; therapies that modify lipoprotein number target apolipoprotein(a); and those that enhance lipoprotein function target apolipoprotein A-I.
- Novel approaches with sustained effects and infrequent dosing regimens might overcome the issues with treatment adherence that are common to therapies requiring more frequent dosing.

Introduction

The burden of atherosclerotic cardiovascular disease (ASCVD) remains high despite the availability of effective therapies targeting modifiable risk factors such as hypertension and high plasma levels of LDL cholesterol. An estimated 17.9 million people died from ASCVD in 2019, representing 32% of all global deaths[1]. Reducing plasma LDL cholesterol levels has been shown to lower the risk of cardiovascular events, and these benefits are constant per unit change in plasma LDL cholesterol level and independent of the individual baseline risk of the patient, presence or absence of comorbidities, baseline lipid profile and approach to LDL cholesterol lowering[2]. A meta-analysis by the Cholesterol Treatment Trialists showed that the relative risk reduction in cardiovascular events with statin therapy was 22% per 1 mmol/l reduction in plasma LDL cholesterol levels[3]. In patients with a high baseline risk of cardiovascular events, this 1 mmol/l absolute lowering of LDL cholesterol levels translates into greater absolute risk reductions. The greater absolute benefit in patients with high or very high risk of cardiovascular events, who constitute a small proportion of patients at risk of cardiovascular disease, is the rationale for using additional, often costly, lipid-lowering therapies alongside first-line therapies such as statins. This approach of combination therapies will be required to achieve the increasingly lower LDL cholesterol goals recommended by the guidelines, which might not be achievable with any single therapy, analogous to hypertension management, in which the use of multidrug combination therapies is the norm. In this regard, several therapies have become available in the past decade, with robust data demonstrating that these strategies to lower plasma LDL cholesterol levels are safe and can reduce the incidence of cardiovascular outcomes. However, these therapies remain underutilized in clinical practice for a variety of reasons.

Even though LDL cholesterol level in the plasma is a good surrogate for the atherogenic cholesterol concentration in the plasma, all apolipoprotein B (apoB)-carrying lipoproteins (such as VLDL, intermediate-density lipoprotein (IDL) and chylomicrons, as well as their cholesterol-rich remnants[4]) are potentially atherogenic[5]. Indeed, in patients with diabetes mellitus, metabolic syndrome or other presentations of insulin resistance, the plasma levels of these triglyceride-rich lipoproteins are elevated, contributing to the risk of ASCVD[6]. In these patients, other lipid measures, such as non-HDL cholesterol or apoB levels, might be more representative of total atherogenic lipid burden than LDL cholesterol levels. Moreover, genetic and observational studies support a causal role of the LDL-like particle lipoprotein(a) (Lp(a)) in ASCVD[7–9]. Whether lowering the plasma levels of Lp(a) with pharmacological therapies translates into similar reductions in the risk of ASCVD to those observed with LDL cholesterol-lowering therapies remains to be established.

In this Review, we discuss current treatment regimens for lowering plasma LDL cholesterol levels to reduce the risk of cardiovascular disease and highlight treatment gaps and challenges, as well as the potential of both novel available and future therapeutic approaches to address them. Finally, we describe the characteristics and mechanisms of action of these novel and emerging therapies. In the context of this Review, novel therapies refer to those that are approved for clinical use to treat dyslipidaemia and/or reduce the risk of cardiovascular events and have mechanisms of action distinct from those of previous therapies. Novel therapies include bempedoic acid, inclisiran, evinacumab and icosapent ethyl. Inevitable questions from clinicians about these strategies include how they work, how safe they are, where they fit into current treatment pathways and how to use them. Potential future therapeutic strategies refer to therapies in development that have not been approved for routine clinical practice, but that might resolve current unmet medical needs by targeting novel pathways or existing pathways through novel approaches.

Current lipid-lowering regimens

National and international guidelines recommend plasma LDL cholesterol as the primary plasma lipid target for reducing the risk of cardiovascular events, recommending the lowest LDL cholesterol level thresholds for those at highest risk[10–12]. Furthermore, guidelines propose a stepwise approach to treatment escalation to achieve LDL cholesterol goals[13].

Statin therapy is recommended as the first-line pharmacological treatment in patients with a risk of ASCVD that is sufficiently high to benefit from pharmacological LDL cholesterol lowering with an acceptable 'number needed to treat' (>5% for 10-year risk of fatal cardiovascular disease[10] and >20% for 10-year risk of a first ASCVD event[10,11]). Statins reduce LDL cholesterol levels by inhibiting HMG-CoA reductase (an enzyme that catalyses the rate-limiting step in cholesterol biosynthesis), which leads to reduced cholesterol levels in the liver and upregulation of LDL receptors (LDLRs) in hepatocytes, thereby increasing the clearance of circulating LDL. The second-line treatment is ezetimibe, either as an add-on to statin therapy or as monotherapy. Ezetimibe, which inhibits cholesterol absorption in the intestine, reduces cardiovascular events in proportion to the absolute reduction in plasma LDL cholesterol levels and the duration of therapy, both as monotherapy and as add-on to a statin[2,14,15]. Moreover, if similar reductions in LDL cholesterol level are achieved with high-intensity statin monotherapy

or with the combination of moderate-intensity statin therapy plus ezetimibe, similar rates of cardiovascular events are observed[16]. PCSK9-targeting treatments should be considered in patients at very high risk of cardiovascular disease and in patients with familial hypercholesterolaemia in the high-risk category[10]. PCSK9 binding to the LDLR promotes the degradation of LDLRs in hepatocytes, thereby decreasing the number of LDLRs on the cell surface. Therefore, blocking the interaction between circulating PCSK9 and LDLRs prevents LDLR degradation and promotes the clearance of circulating LDL cholesterol (Fig. 1).

However, in real-world clinical practice, statin monotherapy is the most frequently used strategy. For example, in the DA VINCI registry[17] in Europe, statin-based monotherapy was used in approximately 84% of patients who received lipid-lowering therapy, resulting in only 33% of patients achieving 2019 ESC/EAS risk-based LDL cholesterol goals[10] (these guidelines lowered the LDL cholesterol target values in three cardiovascular risk categories). Moreover, the first results from the FHSC registry[18] in patients with familial hypercholesterolaemia revealed that among patients with extreme elevations in plasma LDL cholesterol levels from birth, only 2.7% of the patients achieved LDL cholesterol levels <1.8 mmol/l with lipid-lowering medications. Both the DA VINCI[17] and FHSC[18] registries show that combination therapies are underutilized, ranging from 10% combination therapy in patients without familial hypercholesterolaemia to approximately 21% in those with familial hypercholesterolaemia. This finding largely explains the poor attainment of treatment goals with monotherapy in those patients with high starting levels of LDL cholesterol. The reasons behind the poor implementation of guidelines are likely to be multifactorial, including access to and cost of therapies, concerns about safety of the more potent regimens and inability to quantify the benefits of therapy, as well as poor health literacy and perception of medication benefits, and inadequate treatment maintenance among patients[19,20].

Higher intensity with combination therapy

If the average plasma LDL cholesterol level before treatment is 3.0–3.5 mmol/l in the general population[21,22], attaining LDL cholesterol targets as low as 1.8 mmol/l or even 1.4 mmol/l is unlikely to be feasible in the majority of individuals with the use of high-intensity statin monotherapy (which is generally considered to reduce LDL cholesterol levels by approximately 50%), necessitating safe and effective adjunct therapies. Therefore, from a practical standpoint, once cardiovascular risk assessment has been completed and the treatment pathway recommends a very low LDL cholesterol target, combination therapy should be considered early in the decision-making process as a prerequisite to achieve effective plasma LDL cholesterol control.

According to simulations from the SWEDEHEART registry[23], even among patients with a very high risk of cardiovascular events and a recent myocardial infarction, 90% of the patients achieved their guideline recommended LDL cholesterol goal through a combination

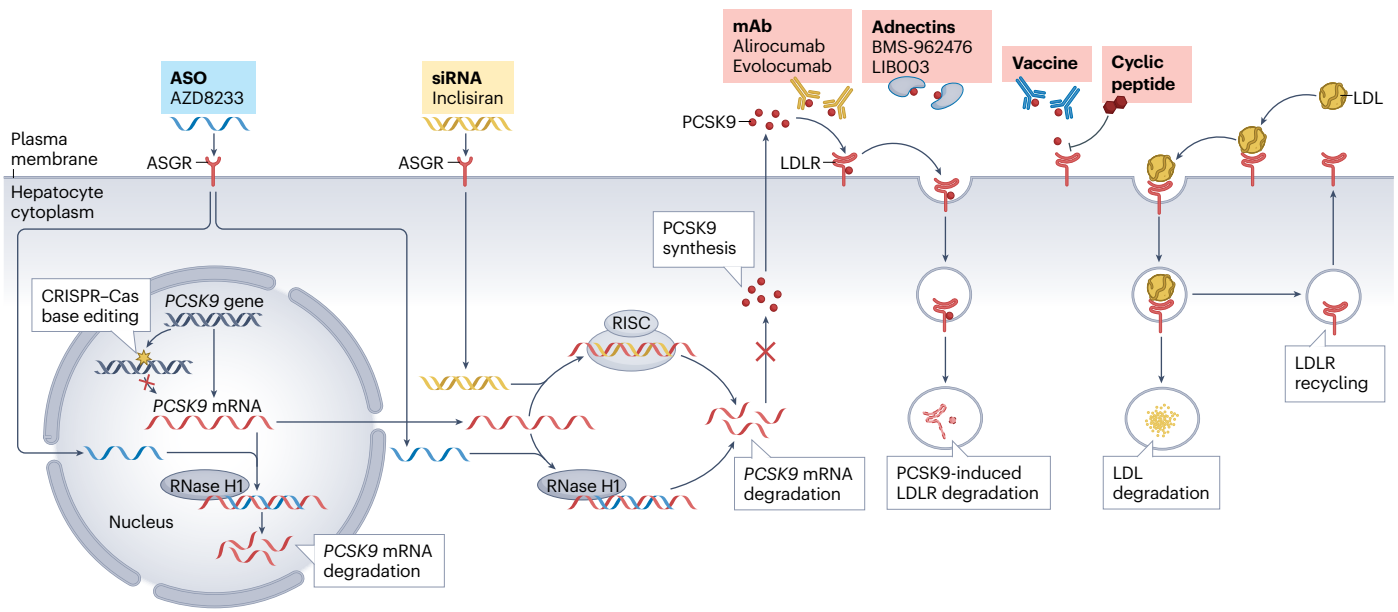

Fig. 1 | Novel and emerging LDL-lowering therapies targeting PCSK9. Binding of an LDL particle to the LDL receptor (LDLR) leads to LDL internalization and subsequent degradation and LDLR recycling to the cell surface. Binding of PCSK9 to the LDLR disrupts the LDLR recycling process and initiates degradation of the whole receptor–ligand complex. Therefore, fewer LDLRs are present at the hepatocyte surface, thereby reducing the hepatocyte capacity to take up circulating LDL particles and hence decreasing LDL clearance. At the gene level, strategies that are currently at preclinical stages of development involve CRISPR–Cas base editing to introduce loss-of-function variants in *PCSK9*, which ultimately leads to a reduction in PCSK9 protein levels. Strategies targeting PCSK9 at the mRNA level include the antisense oligonucleotide (ASO) AZD8233 and the small interfering RNA (siRNA) inclisiran, which are both at clinical trial stages of development. AZD8233 binds to *PCSK9* mRNA in the nucleus and the cytoplasm, causing *PCSK9* mRNA degradation via RNase H1-mediated cleavage, thereby suppressing PCSK9 protein synthesis, whereas inclisiran is loaded into the RNA-induced silencing complex (RISC) in the cytoplasm to induce *PCSK9* mRNA degradation. Both AZD8233 and inclisiran are conjugated to triantennary *N*-acetylgalactosamine, which allows hepatocyte-specific uptake through asialoglycoprotein receptors (ASGR). At the protein level, the therapeutic agents bind to the PCSK9 protein to inhibit its interaction with the LDLR. Therapies approved for clinical use include the monoclonal antibodies (mAbs) evolocumab and alirocumab. Strategies in clinical development include adnectins (BMS-962476 and LIB003), a vaccine and a cyclic peptide.

of a high-intensity statin therapy with ezetimibe and a monoclonal antibody against PCSK9, provided that the patients had residual LDLR function. However, in patients with extremely high pretreatment levels of LDL cholesterol and with little or no LDLR activity, as seen in those with homozygous familial hypercholesterolaemia, maximizing combination therapy with a triple combination of statin, ezetimibe and a PCSK9-targeting medication is insufficient. Previously, the treatment regimen in these patients could only be escalated to lipid apheresis, which does not require functioning LDLRs[24,25]. Attempts at using pharmacotherapy to reduce apoB assembly (and thereby reduce circulating levels of LDL precursors, which would be independent of LDLR) have had mixed results[26]. High costs and adverse effects, such as large and frequent injection-site reactions with mipomersen[27] and an increase in hepatic fat with lomitapide[28], have limited the use of these medications.

A novel therapy, evinacumab, was approved in 2021 by the EMA and the FDA for the treatment of homozygous familial hypercholesterolaemia. Evinacumab is a monoclonal antibody that binds to circulating angiopoietin-related protein 3 (ANGPTL3) and sterically prevents the ANGPTL3-induced inhibition of lipoprotein lipase (LPL) and endothelial lipase (EDL) activity. The increase in LPL activity through evinacumab therapy enhances the hydrolysation of triglycerides. Thereby, evinacumab reduces plasma LDL cholesterol levels independently of the presence of LDLR by promoting the processing of VLDL and the removal of VLDL remnants before the formation of LDL, through an EDL-dependent mechanism[29].

Evinacumab has a recommended dosing of monthly intravenous injections. A phase III trial[29] tested evinacumab in 65 patients with a clinical or genetic diagnosis of homozygous familial hypercholesterolaemia and a mean age of 42 years. Importantly, evinacumab was tested on a background of stable lipid-lowering therapies, including high-intensity statins (77%), ezetimibe (75%), monoclonal antibodies against PCSK9 (77%), lomitapide (25%) and lipid apheresis (34%), with >50% of patients receiving triple combination therapy[29]. Despite intense background therapy, baseline plasma LDL cholesterol levels were high, at 6.59 mmol/l. Monthly intravenous infusion of evinacumab (15 mg per kg of body weight) reduced LDL cholesterol levels from baseline by 49% at 24 weeks compared with placebo, with consistent effects irrespective of background lipid-lowering treatments, including apheresis. Notably, no differences in efficacy were observed between patients with null–null *LDLR* variants and patients with non-null *LDLR* variants[29]. Even evinacumab-treated patients with <2% remaining LDLR function achieved LDL cholesterol reductions of 72% compared with placebo-treated patients, consistent with the proposed mode of action of evinacumab independent of the LDLR[29]. Overall, the frequency of adverse events was similar in the evinacumab and placebo groups[29,30]. Therefore, additional LDL cholesterol lowering by about 50% in patients with extremely high LDL cholesterol levels who are receiving maximally tolerated treatment regimens, including PCSK9-targeting medications, can be achieved by adding either a monthly intravenous regimen of evinacumab or a weekly, subcutaneous, self-administered dose of evinacumab. This strategy, if started early, might mitigate the risk of cardiovascular events to almost the risk levels in the general population.

Reducing adverse effects

Rates of perceived adverse effects are fairly high with statins. Observational studies suggest that 5–10% of individuals taking statins describe an inability to tolerate statins, mostly owing to muscle-related symptoms that range from myalgia with or without elevation of plasma levels of muscle enzymes (creatine kinase) to (rarely) rhabdomyolysis[31]. By contrast, no differences in adverse effects between statin and placebo groups have been found in placebo-controlled trials[32,33]. A meta-analysis published in 2022 including data from 19 randomized, controlled trials found a 4% excess risk of statin-associated muscle symptoms[34]. Moreover, one in 15 reports of muscle pain was associated with statin treatment[34].

Regardless of whether or not these symptoms can be attributed to the statin treatment directly[35], the reputation of these therapies among patients, and possibly care-givers, has been damaged. Through increased organ specificity, adverse effects leading to treatment discontinuation are low with the use of more recent LDL cholesterol-lowering treatments. This finding might change a patient's perception of lipid-lowering therapies in general, by showing that it is not lipid lowering per se that is associated with adverse symptoms, but rather that some therapies can more commonly cause symptoms, related to their kinetics and mode of action. For example, muscle symptoms do not seem to be more frequent with bempedoic acid than with placebo[36]. Bempedoic acid is a small-molecule inhibitor of ATP citrate synthase (ACLY), an enzyme that acts upstream of HMG-CoA reductase (which is targeted by statins). ACLY converts citrate plus CoA into oxaloacetate and acetyl-CoA, which is the substrate for fatty acid and cholesterol synthesis. Similar to statins, which also interfere with cholesterol biosynthesis, inhibition of ACLY by bempedoic acid decreases cytosolic cholesterol concentration, leading to the activation of the transcription factor sterol regulatory element-binding protein, which increases *LDLR* expression and thus the number of LDLRs on the hepatocyte surface, thereby enhancing the clearance of circulating LDL particles. Bempedoic acid is administered orally as a prodrug, at a dose of 180 mg per day, and requires intracellular activation by the liver-specific enzyme long-chain fatty acid transport protein 2. This enzyme is largely absent in skeletal muscle, which might potentially mitigate muscle-related symptoms associated with bempedoic acid therapy, despite initiating the same cascade of intracellular cholesterol depletion and LDLR upregulation as does statin therapy. Therefore, bempedoic acid might be an effective LDL cholesterol-lowering therapy in patients with statin intolerance, as has been shown in the CLEAR Serenity and CLEAR Tranquility trials[37,38]. In these trials, bempedoic acid treatment reduced LDL cholesterol levels by 24.5% compared with placebo in the absence of statin background therapy[39]. However, bempedoic acid has some adverse effects. In the CLEAR Serenity trial[37], patients treated with bempedoic acid had no increase in muscle-related adverse effects compared with those receiving placebo, but had higher rates of gout (1.7% versus 0.9%). In the CLEAR Outcomes trial[40] in a patient population with established or at high risk of cardiovascular disease and statin intolerance (baseline plasma LDL cholesterol level ~139 mg/dl (3.59 mmol/l)), bempedoic acid treatment reduced plasma LDL cholesterol levels by approximately 20% compared with placebo. In addition, compared with placebo, bempedoic acid treatment reduced the primary end point of cardiovascular death, non-fatal myocardial infarction, non-fatal stroke or coronary revascularization by 13% and the composite of cardiovascular death, non-fatal myocardial infarction or non-fatal stroke by 15%[40].

Given that lipid-modulating treatment for the prevention of ASCVD is a lifelong approach, for a therapy to be acceptable it needs to have a good safety profile and be well tolerated. Safety and tolerability have, in part, been improved through the current approach to drug development. Small interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs) are designed for a genetically determined

treatment target, thereby potentially improving the specificity and efficacy with a lower likelihood of off-target effects than conventional therapies. Moreover, specific molecular alterations to achieve greater stability and better organ specificity can further reduce off-target effects. For instance, conjugation of the siRNA or ASO with triantennary *N*-acetylgalactosamine (GalNAc) facilitates active liver-specific uptake through asialoglycoprotein receptors in hepatocytes[41]. In the case of volanesorsen, an ASO targeting *APOC3*, its GalNAc-conjugated form (olezarsen) significantly improved safety, which is discussed in more detail below.

Increasing treatment adherence

Small-molecule drugs, such as statins, ezetimibe or bempedoic acid, have a short half-life and must be taken daily. Therefore, optimal treatment adherence is required with these medications to achieve the desired LDL cholesterol reduction. However, in clinical reality, adherence to statin therapy is poor and declines over time[42,43]. In primary care, only 61% of patients with high cardiovascular risk adhered to therapy for 3 months after the first prescription of a statin, and only 55% adhered to therapy for 6 months[44]. Poor treatment adherence to lipid-lowering therapy impairs the likelihood of achieving the optimal LDL cholesterol target and has a detrimental effect on the prevention of ASCVD. In secondary prevention, a significant reduction in recurrent myocardial infarction rates and mortality could only be shown with a >80% adherence to statin therapy[45].

Clinical trial data indicate good adherence rates in patients who self-administer monoclonal antibodies against PCSK9 (ref. 46). However, reports from clinical practice have shown that acceptable adherence rates, even for antibody therapies, are achieved in only about 30% of patients within the first year of starting therapy[47]. Therefore, new long-lasting therapies and modes of application that reduce the treatment burden for the patient are highly relevant. For example, for inclisiran (a siRNA targeting *PCSK9*), only two injections per year provide an additional 50% reduction in LDL cholesterol levels on a background of statin therapy[48]. The resulting simplified, but high-intensity treatment regimen could increase treatment adherence[49] and, as data from the ORION-1 trial[50] show, might also reduce the intraindividual variability in LDL cholesterol levels over time that has been observed with statin therapy[51]. Low intraindividual LDL cholesterol variability over time is associated with greater cardiovascular benefits[52]. Therefore, long-lasting therapies such as inclisiran might help to achieve even greater reductions in the risk of cardiovascular events compared with treatments with a similar percentage of LDL cholesterol-lowering effectiveness but with higher inherent on-treatment LDL cholesterol variability, such as small-molecule drugs that require daily dosing (for example, high-intensity statins). Furthermore, the infrequent dosing regimen combined with administration by a health-care professional might help to achieve almost optimal treatment adherence and, therefore, maximize the cardiovascular benefits[53].

Whether the LDL cholesterol-lowering effect of inclisiran also leads to a reduction in ASCVD events will be investigated in the ORION-4 cardiovascular outcomes trial[54]. This randomized, placebo-controlled trial is currently ongoing and aims to recruit approximately 15,000 participants with a history or evidence of stable ASCVD (previous myocardial infarction or ischaemic stroke, or peripheral artery disease defined by previous lower limb artery revascularization or aortic aneurysm repair) and aged ≥40 years for men and ≥55 years for women, and will have a follow-up of 5 years. Given that this trial is an event-driven study, >1,500 assessed cardiovascular events will be reported, which will provide robust estimates of the benefit of inclisiran therapy for cardiovascular outcomes.

Beyond targeting LDL: omega-3 fatty acids

Current lipid-targeting approaches for the prevention of ASCVD focus on lowering LDL cholesterol levels. Other potential approaches include using omega-3 fatty acids, which have been shown to reduce the plasma levels of triglycerides and triglyceride-rich lipoproteins, and have shown mixed, but promising results in cardiovascular outcome trials. Data from an open-label, randomized trial[55] indicated that in Japanese patients with hypercholesterolaemia, treatment with an eicosapentaenoic acid (EPA; 1,800 mg of pure icosapent ethyl per day) reduced cardiovascular events by 19% after a mean follow-up of 4.6 years compared with placebo. However, trial limitations included the PROBE design (open interventional design with blinded clinical end point assessment) and the high levels of EPA at baseline in the study population owing to dietary intake of EPA, mainly though intake of fish. In the REDUCE-IT trial[56] in patients with hypertriglyceridaemia and established cardiovascular disease or with diabetes and additional risk factors, treatment with 4 g of icosapent ethyl per day reduced cardiovascular events by 25% after a median of 4.9 years compared with placebo. Furthermore, the EVAPORATE trial[57] data suggest that treatment with icosapent ethyl slows atherosclerosis progression and potentially induces plaque regression in patients with ASCVD and hypertriglyceridaemia who are receiving statins.

These results differ markedly from the findings of the ASCEND[58], VITAL[59] and STRENGTH[60] clinical trials, which did not find a beneficial effect of supplementation with omega-3 fatty acids in reducing the risk of cardiovascular disease. The ASCEND trial[58] included patients with type 2 diabetes and without ASCVD, whereas the VITAL trial[59] assessed men aged ≥50 years and women aged ≥55 years without ASCVD, and both tested low-dose formulations of EPA plus docosahexaenoic acid (DHA). The STRENGTH trial[60] investigated the efficacy of a mixture of omega-3 fatty acids (EPA and DHA) compared with corn oil for the secondary prevention of ASCVD in patients receiving statin therapy and for the primary prevention of ASCVD in patients at high risk of ASCVD who had high triglyceride levels and low HDL cholesterol levels.

Three main hypotheses might account for the contrasting results among the different trials. First, high EPA levels in plasma or a certain threshold of EPA in plasma (~100 μg/ml) might be required to provide a beneficial effect on cardiovascular outcomes, as achieved in the REDUCE-IT trial[56] but not in the STRENGTH trial[61]. Second, the effects of EPA and DHA differ, and the latter might have adverse effects on cardiovascular events, potentially outweighing the beneficial effect of EPA therapy in the STRENGTH trial. However, secondary analysis of the STRENGTH trial data showed no association between cardiovascular end points and the achieved levels of EPA and DHA in plasma[62]. Third, the placebo used in the REDUCE-IT trial, a mineral oil, has been shown to increase inflammatory biomarkers associated with ASCVD and reduce the absorption of statins, thereby causing an increase in LDL cholesterol levels[63]. By contrast, the corn oil placebo used in the STRENGTH trial had neutral effects. Nevertheless, the observed beneficial effect of EPA in these clinical trials was probably real, but its magnitude is uncertain and might have been overestimated in the REDUCE-IT trial.

The exact mechanisms of action of omega-3 fatty acids in reducing levels of triglyceride-rich lipoproteins in the plasma are not clear. In humans, EPA and DHA might reduce plasma triglyceride-rich lipoprotein levels mainly through two mechanisms: by reducing hepatic production of VLDL and by increasing postprandial LPL activity[64].

Novel and emerging therapies

The characteristics of novel and emerging therapies for modulating plasma lipid levels and their effects on the plasma levels of different lipoproteins and the risk of cardiovascular events are summarized in Table 1. Molecular pathways can be targeted therapeutically at three levels: the protein, the mRNA and the gene. At the protein level, small-molecule drugs or monoclonal antibodies can inhibit the action of the protein either by affecting the binding of the protein to a relevant receptor or by sequestering circulating proteins, thereby reducing the amount of freely available protein to exert its biological effects. At the mRNA level, ASOs and siRNAs trigger the degradation of target mRNA to prevent translation into the protein[65]. ASOs are single-stranded RNA or DNA sequences that are complementary to target mRNA and bind through Watson–Crick base-pair interactions to the target transcripts in the nucleus and cytoplasm. The non-sequence-specific endonuclease RNase H1 cleaves the mRNA strand, thereby preventing translation into the protein. siRNAs are single-stranded or double-stranded RNAs with a guide strand that is complementary to a target mRNA. siRNAs induce the endogenous process of RNA interference in the cytoplasm. The siRNA is loaded in the RNA-induced silencing complex (RISC) and the antisense (guide) strand directs the RISC to the target mRNA, which is then degraded by the complex. Specific molecular modifications to the siRNA can modulate its stability within the complex, thereby facilitating the degradation of multiple mRNAs and enabling a long duration of efficacy (up to several months with a single dose). At the gene level, two approaches are currently being explored in the context of lipid-lowering therapies: CRISPR–Cas base editing and CRISPR–Cas gene editing.

Targeting PCSK9

Although PCSK9-targeting therapeutics lower LDL cholesterol levels by increasing the number of LDLRs on the cell surface, their mechanism of action is very different from that of statins or bempedoic acid (Fig. 1). Extracellular PCSK9 regulates LDLR recycling. Binding of PCSK9 to LDLR prevents normal recycling of the LDLR and instead targets the LDLR–PCSK9 complex for lysosomal degradation. Inhibition of PCSK9 leads to increased LDLR recycling rates and prolonged LDLR functional lifespan[66–68]. Therefore, therapies directed at inhibiting PCSK9 or decreasing PCSK9 levels are a logical strategy for lowering circulating LDL cholesterol levels.

The functions of PCSK9 beyond its role in LDLR degradation, especially its intracellular functions, are not as well understood. PCSK9 regulates lipid homeostasis in hepatocytes, and high levels of PCSK9 have been shown to be associated with an increased risk of non-alcoholic fatty liver disease (NAFLD)[69]. Most preclinical studies suggest a pro-steatotic role of PCSK9 (refs. 70,71). However, PCSK9 in the endoplasmic reticulum acts as a chaperone protecting against endoplasmic reticulum stress, which should attenuate liver damage[69]. In addition, the results from clinical studies are conflicting. One study found that hepatic PCSK9 expression was inversely correlated with steatosis in patients with obesity[72]. By contrast, another study found that in patients with obesity who underwent bariatric surgery, higher circulating PCSK9 levels were associated with increased steatosis[73]. Therefore, the effect of PCSK9 might depend on its cellular localization or the diet and disease state of the individual. In addition, the different therapeutic approaches targeting PCSK9 result in different circulating and intracellular levels of PCSK9. The first PCSK9-targeted medications approved for clinical use were monoclonal antibodies, followed by the siRNA inclisiran. The main difference between the antibody approach and the siRNA approach is that the first inhibits the interaction between PCSK9 and the LDLR and leads to a compensatory increase in plasma PCSK9 levels[74], whereas the latter leads to decreased plasma PCSK9 levels by lowering PCSK9 synthesis rates[50]. Further research into whether these changes translate into clinically relevant effects, such as the occurrence of NAFLD, is warranted.

All currently available PCSK9-targeting approaches lead to further reductions in plasma LDL cholesterol levels of >50% in addition to the reductions achieved with statin therapy[48,75]. Numerically, monoclonal antibodies against PCSK9 achieve greater percentage reductions than the currently available siRNA inclisiran, but head-to-head comparisons would be required to confirm within-class differences in efficacy.

PCSK9 inhibitors reduce not only LDL cholesterol levels, but also cholesterol carried in triglyceride-rich lipoproteins[5,76,77], although the achieved reductions in triglyceride-rich lipoprotein levels are more modest than those achieved with statin therapy. A study that compared the effect of atorvastatin and evolocumab on postprandial triglyceride-rich lipoprotein metabolism after ingestion of an oral fat load in 80 healthy men with normolipidaemia found that evolocumab reduced the concentrations of apoB-100-carrying triglyceride-rich lipoproteins, such as VLDL, but not of apoB-48-carrying lipoproteins (which are mostly chylomicrons)[78]. By contrast, atorvastatin reduced both apoB-100 and apoB-48 concentrations. These differences might be explained by additional inhibitory effects of atorvastatin on apolipoprotein C-III (apoC-III) and ANGPTL3, two inhibitors of LPL, because the hepatic removal of apoB-48-carrying lipoproteins requires LPL-dependent lipolysis. PCSK9-targeting medications affect triglyceride-rich lipoprotein levels only through stimulation of hepatic LDLR-mediated clearance, and thus have no effect on chylomicron removal.

Ongoing efforts in the development of PCSK9-targeting medications mainly focus on increasing its inhibitory effect and the duration of the effect, improving the formulations to reduce the volume of injectables, increasing the ease of use to enable self-administration, and alternative modes of application, especially oral formulations.

Adnectins targeting PCSK9: BMS-962476 and LIB003. Adnectins act at the protein level and are synthetic polypeptides that bind with high affinity and specificity to their target[79]. Currently, two adnectins targeting PCSK9 are under development: BMS-962476 and LIB003. Similar to monoclonal antibodies against PCSK9, these adnectins bind to PCSK9 and inhibit the interaction between PCSK9 and the LDLR. Therefore, the LDLR is not guided towards lysosomal degradation after internalization into the hepatocyte and is instead recycled, leading to an increased number of LDLRs on the surface of hepatocytes and thus increased uptake of circulating LDL (Fig. 1). BMS-962476 reduced LDL cholesterol concentration in plasma by up to 48% between day 4 and day 14 after a single subcutaneous administration[80], suggesting the need for a dosing regimen of injections every 2 weeks.

LIB003 is a recombinant fusion protein of a PCSK9-binding adnectin plus human serum albumin. Linkage of albumin to the adnectin increases the half-life to up to 12–15 days, thereby allowing decreased dosing frequency. Subcutaneous injection of 300 mg LIB003 every 4 weeks led to a maximum LDL cholesterol reduction of 77% at week 12 compared with placebo in patients with plasma LDL cholesterol levels >2.07 mmol/l at baseline who were already receiving maximally tolerated statin therapy[81]. At the 36-week, open-label, extension phase of the study, LDL cholesterol levels remained continuously reduced by 60% from baseline[82].

Table 1 | Efficiency and efficacy of novel and emerging lipid-modulating therapies

Drug	Molecular target	Drug type	Dosage	Phase of development	Change in plasma level (%)					HR (95% CI) for MACE
					LDL cholesterol	Non-HDL cholesterol	VLDL cholesterol	HDL cholesterol	Lp(a)	
Bempedoic acid	ACLY	Small molecule	180 mg once per day; oral	III–IV	−18.1 (ref. 36)	−13.3 (ref. 36)	NR	−1.8 (ref. 194)	NS[36]	0.87 (0.79–0.96)[40]
Inclisiran	*PCSK9*	siRNA	300 mg every 6 months; SC injection	III–IV	−49.9 to −52.3 (ref. 48)	−46 (ref. 77)	−16 (ref. 77)	+9 (ref. 77)	−25 (ref. 77)	ORION 4 trial[195], ongoing
AZD8233	*PCSK9*	ASO	Oral, SC injection	I	−68 (ref. 83)	NR	NR	NR	NR	NA
BMS-962476	PCSK9	Adnectin	Assumed to be 0.3 mg per kg of body weight every 2 weeks; SC injection	I	−48 (ref. 80)	NR	NR	NR	NR	NA
LIB003	PCSK9	Adnectin	300 mg once per month; SC injection	II–III	−60 to −77 (refs. 81,82)	NR	NR	NR	−29 (ref. 82)	NA
Obicetrapib	CETP	Small molecule	10 mg once per day; oral	II	−45.3 (ref. 143)	NR	NR	+179.0 (ref. 143)	−33.4 (ref. 143)	Planned clinical trial[144]
Icosapent ethyl	Unknown	Omega-3 fatty acid	2 g twice per day; oral	III–IV	−11.4 (ref. 56)	−13.1 (ref. 56)	NR	−2.5 (ref. 56)	NR	0.75 (0.68–0.83)[56]
Omega-3 fatty acids (DHA and EPA)	Unknown	Omega-3 fatty acid	4 g once per day; oral	III	−2.3 (ref. 60)	−5 (ref. 60)	NR	NS[60]	NR	0.99 (0.90–1.09)[60]
Volanesorsen	*APOC3*	ASO	285 mg every week or every 2 weeks; SC injection	III–IV	+130 (ref. 107)	−46 (ref. 107)	−58 (ref. 107)	+46 (ref. 107)	NR	NA
Olezarsen	*APOC3*	ASO	50 mg every 4 weeks; SC injection	II–III	−1 to +23 (ref. 110)	−20 (ref. 110)	−58 (ref. 110)	+30 (ref. 110)	NR	NA
Evinacumab	ANGPTL3	Monoclonal antibody	15 mg per kg of body weight once per month or 300 mg once per week; SC injection	III–IV	−52.9 (ref. 30)	−53.8 (ref. 30)	NR	−30.3 (ref. 30)	−11.9 (ref. 30)	NA
Vupanorsen	*ANGPTL3*	ASO	80 mg once per month; SC injection	II	−7 (ref. 125)	−18 (ref. 125)	−38 (ref. 125)	−24 (ref. 125)	NS[125]	NA
ARO-ANG3	*ANGPTL3*	siRNA	300 mg once per month; SC injection	I–II	−54 (ref. 196)	NR	−65 (ref. 131)	−37 (ref. 131)	NR	NA
Pelacarsen	*LPA*	ASO	20 mg once per week or 80 mg once per month; SC injection	III	−15.2 (ref. 167)	NR	NR	+3.7 (ref. 167)	−80 (ref. 167)	Lp(a) HORIZON trial[169], ongoing
Olpasiran	*LPA*	siRNA	225 mg every 12 weeks	I–II	−24.8 (ref. 171)	NR	NR	NR	−101.1 (ref. 171)	NA
LY3819469	*LPA*	siRNA	To be defined	I	NR	NR	NR	NR	NR	NR
SLN360	*LPA*	siRNA	To be defined	Preclinical	NR	NR	NR	NR	NR	NR
CSL112	ApoA-I	Plasma-derived apoA-I	6 g once per week; IV infusion	II–III	NR	NR	NR	NR	NR	AEGIS II trial[191], ongoing
ACP-501	LCAT	Recombinant human LCAT	Single IV infusion of 13.5 mg per kg of body weight	I	NR	NR	NR	+44[193]	NR	NR

ACLY, ATP citrate lyase; ANGPTL3, angiopoietin-related protein 3; apoA-I, apolipoprotein A-I; ASO, antisense oligonucleotide; CETP, cholesteryl ester transfer protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IV, intravenous; LCAT, lecithin–cholesterol acyltransferase; Lp(a), lipoprotein(a); MACE, major adverse cardiovascular events; NA, not applicable; NR, not reported; NS, not significant; PCSK9, proprotein convertase subtilisin/kexin type 9; SC, subcutaneous; siRNA, small interfering RNA.

ASO targeting *PCSK9*: AZD8233. In contrast to siRNAs, which act only in the cytoplasm, ASOs act in the nucleus and cytoplasm. AZD8233 (also known as ION 449) is a GalNAc-conjugated ASO with constrained ethyl chemistry targeting *PCSK9* mRNA[83]. A single subcutaneous injection of 90 mg of AZD8233 reduced LDL cholesterol levels by 68% from baseline[83]. The effect remained stable for a month, after which LDL cholesterol levels returned to baseline levels by 16 weeks. In the phase IIb ETESIAN trial[84], the same dose of AZD8233 was administered on days 1, 8, 29 and 57 in 119 patients receiving statin therapy who had plasma LDL cholesterol levels of ≥70 mg/dl (1.8 mmol/l) and <190 mg/dl (4.9 mmol/l), and fasting triglyceride levels of <400 mg/dl (4.57 mmol/l). AZD8233 therapy reduced LDL cholesterol by up to 79% at week 12 compared with placebo. These reductions are, so far, numerically the highest LDL cholesterol reductions achieved among all the PCSK9-targeting medications reported to date.

A new formulation of AZD8233 with sodium caprate, called AZD6615, can be administered orally and has been shown to have sufficient bioavailability in animal models with a once per day dosing[83]. This dosing regimen would offer an alternative to patients who prefer taking a tablet daily over biweekly or monthly injections. However, further development of AZD6615 was recently halted, although the reasons have not been publicly disclosed.

The increased duration of effects achieved with medications that act on gene expression compared with small-molecule drugs has the advantage of decreased dosing frequencies. However, the long tissue half-life also extends the duration of potential adverse effects. Therefore, a strategy to rapidly reverse the activity of ASOs or siRNAs might be warranted in certain situations. This reversal has been achieved in experimental models through the injection of complementary sense oligonucleotides that act as a synthetic target with high affinity for the siRNA or ASO[85,86].

Cyclic peptides targeting PCSK9. Another approach to PCSK9 inhibition with oral administration is the use of cyclic peptides[87]. These small molecules are developed through a structure-based design. Cyclic peptides targeting PCSK9 are still in the early clinical phases, but the key barriers in the development of these cyclic peptides have already been overcome, such as improving their molecular stabilization to protect them against gut proteases and reducing their molecular weight. In a phase IIb, dose-finding study[88], treatment with the oral PCSK9 inhibitor MK-0616 produced dose-dependent reductions in plasma LDL cholesterol levels of 41.2–60.9% between baseline and week 8. This oral PCSK9 inhibitor is going to be tested in a phase III trial to assess its effects on LDL cholesterol lowering and cardiovascular outcomes. The potential cost of MK-0616 is likely to be lower than that of injectable anti-PCSK9 therapies. Therefore, this drug fills the gap for patients who require additional LDL cholesterol lowering despite already receiving currently available oral therapies (statins plus other oral agents) but who cannot access injectable PCSK9-lowering therapies.

Vaccines targeting PCSK9. Approaches that use PCSK9-derived peptides to stimulate an immune response against PCSK9 are still in preclinical development but have shown promising results in animal models[89,90]. The anti-PCSK9 vaccine decreased PCSK9 plasma concentrations by about 60% compared with concentrations in controls and lasted for at least 16 weeks after prime immunization[91]. Two peptide vaccines, AT04A and AT06A, have been assessed in a phase I trial[92] in 72 healthy participants. All participants received initial immunization in weeks 0, 4 and 8, followed by a booster in week 60. Only AT04A induced an LDL cholesterol reduction of clinical relevance, which was numerically moderate compared with other PCSK9-targeting approaches, with a maximum reduction of 13.3% at week 70 and a mean reduction of 7.2% over the whole study period of 90 weeks[92].

***PCSK9* gene editing.** Permanent suppression of PCSK9 might be achieved through CRISPR–Cas gene editing. CRISPR base editors have been used to successfully introduce a loss-of-function mutation in *PCSK9* in cynomolgus monkeys[93]. In this strategy, the CRISPR base editor and a guide RNA are loaded into lipid nanoparticles that enable liver-specific delivery. The guide RNA strand binds to the target DNA strand through complementary base pairing, which leads to displacement of a small segment of single-stranded DNA in an R-loop[94]. Within the R-loop, the exposed DNA bases can then be modified by the base editor. Sustained reductions in circulating PCSK9 and LDL cholesterol levels of 90% and 60%, respectively, were achieved over the observation period of 8 months after a single injection of the lipid nanoparticles[93].

Targeting apoC-III

Plasma levels of LDL cholesterol and triglyceride-rich lipoprotein cholesterol are regulated through LDLR-dependent and LDLR-independent pathways. The latter contribute to lipoprotein modification and lipoprotein maturation[95]. Among the factors regulating these processes is apoC-III. The glycoprotein apoC-III is predominantly synthesized in the liver and, to a small extent, in the intestine[96]. ApoC-III is one of the major components of triglyceride-rich lipoproteins and, to a lesser extent, of HDL[97]. ApoC-III has a unique role in VLDL metabolism because it is involved in both the formation and composition of VLDL particles and their reuptake in the liver[98].

Increased intracellular concentrations of apoC-III in hepatocytes lead to increased formation of cytosolic lipid droplets and the accumulation of pre-VLDL and VLDL particles in the endoplasmic reticulum and the Golgi apparatus[99]. Although in vitro studies suggest an involvement of apoC-III in VLDL production[100], a kinetics study showed that individuals with *APOC3* loss-of-function variants have unaltered VLDL production rates compared with individuals without loss-of-function variants[101]. Conversely, extracellular apoC-III influences the clearance of triglyceride-rich lipoproteins through inhibition of LPL-mediated lipolysis of VLDL triglycerides and a currently unknown LPL-independent mechanism that interferes with hepatic uptake of VLDL and triglyceride-rich remnant particles[102,103]. In summary, higher circulating apoC-III levels lead to higher circulating levels of atherogenic triglyceride-rich lipoproteins.

Findings from genetic studies support the pro-atherogenic role of apoC-III and suggest an association with cardiovascular mortality[104]. Loss-of-function variants in *APOC3* are associated with lower plasma triglyceride levels and with a reduced risk of ASCVD[104]. Individuals with a genetically determined reduction in apoC-III production caused by a R19X variant in *APOC3* have a 50% lower concentration of apoC-III in plasma and twice the clearance rate of triglyceride-rich apoB-100-carrying lipoproteins compared with individuals without this variant[102]. Another study in individuals with a loss-of-function variant in *APOC3* showed that the low risk of cardiovascular disease associated with this variant could be explained by the presence of lower plasma concentrations of triglyceride-rich lipoproteins in carriers of the variant than in non-carriers[105]. This effect remained significant even after correction for lipid-lowering therapy. It has been proposed that in addition to promoting the formation of triglyceride-rich lipoproteins, apoC-III

might also facilitate the development of atherosclerosis by increasing arterial wall inflammation[106].

Pharmacological inhibition of apoC-III has shown great efficacy in reducing plasma triglyceride levels[107–110] (Fig. 2). Therefore, the initial clinical development of these therapies focused on patients with familial chylomicronaemia syndrome, who have genetically determined high triglyceride levels in plasma that are often caused by genetic variants in the *LPL* gene. Patients with this condition have an increased risk of pancreatitis but not of ASCVD events compared with the general population[111]. Therefore, clinical trials in this patient population did not assess cardiovascular outcomes.

ASOs targeting *APOC3*: volanesorsen and olezarsen. The first apoC-III-targeting medication approved for clinical use (for the treatment of familial chylomicronaemia syndrome) was volanesorsen, an unconjugated ASO that selectively binds to *APOC3* mRNA (Fig. 2). The triglyceride-lowering efficiency of volanesorsen has been assessed in three phase III trials: APPROACH[107], involving patients with familial chylomicronaemia syndrome; COMPASS[108], involving patients with severe multifactorial hyperchylomicronaemia (fasting plasma triglyceride levels >500 mg/dl (5.65 mmol/l)) or familial chylomicronaemia syndrome; and BROADEN[105], involving patients with familial partial lipodystrophy.

The APPROACH trial[107] involved 66 patients with familial chylomicronaemia syndrome who were randomly assigned 1:1 to receive volanesorsen or placebo. Volanesorsen treatment reduced plasma levels of triglycerides by 77% and of apoC-III by 84% after 3 months compared with an increase of 18% and 6%, respectively, in the placebo group[107]. Furthermore, levels of non-HDL cholesterol, VLDL cholesterol and apoB-48 decreased by 46%, 58% and 76%, respectively, with volanesorsen compared with increases of 12%, 15% and 14%, respectively, with placebo. However, HDL cholesterol, LDL cholesterol and apoB levels increased by 46%, 136% and 20%, respectively, in the treatment group compared with 7%, 6% and 4%, respectively, in the placebo group. Similar reductions in the plasma levels of triglycerides, triglyceride-rich lipoproteins and non-HDL were obtained in the COMPASS trial[108], which included 114 participants. However, the increases in LDL cholesterol level were less pronounced in the COMPASS trial than in the APPROACH trial, and apoB levels remained unaltered[108].

The discordant changes in non-HDL cholesterol and apoB levels indicate a shift towards higher particle numbers but with overall lower cholesterol content. Furthermore, the rise in LDL cholesterol and HDL cholesterol levels suggests a shift of cholesterol between the lipoprotein subfractions. Therefore, volanesorsen effectively lowered the levels of cholesterol in triglyceride-rich lipoprotein subfractions. However, its effect on the risk of ASCVD events remains uncertain, given that higher particle numbers are associated with a higher likelihood of the lipoprotein particles infiltrating the arterial walls, which might promote atherosclerotic plaque formation even though the cholesterol content of the infiltrating particles is lower overall.

The main disadvantage of volanesorsen is treatment-associated thrombocytopenia[107]. A genetic analysis suggests that the thrombocytopenia is an off-target effect, given that no differences in platelet counts were apparent between individuals with genetically determined low apoC-III levels and individuals with normal apoC-III levels[112]. To increase the safety and tolerability of the approach through increased organ specificity, a GalNAc-conjugated ASO targeting *APOC3*, olezarsen, was developed[110]. Olezarsen (previously known as AKCEA-APOCIII-LRx) differs structurally from volanesorsen only in its GalNAc moiety.

In a phase II, dose-ranging trial[110], including 114 patients with fasting triglyceride levels of 200–500 mg/dl (2.26–5.65 mmol/l) and

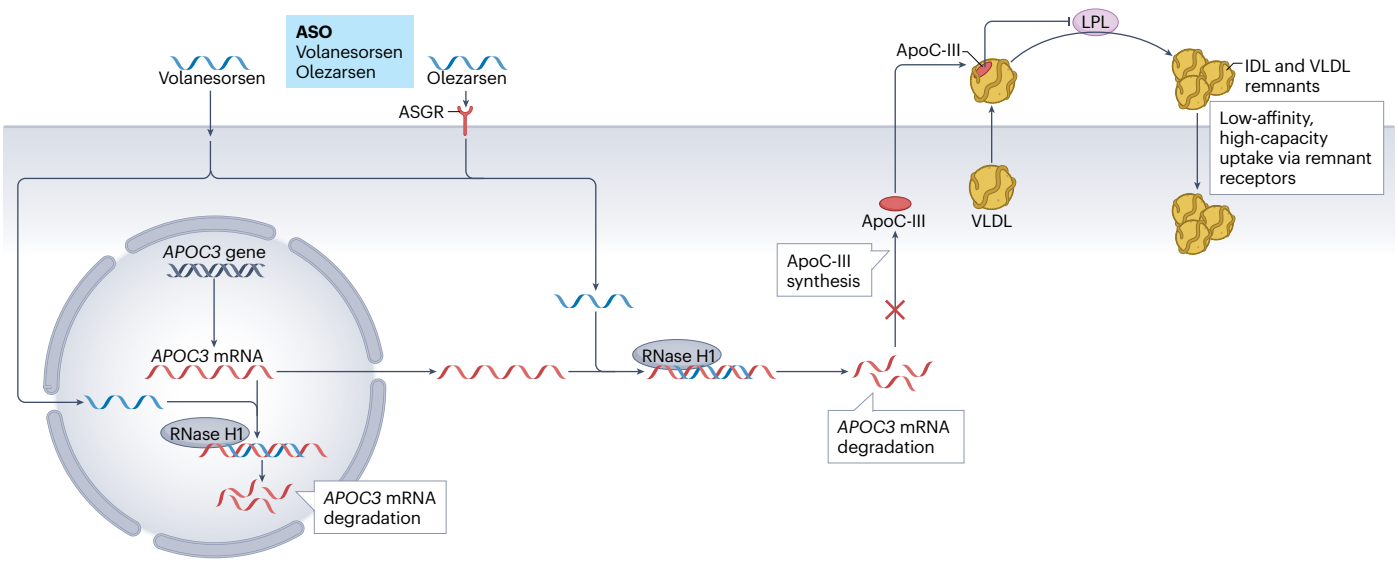

Fig. 2 | Novel and emerging lipid-lowering therapies targeting apoC-III. Apolipoprotein C-III (apoC-III) is an apolipoprotein present in triglyceride-rich lipoproteins. In plasma, apoC-III is predominantly bound to VLDL and HDL. ApoC-III inhibits lipoprotein lipase (LPL)-dependent lipolysis of VLDL particles to intermediate-density lipoprotein (IDL) and VLDL remnants and the uptake of these remnants via hepatic receptors, thus decreasing the clearance of these particles. Therapies targeting apoC-III that are currently approved for clinical use or in clinical development are the antisense oligonucleotides (ASO) volanesorsen (approved for the treatment of familial chylomicronaemia syndrome) and olezarsen. These ASOs bind to *APOC3* mRNA, which encodes apoC-III, in the nucleus and the cytoplasm to induce *APOC3* mRNA degradation and thereby suppress apoC-III synthesis. The ASO olezarsen is conjugated to triantennary *N*-acetylgalactosamine, which allows hepatocyte-specific uptake mediated by asialoglycoprotein receptors (ASGR).

established ASCVD or with high risk of ASCVD, olezarsen reduced triglyceride levels by 29% with the 10 mg dose administered every 4 weeks and by 66% with the 50 mg dose every 4 weeks after 25–27 weeks of treatment compared with placebo. The changes in LDL cholesterol levels were dose-independent, ranging from a 1% decrease to a 23% increase. With the highest olezarsen dose, non-HDL cholesterol, VLDL cholesterol and apoB levels were reduced by 20%, 58% and 10%, respectively, compared with placebo[110]. As intended, platelet counts remained unaltered with olezarsen treatment. The most common adverse effects in the intervention group were injection-site erythema (15.6%) and arthralgia (12.2%) compared with 0% for both in the placebo group.

Therefore, olezarsen can effectively lower apoC-III and triglyceride levels in patients with clinical conditions with a phenotype of elevated triglyceride concentrations in plasma, such as secondary severe or mild hypertriglyceridaemia, multifactorial chylomicronaemia syndrome and familial chylomicronaemia syndrome. Whether the modest reductions in the plasma levels of apoB and non-HDL cholesterol translate into significant reductions in the risk of cardiovascular disease remains to be established.

Targeting ANGPTL3

ANGPTL3 is an inhibitor of LPL and EDL and is mainly produced in the liver (Fig. 3). Loss-of-function variants in *ANGPTL3* are associated with a 9–12% reduction in LDL cholesterol levels and a 17–27% reduction in triglyceride levels, and a 34–41% lower risk of coronary artery disease compared with individuals without this variant[113,114]. Reductions in the plasma levels of free ANGPTL3 lead to higher LPL and EDL activity[115,116]. Unlike apoC-III, ANGPTL3 seems to work only through an LPL-dependent mechanism, and is therefore less effective than apoC-III-targeting therapies or ineffective in patients with a loss-of-function variant in *LPL* causing familial chylomicronaemia syndrome. Elevated LPL and EDL activity increases the influx of free fatty acids into muscles during the fasting state and lipogenesis in the adipose tissue during the fed state for storage, as shown in animal models[117,118]. LPL and EDL hydrolyse triglycerides in VLDL particles, facilitating IDL and LDL formation. Furthermore, ANGPTL3-targeting therapies increase LDL cholesterol and remnant cholesterol uptake into the liver, while simultaneously decreasing hepatic VLDL synthesis[119].

However, ANGPTL3 on its own has a low capacity to inhibit LPL and is expressed independently of nutritional state (fed or fasted)[120–122]. ANGPTL3 exerts its effect through its interaction with ANGPTL4 and ANGPTL8 (ref. 123). Through the interplay of these proteins, triglyceride partitioning is orchestrated according to nutritional state[122]. ANGPTL4 is mainly expressed in adipose tissue during the fasting state and inhibits LPL locally, therefore funnelling triglycerides to oxidative tissues[122]. In the fed state, ANGPTL8 is expressed in the liver and forms a complex with ANGPTL3 (ref. 122). This complex is secreted and is >100-fold more potent at inhibiting LPL than ANGPTL3 alone[124]. Furthermore, ANGPTL8 in adipose tissue forms a complex with free ANGPTL4. The ANGPTL4–ANGPTL8 complex has low capacity to inhibit LPL and might also attenuate the LPL inhibition mediated by circulating ANGPTL3–ANGPTL8 complexes[122].

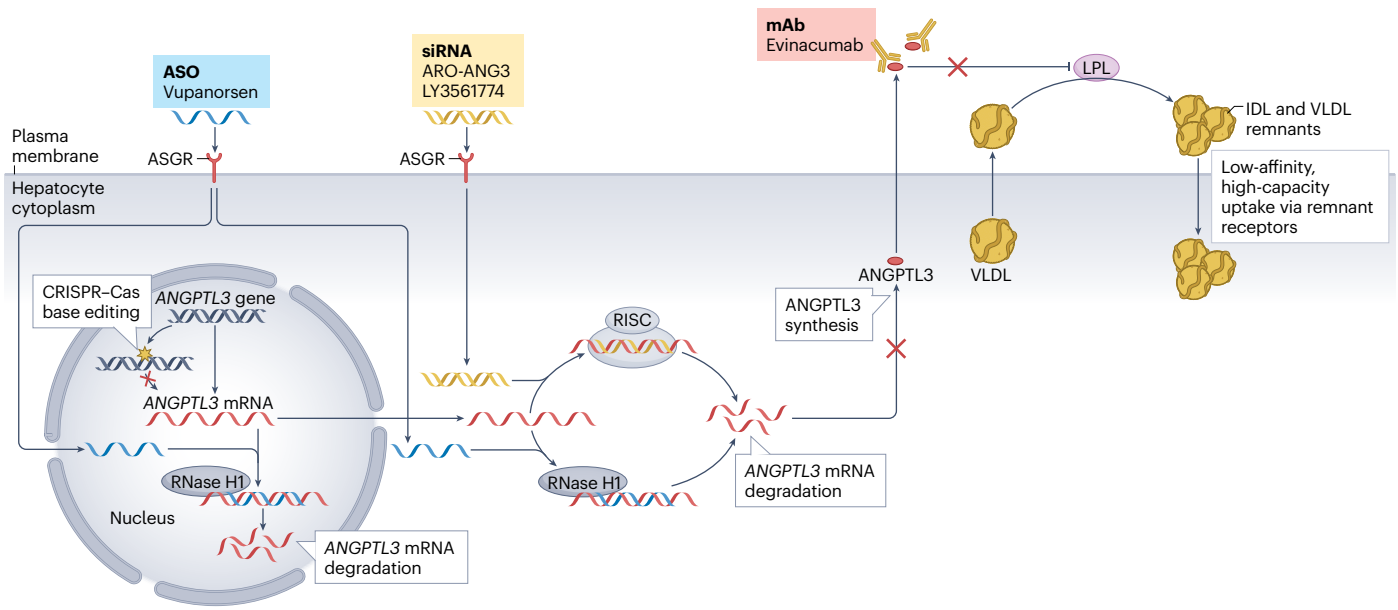

Fig. 3 | Novel and emerging lipid-lowering therapies targeting ANGPTL3. Angiopoietin-related protein 3 (ANGPTL3) is a circulating inhibitor of lipoprotein lipase (LPL) (and endothelial lipase). Therefore, ANGPTL3 inhibits the lipolysis of VLDL particles to intermediate-density lipoprotein (IDL) and VLDL remnants and their uptake via hepatic receptors, thus decreasing the clearance of these particles. Therapies targeting ANGPTL3 at the gene level are currently at preclinical stages and involve using CRISPR–Cas base editing to introduce loss-of-function mutations in the *ANGPTL3* gene to prevent production of the protein. At the mRNA level, strategies being assessed at clinical trial stages include the antisense oligonucleotide (ASO) vupanorsen and the small interfering RNAs (siRNAs) ARO-ANG3 and LY3561774. Vupanorsen binds to *ANGPTL3* mRNA in the nucleus and the cytoplasm, causing its degradation via RNase H1-mediated cleavage and thereby suppressing ANGPTL3 protein synthesis. ARO-ANG3 and LY3561774 induce *ANGPTL3* mRNA degradation in the cytoplasm mediated by the RNA-induced silencing complex (RISC). Both vupanorsen and the siRNAs are conjugated to triantennary *N*-acetylgalactosamine to enable hepatocyte-specific uptake through asialoglycoprotein receptors (ASGR). At the protein level, the monoclonal antibody (mAb) evinacumab, which has been approved for clinical use, binds to ANGPTL3 to block its inhibitory effect on LPL.

ASO targeting *ANGPTL3*: vupanorsen. Vupanorsen is a GalNAc-conjugated ASO targeting hepatic *ANGPTL3* mRNA[119] (Fig. 3). In a double-blind, placebo-controlled, dose-ranging phase II study[125] among 105 patients with diabetes, hepatic steatosis and hypertriglyceridaemia, monthly subcutaneous injections of vupanorsen significantly reduced plasma triglyceride and VLDL cholesterol levels by 44% and 38%, respectively, compared with placebo. However, its LDL cholesterol-lowering effect was modest at 7%. In the dose-finding, phase IIb TRANSLATE-TIMI 70 trial[126] that included 286 patients with elevated non-HDL cholesterol and triglyceride levels who were receiving a stable dose of statin, vupanorsen injections twice per week reduced non-HDL cholesterol and triglyceride levels at 24 weeks, with a maximum reduction of 27.7% and 56.8%, respectively, compared with placebo. No clinically meaningful reductions in LDL cholesterol and apoB levels were observed, and a dose-dependent reduction was only apparent for triglycerides but not for non-HDL chloesterol[126]. Furthermore, changes in non-HDL cholesterol levels did not correlate with apoB changes, suggesting that the observed reduction in non-HDL cholesterol levels reflected a lower triglyceride content in triglyceride-rich lipoproteins rather than a decrease in particle number. If the potential cardiovascular benefit of targeting ANGPTL3 were solely mediated through reducing the number and cholesterol content of apoB-carrying lipoproteins, vupanorsen might have a less favourable effect than other medications within this class. However, the effect of these changes on atherosclerosis and ASCVD event rates remains to be established.

Moreover, vupanorsen treatment increased hepatic fat content in a dose-dependent manner[126]. By contrast, loss-of-function variants in *ANGPTL3* are not associated with liver disease, and treatment with an *ANGPTL3*-targeting ASO in mice reduced liver triglyceride content[119,127]. Moreover, the siRNA ARO-ANG3 targeting *ANGPTL3* did not increase liver fat content in patients with hepatic steatosis[128]. Therefore, the hepatic targeting of ANGPTL3 per se might not cause hepatic lipid accumulation but might be an off-target effect of vupanorsen. This adverse effect raised concerns about the safety of vupanorsen, and further development of this medication was stopped.

siRNAs targeting *ANGPTL3*: ARO-ANG3 and LY3561774. ARO-ANG3 is a GalNAc-conjugated siRNA targeting *ANGPTL3* mRNA (Fig. 3). This siRNA has been tested in phase I clinical studies in healthy volunteers, patients with hypertriglyceridaemia and patients with familial chylomicronaemia syndrome, and in these studies individuals with hypercholesterolaemia who were receiving stable doses of statin (with or without ezetimibe) had mean reductions in LDL cholesterol levels of 39–42% and mean reductions in triglyceride levels of 79%[129]. In an open-label trial including 17 patients with heterozygous familial hypercholesterolaemia, ascending doses of ARO-ANG3 (100 mg, 200 mg and 300 mg) led to increasing reductions in plasma LDL cholesterol levels (by 23%, 30% and 37%, respectively) from baseline to 16 weeks after treatment initiation[130]. In healthy volunteers, ARO-ANG3 administered by subcutaneous injection on days 1 and 29 reduced LDL cholesterol levels from baseline by 45–54% at 4–6 weeks after the second administration, with the effects lasting up to 16 weeks after the last dose[130,131]. The *ANGPTL3*-targeting siRNA LY3561774 is currently being assessed in a phase I trial in 74 patients with plasma triglyceride levels of 1.71–5.72 mmol/l and plasma LDL cholesterol levels of ≥1.8 mmol/l[132].

The remarkable differences in the LDL cholesterol-lowering capacity between the monoclonal antibody (evinacumab), the siRNAs and the ASO targeting ANGPTL3 are not fully understood. Genetic studies suggest the need for an almost complete inhibition of ANGPTL3 to achieve substantial LDL cholesterol reductions[113,114]. Further studies are ongoing[133,134] to show whether the siRNA approach provides similar sustained durable efficacy in reducing ANGPTL3 with twice-yearly dosing to that observed for PCSK9-lowering with inclisiran.

***ANGPTL3* gene editing.** Approaches to lower *ANGPTL3* expression by gene editing are still at the preclinical stage. CRISPR–Cas technology was successfully applied in a mouse model to introduce loss-of-function mutations in *ANGPTL3*, which significantly reduced plasma LDL cholesterol levels compared with those in control mice[135]. This approach has been replicated in a model of homozygous familial hypercholesterolaemia in non-human primates. CRISPR-based *ANGPTL3* editing with two different formulations induced a 94% ($n = 3$) and a 97% ($n = 3$) reduction in ANGPTL3 levels in blood, respectively, and a 35% reduction in LDL cholesterol levels from baseline[136]. An increase in editing potency and consequently further reductions in circulating ANGPTL3 levels were achieved through GalNAc conjugation of the lipid nanoparticle carrying the CRISPR–Cas platform compared with standard lipid nanoparticles[137].

Targeting CETP

Cholesteryl ester transfer protein (CETP) facilitates the transport of cholesterol from HDL to LDL and VLDL, and increases the transfer of triglycerides from VLDL to LDL and HDL[138]. These actions result in decreased plasma HDL cholesterol levels and increased plasma LDL cholesterol levels, which promote the development of atherosclerosis and ASCVD[2]. Mendelian randomization studies suggested that *CETP* variants resulting in reduced CETP function have anti-atherosclerotic effects[139–141].

The field of clinical trial research to assess the cardiovascular benefits of CETP inhibitors has been a steep learning curve. These trials showed that the cardiovascular benefits of CETP inhibitors relate to the absolute magnitude of reduction in LDL cholesterol levels in plasma (determined by treatment potency and the starting levels of LDL cholesterol and apoB)[142] and the duration of treatment, with half the relative benefit observed at 1 year versus at later years, meaning that longer trials are needed to observe benefits. Therefore, we not only need a therapy without off-target adverse effects (which offset the potential benefits of LDL cholesterol lowering) but also the right trial design involving patients with a high enough threshold of baseline LDL cholesterol level, a potent therapy that induces a large enough absolute lowering of LDL cholesterol levels, and a sufficiently long period of follow-up over which a meaningful reduction in clinical events might be demonstrated.

Obicetrapib. Obicetrapib is a small molecule that inhibits CETP, leading to a decreased transport of cholesterol from HDL to LDL and VLDL and decreased transfer of triglycerides from VLDL to LDL and HDL. The clinical development of the CETP inhibitor obicetrapib had been halted but has now been restarted, and the data show that this drug potently lowers LDL cholesterol levels, is safe and has a short half-life[143,144]. An early trial in patients with mild dyslipidaemia showed that obicetrapib reduced plasma apoB and LDL cholesterol levels by 33.7% and 45.3%, respectively, from baseline to 12 weeks of treatment and was well-tolerated[143]. Obicetrapib has now been assessed in the ROSE trial[144] as an adjunct to high-intensity statin treatment in 120 patients with dyslipidaemia. Treatment with 10 mg of obicetrapib per day for 8 weeks significantly decreased the levels of LDL cholesterol by 51%, apoB by 30% and non-HDL cholesterol by 44% compared with placebo[144].

The ongoing phase II OCEAN trial[145] will assess obicetrapib as a combination therapy with ezetimibe in patients with LDL cholesterol levels of >1.8 mmol/l and >2.5 mmol/l. The PREVAIL trial[146], a cardiovascular outcomes study to evaluate obicetrapib in patients with ASCVD, started recruitment in 2022 and will follow about 9,000 participants over the next 4 years.

In a population with high baseline plasma LDL cholesterol levels, a sufficiently large reduction in LDL cholesterol levels should be achievable with obicetrapib therapy and would probably show a cardiovascular benefit after a sufficiently long follow-up (for example, a median of 3–4 years). This result, if observed, would considerably broaden the range and the potency of oral LDL cholesterol-lowering treatment regimens available for combination with high-intensity statin therapy.

Other CETP inhibitors. Other CETP inhibitors that were under development did not demonstrate cardiovascular benefits in clinical trials, owing to adverse effects, insufficient LDL cholesterol lowering or limited follow-up[147]. Therefore, the lack of cardiovascular benefit might be compound-specific and related to trial design. For example, the CETP inhibitor torcetrapib had toxic off-target effects and was associated with increased mortality in 15,067 patients with a high risk of cardiovascular disease compared with placebo[148,149]. The adverse effects were mediated by increasing aldosterone production and thereby blood pressure, which potentially attenuated any benefits of very modest absolute reductions in LDL cholesterol levels[148,149]. In the dal-OUTCOMES trial[150], the CETP inhibitor dalcetrapib showed only minimal LDL cholesterol reductions and, therefore, a neutral effect on cardiovascular outcomes, but was shown to be safe. Post-hoc genome-wide association analysis of the dal-OUTCOMES data suggested a 39% relative risk reduction in patients with a single-nucleotide polymorphism in the *ADCY9* gene[151]. However, in the randomized, placebo-controlled dal-GenE outcomes trial[152], which included 6,147 individuals with the variant in *ADCY9*, dalcetrapib treatment did not significantly reduce the rate of cardiovascular events compared with placebo after a mean follow-up of 3 years, and only showed a trend for the prevention of myocardial infarction. The trial of evacetrapib was stopped for futility after approximately 2.3 years of follow-up, with a 31.1% decrease in plasma LDL cholesterol level but only an approximately 25 mg/dl (0.65 mmol/l) absolute reduction[153]. By contrast, the largest and longest trial of the CETP inhibitor anacetrapib showed that this drug lowered LDL cholesterol levels by 17% as measured through β-quantification, compared with the 41% estimated through a direct assay[154]. The study included approximately 30,000 individuals with a high risk of cardiovascular events and a starting LDL cholesterol level in plasma of 61 mg/dl (1.58 mmol/l), and had a median follow-up of 4.1 years. The observed absolute difference in plasma LDL cholesterol levels between the anacetrapib and placebo groups was 11 mg/dl (0.3 mmol/l), and anacetrapib yielded a reduction in the risk of major coronary events of 6% compared with placebo[154]. However, further development of anacetrapib was stopped because of an extremely long elimination half-life of up to 80 h after 14 days of dosing, with detectable concentrations of anacetrapib at 2–4 years after 76 weeks of treatment[155], and its accumulation in adipose tissue[154,156].

Targeting Lp(a)

Lp(a) is formed by the binding of apolipoprotein(a) (apo(a)) to apoB-carrying lipoproteins. Lp(a) concentrations in the plasma are mainly determined by the expression levels of *LPA*, which encodes apo(a)[157], meaning that plasma Lp(a) levels remain remarkably constant throughout an individual's life and are not influenced meaningfully by diet and lifestyle[158]. Therefore, reductions in plasma Lp(a) levels can be achieved by targeting apo(a) synthesis (Fig. 4).

Therapies targeting PCSK9, irrespective of the therapeutic approach, have been shown to lower Lp(a) levels by 20–28% compared

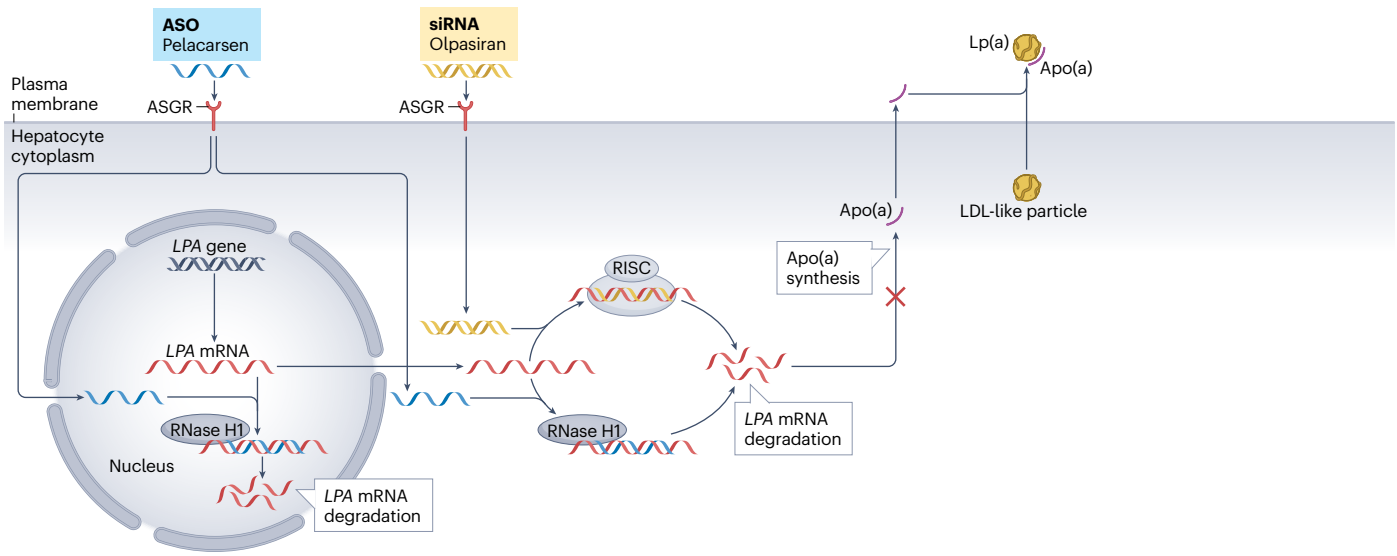

Fig. 4 | Emerging lipid-lowering therapies targeting Lp(a). Therapeutic strategies to lower plasma lipoprotein(a) (Lp(a)) levels that are currently being tested in clinical trials include an antisense oligonucleotide (ASO) and a small interfering RNA (siRNA). The ASO pelacarsen binds to *LPA* mRNA in the nucleus and the cytoplasm, causing RNase H1-mediated *LPA* mRNA degradation. The siRNA olpasiran binds to *LPA* mRNA in the cytoplasm to induce *LPA* mRNA degradation through the RNA-induced silencing complex (RISC). Degradation of *LPA* mRNA suppresses the translation and synthesis of apolipoprotein(a) (apo(a)), which in turn prevents the formation of Lp(a). Pelacarsen and olpasiran are conjugated to triantennary *N*-acetylgalactosamine, which confers hepatocyte-specific uptake through asialoglycoprotein receptors (ASGR).

with placebo[159–161]. Given that these therapies significantly lower LDL cholesterol levels, whether these reductions in Lp(a) levels confer additional cardiovascular benefit beyond that associated with LDL cholesterol lowering is uncertain[162]. Two large cardiovascular outcome trials in patients with stable coronary artery disease and recent acute coronary syndrome have provided discordant results[163,164]. In patients with stable coronary artery disease, absolute and percentage reductions in plasma Lp(a) levels induced by the PCSK9 inhibitor evolocumab did not confer additional cardiovascular benefit beyond that achieved through the LDL cholesterol reduction[163]. Rather, individuals with higher plasma Lp(a) levels were at higher risk of cardiovascular events and, therefore, derived greater absolute benefits with evolocumab therapy[163]. By contrast, reductions in Lp(a) levels seemed to confer cardiovascular benefits independently of LDL cholesterol lowering with the PCSK9 inhibitor alirocumab in patients with recent acute coronary syndrome[164]. Therefore, with PCSK9-targeting agents, it is not possible to determine whether Lp(a) lowering per se independently of other effects reduces the risk of cardiovascular events. However, three drugs specifically aimed at lowering Lp(a) levels by targeting hepatic production of apo(a) are in advanced development: pelacarsen, olpasiran and LY3819469.

ASO targeting *LPA*: pelacarsen. Pelacarsen (also known as ISIS-APO(a)Rx, IONIS-APO(a)-LRx, AKCEA-APO(a)-LRx and TQJ230) is an ASO with a GalNAc conjugation targeting *LPA* mRNA (Fig. 4). Phase II clinical trials[165,166] indicate that pelacarsen is generally safe and lowers plasma Lp(a) levels in a dose-dependent manner. Among 286 patients with established ASCVD and elevated Lp(a) levels (>60 mg/dl (150 nmol/l)), the greatest reductions in Lp(a) levels were observed with dosing regimens of 60 mg per month or 20 mg per week, which achieved reductions of 72% and 80%, respectively[167]. With the latter dosing, 98% of patients attained the Lp(a) levels of <50 mg/dl (125 nmol/l) formerly recommended by European[168] and North American[11] guidelines. The ongoing cardiovascular outcome trial Lp(a)HORIZON[169], which is testing a dose of 80 mg pelacarsen per month in patients with ASCVD and Lp(a) levels >150 nmol/l, could provide conclusive evidence on a causal role of Lp(a) in ASCVD and whether modification of Lp(a) concentrations prevents cardiovascular events.

siRNAs targeting *LPA*: olpasiran and LY3819469. Olpasiran (AMG890) is a siRNA targeting *LPA* mRNA that has been investigated in phase I and phase II studies. In the phase I trial[170], which included 64 patients with elevated Lp(a) levels (≥70 nmol/l to ≤199 nmol/l and ≥200 nmol/l), a single dose of olpasiran reduced plasma Lp(a) levels by up to 94% on day 113. In the phase II, dose-finding trial[171], a placebo-adjusted Lp(a) reduction of 101.1% was achieved with the highest dose of olpasiran (225 mg every 12 weeks). These data suggest that significant and sustained long-term reductions in Lp(a) concentrations in plasma are achievable with twice-yearly dosing, similar to inclisiran, which might translate into substantial cardiovascular benefit if Lp(a) is confirmed to be a causal factor in ASCVD events. LY3819469 is another siRNA against *LPA* under development. A phase I trial[172] was completed in November 2022.

Finally, SLN360 is another siRNA targeting *LPA* mRNA. In a phase I study[173], single ascending doses (30 mg, 100 mg, 300 mg and 600 mg) of SLN360 led to reductions in plasma Lp(a) levels from baseline to 150 days of 46–96% among 32 patients with plasma Lp(a) levels >150 nmol/l and without established ASCVD. The therapy was well-tolerated.

Targeting HDL cholesterol

An inverse association between plasma HDL cholesterol levels and the risk of ASCVD has been reported in epidemiological studies[174–177]. However, a meta-analysis showed that a therapeutically induced increase in HDL cholesterol levels did not reduce cardiovascular morbidity and mortality[178]. Furthermore, Mendelian randomization studies did not provide compelling evidence that HDL cholesterol is causally associated with the risk of ASCVD[179–181]. This finding might be partly explained by the non-linear, U-shaped association between plasma HDL cholesterol levels and ASCVD events[182–184], which limits Mendelian randomization models, but is most likely due to the heterogeneity and various dynamic functions of different HDL particles.

Low HDL cholesterol levels in plasma are commonly found in patients with cardiometabolic conditions, such as insulin resistance, metabolic syndrome and type 2 diabetes, and are often associated with hypertriglyceridaemia[99]. Therefore, plasma HDL cholesterol levels could be thought of as a stable marker — similar to HbA1c for glycaemia — for mixed dyslipidaemia, which in turn is associated with an increased risk of ASCVD events[185]. However, in contrast to targeting LDL cholesterol, the goal of raising HDL cholesterol levels might be an oversimplification because this approach neglects the fact that HDL is more complex than LDL, with 16 described subgroups and a variety of functions that are still not fully understood. HDL functions are determined by the HDL composition of lipids, proteins and microRNAs[185]. The spectrum of functions includes modulation of lipid metabolism to proteolysis, haemostasis, immunity, complement activation and inflammation[185]. At least 219 non-randomly distributed proteins are associated with HDL, and their post-translational modification adds to the complexity and dynamics of HDL functions[185].

Nevertheless, HDL is the key lipoprotein in reverse cholesterol transport and has other atheroprotective functions[185]. Therefore, mechanistic plausibility for targeting HDL remains and is driving further development of HDL-targeting therapeutics. These approaches restore or substitute HDL particle function, so far with mixed outcomes in clinical trials.

CSL112. CSL112 is a lipoprotein composed of human plasma-derived apolipoprotein A-I (apoA-I) and phosphatidylcholine and stabilized with sucrose. ApoA-I is the major protein component of HDL and optimizes the function of HDL in reverse cholesterol transport from the periphery to the liver by increasing the HDL-mediated cholesterol efflux capacity. An increase in cholesterol efflux from macrophages in the atherosclerotic plaque might lead to a reduction in plaque vulnerability and, consequently, a decrease in the rates of recurrent cardiovascular events.

In 1,258 patients with a recent myocardial infarction, treatment with CSL112 significantly increased cholesterol efflux capacity of HDL particles, an ex vivo measure of HDL function, compared with placebo[186]. The increased cholesterol efflux capacity mediated by CSL112 contributes to atherosclerotic plaque stability by attenuating inflammation in the plaque, macrophage recruitment to the plaque and apoptosis of foam cells, reducing plaque and necrotic lipid core size, and increasing collagen expression and plaque remodelling[187].

Previous approaches targeting HDL by using apoA-I infusion therapies, such as the pre-β-HDL-like particles apoA-I Milano and CER-001 (that both contain recombinant apoA-I), did not demonstrate robust plaque regression[188,189]. However, treatment with CSL112 induced a greater increase in cholesterol efflux capacity (330%) compared with the increase induced by apoA-I Milano (80–90%) or CER-001 (6%)[187].

Furthermore, CSL112, but not apoA-I Milano or CER-001, can activate lecithin–cholesterol acyltransferase (LCAT), promoting the maturation of HDL particles and their hepatic clearance[190]. Whether the CSL112 approach to increasing cholesterol efflux capacity translates into a reduction in cardiovascular events is being assessed in the ongoing AEGIS II trial[191]. Nevertheless, clinical implementation of this approach would be challenging and restricted to patients with a recent myocardial infarction, because it requires a 60-min intravenous administration within 5 days of the event, followed by infusions once per week for 4 weeks and, therefore, requires additional effort by clinics and outpatient departments.

ACP-501. A different approach to increasing reverse cholesterol transport is through infusion of recombinant human LCAT. LCAT catalyses the esterification of cholesterol, which is part of the HDL maturation process and increases the capacity of HDL to remove additional cholesterol from peripheral tissues[192]. In a phase I trial[193], infusion of ACP-501, which is a recombinant human LCAT, increased plasma HDL cholesterol levels in patients with coronary heart disease and low HDL cholesterol levels at baseline, inducing maximal increases in HDL cholesterol levels of about 42%. Any beneficial effects on prevention of cardiovascular disease remain to be established.

Conclusions

For almost four decades, lipid management relied primarily on the use of small-molecule drugs. Genetic and population studies identified variants in novel genes (such as *PCSK9* and *ANGPTL3*) that contribute to atherosclerosis, which led to the identification of potential new treatment targets. Moreover, with these studies, the pathways associated with the treatment targets are better understood and some adverse effects can be anticipated at early stages. Parallel advances in drug development, such as monoclonal antibodies, siRNAs and ASOs (which can be specifically designed to act on the identified target), have facilitated improved target organ specificity and increased the duration of action of the therapies. Therefore, emerging lipid-lowering therapies, acting at different levels from the protein to the gene and targeting different pathways, provide unprecedented opportunities for personalize care, address the residual risk of cardiovascular disease and improve medication adherence depending on the mode of action of the therapy.

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References

1. World Health Organization. Cardiovascular diseases (CVDs) *WHO* https://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) (2021).
2. Ference, B. A. 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.* **38**, 2459–2472 (2017).
3. Trialists, C. T. 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* **380**, 581–590 (2012).
4. Duran, E. K. & Pradhan, A. D. Triglyceride-rich lipoprotein remnants and cardiovascular disease. *Clin. Chem.* **67**, 183–196 (2021).
5. Ginsberg, H. N. et al. Triglyceride-rich lipoproteins and their remnants: metabolic insights, role in atherosclerotic cardiovascular disease, and emerging therapeutic strategies – a consensus statement from the European Atherosclerosis Society. *Eur. Heart J.* **42**, 4791–4806 (2021).
6. Chait, A. et al. Remnants of the triglyceride-rich lipoproteins, diabetes, and cardiovascular disease. *Diabetes* **69**, 508–516 (2020).
7. Nordestgaard, B. G. et al. Lipoprotein(a) as a cardiovascular risk factor: current status. *Eur. Heart J.* **31**, 2844–2853 (2010).
8. Clarke, R. et al. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. *N. Engl. J. Med.* **361**, 2518–2528 (2009).
9. Burgess, S. et al. Association of LPA variants with risk of coronary disease and the implications for lipoprotein(a)-lowering therapies: a Mendelian randomization analysis. *JAMA Cardiol.* **3**, 619–627 (2018).
10. Mach, F. et al. 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk: The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS). *Eur. Heart J.* **41**, 111–188 (2019).
11. Grundy, S. M. et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol. *J. Am. Coll. Cardiol.* **73**, e285–e350 (2019).
12. National Institute for Health and Care Excellence. Cardiovascular disease: risk assessment and reduction, including lipid modification. Clinical guideline CG181. *NICE* https://www.nice.org.uk/guidance/cg181 (2016).
13. Lloyd-Jones, D. M. et al. 2022 ACC expert consensus decision pathway on the role of nonstatin therapies for LDL-cholesterol lowering in the management of atherosclerotic cardiovascular disease risk. *J. Am. Coll. Cardiol.* **80**, 1366–1418 (2022).
14. Ouchi, Y. et al. Ezetimibe lipid-lowering trial on prevention of atherosclerotic cardiovascular disease in 75 or older (EWTOPIA 75). *Circulation* **140**, 992–1003 (2019).
15. Cannon, C. P. et al. Ezetimibe added to statin therapy after acute coronary syndromes. *N. Engl. J. Med.* **372**, 2387–2397 (2015).
16. Kim, B.-K. et al. Long-term efficacy and safety of moderate-intensity statin with ezetimibe combination therapy versus high-intensity statin monotherapy in patients with atherosclerotic cardiovascular disease (RACING): a randomised, open-label, non-inferiority trial. *Lancet* **400**, 380–390 (2022).
17. Ray, K. K. et al. EU-wide cross-sectional observational study of lipid-modifying therapy use in secondary and primary care: the DA VINCI study. *Eur. J. Prev. Cardiol.* **28**, 1279–1289 (2020).
18. Vallejo-Vaz, A. J. et al. Global perspective of familial hypercholesterolaemia: a cross-sectional study from the EAS Familial Hypercholesterolaemia Studies Collaboration (FHSC). *Lancet* **398**, 1713–1725 (2021).
19. Kotseva, K. et al. Primary prevention efforts are poorly developed in people at high cardiovascular risk: a report from the European Society of Cardiology EUROObservational Research Programme EUROASPIRE V survey in 16 European countries. *Eur. J. Prev. Cardiol.* **28**, 370–379 (2021).
20. De Backer, G. et al. Management of dyslipidaemia in patients with coronary heart disease: results from the ESC-EORP EUROASPIRE V survey in 27 countries. *Atherosclerosis* **285**, 135–146 (2019).
21. Anderson, K. M., Wilson, P. W., Garrison, R. J. & Castelli, W. P. Longitudinal and secular trends in lipoprotein cholesterol measurements in a general population sample. The Framingham Offspring Study. *Atherosclerosis* **68**, 59–66 (1987).
22. Gómez Gerique, J. A. et al. Improvement of serum lipids concentration in a general population historical cohort. Why?. *Clín. Investig. Arterioscler.* **29**, 239–247 (2017).
23. Allahyari, A. et al. Application of the 2019 ESC/EAS dyslipidaemia guidelines to nationwide data of patients with a recent myocardial infarction: a simulation study. *Eur. Heart J.* **41**, 3900–3909 (2020).
24. Cuchel, M. et al. Homozygous familial hypercholesterolaemia: new insights and guidance for clinicians to improve detection and clinical management. a position paper from the Consensus Panel on Familial Hypercholesterolaemia of the European Atherosclerosis Society. *Eur. Heart J.* **35**, 2146–2157 (2014).
25. Pirillo, A., Catapano, A. L. & Norata, G. D. Monoclonal antibodies in the management of familial hypercholesterolemia: focus on PCSK9 and ANGPTL3 inhibitors. *Curr. Atheroscler. Rep.* **23**, 79 (2021).
26. Fogacci, F. et al. Efficacy and safety of mipomersen: a systematic review and meta-analysis of randomized clinical trials. *Drugs* **79**, 751–766 (2019).
27. Raal, F. J. et al. Mipomersen, an apolipoprotein B synthesis inhibitor, for lowering of LDL cholesterol concentrations in patients with homozygous familial hypercholesterolaemia: a randomised, double-blind, placebo-controlled trial. *Lancet* **375**, 998–1006 (2010).
28. Cuchel, M. et al. Efficacy and safety of a microsomal triglyceride transfer protein inhibitor in patients with homozygous familial hypercholesterolaemia: a single-arm, open-label, phase 3 study. *Lancet* **381**, 40–46 (2013).
29. Raal, F. J. et al. Evinacumab for homozygous familial hypercholesterolemia. *N. Engl. J. Med.* **383**, 711–720 (2020).
30. Rosenson, R. S. et al. Evinacumab in patients with refractory hypercholesterolemia. *N. Engl. J. Med.* **383**, 2307–2319 (2020).
31. Ahmad, Z. Statin intolerance. *Am. J. Cardiol.* **113**, 1765–1771 (2014).
32. Finegold, J. A., Manisty, C. H., Goldacre, B., Barron, A. J. & Francis, D. P. What proportion of symptomatic side effects in patients taking statins are genuinely caused by the drug? Systematic review of randomized placebo-controlled trials to aid individual patient choice. *Eur. J. Prev. Cardiol.* **21**, 464–474 (2020).
33. Gupta, A. et al. Adverse events associated with unblinded, but not with blinded, statin therapy in the Anglo-Scandinavian Cardiac Outcomes Trial – Lipid-Lowering Arm (ASCOT-LLA): a randomised double-blind placebo-controlled trial and its non-randomised non-blind extension phase. *Lancet* **389**, 2473–2481 (2017).
34. Reith, C. et al. Effect of statin therapy on muscle symptoms: an individual participant data meta-analysis of large-scale, randomised, double-blind trials. *Lancet* **400**, 832–845 (2022).
35. Howard, J. P. et al. Side effect patterns in a crossover trial of statin, placebo, and no treatment. *J. Am. Coll. Cardiol.* **78**, 1210–1222 (2021).

36. Ray, K. K. et al. Safety and efficacy of bempedoic acid to reduce LDL cholesterol. *N. Engl. J. Med.* **380**, 1022–1032 (2019).
37. Laufs, U. et al. Efficacy and safety of bempedoic acid in patients with hypercholesterolemia and statin intolerance. *J. Am. Heart Assoc.* **8**, e011662 (2019).
38. Ballantyne, C. M. et al. Efficacy and safety of bempedoic acid added to ezetimibe in statin-intolerant patients with hypercholesterolemia: a randomized, placebo-controlled study. *Atherosclerosis* **277**, 195–203 (2018).
39. Banach, M. et al. Association of bempedoic acid administration with atherogenic lipid levels in phase 3 randomized clinical trials of patients with hypercholesterolemia. *JAMA Cardiol.* **5**, 1124–1135 (2020).
40. Nissen, S. E. et al. Bempedoic acid and cardiovascular outcomes in statin-intolerant patients. *N. Engl. J. Med.* https://doi.org/10.1056/NEJMoa2215024 (2023).
41. Debacker, A. J., Voutila, J., Catley, M., Blakey, D. & Habib, N. Delivery of oligonucleotides to the liver with GalNAc: from research to registered therapeutic drug. *Mol. Ther.* **28**, 1759–1771 (2020).
42. Lansberg, P., Lee, A., Lee, Z.-V., Subramaniam, K. & Setia, S. Nonadherence to statins: individualized intervention strategies outside the pill box. *Vasc. Health Risk Manag.* **14**, 91 (2018).
43. Laufs, U., Karmann, B. & Pittrow, D. Atorvastatin treatment and LDL cholesterol target attainment in patients at very high cardiovascular risk. *Clin. Res. Cardiol.* **105**, 783–790 (2016).
44. Guglielmi, V. et al. Effectiveness of adherence to lipid lowering therapy on LDL-cholesterol in patients with very high cardiovascular risk: a real-world evidence study in primary care. *Atherosclerosis* **263**, 36–41 (2017).
45. Wei, L. et al. Adherence to statin treatment and readmission of patients after myocardial infarction: a six year follow up study. *Heart* **88**, 229–233 (2002).
46. Farnier, M. et al. Long-term treatment adherence to the proprotein convertase subtilisin/kexin type 9 inhibitor alirocumab in 6 ODYSSEY phase III clinical studies with treatment duration of 1 to 2 years. *J. Clin. Lipidol.* **11**, 986–997 (2017).
47. Zafrir, B., Egbaria, A., Stein, N., Elis, A. & Saliba, W. PCSK9 inhibition in clinical practice: treatment patterns and attainment of lipid goals in a large health maintenance organization. *J. Clin. Lipidol.* **15**, 202–211.e2 (2021).
48. Ray, K. K. et al. Two phase 3 trials of inclisiran in patients with elevated LDL cholesterol. *N. Engl. J. Med.* **382**, 1507–1519 (2020).
49. Choudhry, N. K. et al. The implications of therapeutic complexity on adherence to cardiovascular medications. *Arch. Intern. Med.* **171**, 814–822 (2011).
50. Ray, K. K. et al. Inclisiran in patients at high cardiovascular risk with elevated LDL cholesterol. *N. Engl. J. Med.* **376**, 1430–1440 (2017).
51. Ray, K. K. 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.* **4**, 1067–1075 (2019).
52. Bangalore, S., Breazna, A., DeMicco, D. A., Wun, C.-C. & Messerli, F. H. Visit-to-visit low-density lipoprotein cholesterol variability and risk of cardiovascular outcomes. *J. Am. Coll. Cardiol.* **65**, 1539–1548 (2015).
53. Brandts, J. & Ray, K. K. Low density lipoprotein cholesterol-lowering strategies and population health: time to move to a cumulative exposure model. *Circulation* **141**, 873–876 (2020).
54. Stoekenbroek, R. M., Kallend, D., Wijngaard, P. L. & Kastelein, J. J. Inclisiran for the treatment of cardiovascular disease: the ORION clinical development program. *Future Cardiol.* **14**, 433–442 (2018).
55. Yokoyama, M. et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet* **369**, 1090–1098 (2007).
56. Bhatt, D. L. et al. Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. *N. Engl. J. Med.* **380**, 11–22 (2019).
57. Budoff, M. J. et al. Effect of icosapent ethyl on progression of coronary atherosclerosis in patients with elevated triglycerides on statin therapy: final results of the EVAPORATE trial. *Eur. Heart J.* **41**, 3925–3932 (2020).
58. The ASCEND Study Collaborative Group. Effects of n-3 fatty acid supplements in diabetes mellitus. *N. Engl. J. Med.* **379**, 1540–1550 (2018).
59. Manson, J. E. et al. Marine n-3 fatty acids and prevention of cardiovascular disease and cancer. *N. Engl. J. Med.* **380**, 23–32 (2018).
60. Nicholls, S. J. et al. Effect of high-dose omega-3 fatty acids vs corn oil on major adverse cardiovascular events in patients at high cardiovascular risk: the STRENGTH randomized clinical trial. *JAMA* **324**, 2268–2280 (2020).
61. Mason, R. P. & Eckel, R. H. Mechanistic insights from REDUCE-IT STRENGTHen the case against triglyceride lowering as a strategy for cardiovascular disease risk reduction. *Am. J. Med.* **134**, 1085–1090 (2021).
62. Nissen, S. E. et al. Association between achieved ω-3 fatty acid levels and major adverse cardiovascular outcomes in patients with high cardiovascular risk: a secondary analysis of the STRENGTH trial. *JAMA Cardiol.* **6**, 910–917 (2021).
63. US Food and Drug Administration. *FDA Briefing Document: Endocrinologic and Metabolic Drugs Advisory Committee Meeting* (FDA, 2019).
64. Oscarsson, J. & Hurt-Camejo, E. Omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and their mechanisms of action on apolipoprotein B-containing lipoproteins in humans: a review. *Lipids Health Dis.* **16**, 149 (2017).
65. Carthew, R. W. & Sontheimer, E. J. Origins and mechanisms of miRNAs and siRNAs. *Cell* **136**, 642–655 (2009).
66. Chan, J. C. Y. et al. A proprotein convertase subtilisin/kexin type 9 neutralizing antibody reduces serum cholesterol in mice and nonhuman primates. *Proc. Natl Acad. Sci. USA* **106**, 9820–9825 (2009).
67. Frank-Kamenetsky, M. et al. Therapeutic RNAi targeting PCSK9 acutely lowers plasma cholesterol in rodents and LDL cholesterol in nonhuman primates. *Proc. Natl Acad. Sci. USA* **105**, 11915–11920 (2008).
68. Zhang, D.-W. et al. Binding of proprotein convertase subtilisin/kexin type 9 to epidermal growth factor-like repeat A of low density lipoprotein receptor decreases receptor recycling and increases degradation. *J. Biol. Chem.* **282**, 18602–18612 (2007).
69. Lebeau, P. F., Platko, K., Byun, J. H., Makda, Y. & Austin, R. C. The emerging roles of intracellular PCSK9 and their implications in endoplasmic reticulum stress and metabolic diseases. *Metabolites* **12**, 215 (2022).
70. Lebeau, P. F. et al. Diet-induced hepatic steatosis abrogates cell-surface LDLR by inducing de novo PCSK9 expression in mice. *J. Biol. Chem.* **294**, 9037–9047 (2019).
71. Demers, A. et al. PCSK9 induces CD36 degradation and affects long-chain fatty acid uptake and triglyceride metabolism in adipocytes and in mouse liver. *Arterioscler. Thromb. Vasc. Biol.* **35**, 2517–2525 (2015).
72. Emma, M. R. et al. Hepatic and circulating levels of PCSK9 in morbidly obese patients: relation with severity of liver steatosis. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **1865**, 158792 (2020).
73. Ruscica, M. et al. Liver fat accumulation is associated with circulating PCSK9. *Ann. Med.* **48**, 384–391 (2016).
74. Gibbs, J. P. et al. Impact of target-mediated elimination on the dose and regimen of evolocumab, a human monoclonal antibody against proprotein convertase subtilisin/kexin type 9 (PCSK9). *J. Clin. Pharmacol.* **57**, 616–626 (2017).
75. Dicembrini, I., Giannini, S., Ragghianti, B., Mannucci, E. & Monami, M. Effects of PCSK9 inhibitors on LDL cholesterol, cardiovascular morbidity and all-cause mortality: a systematic review and meta-analysis of randomized controlled trials. *J. Endocrinol. Investig.* **42**, 1029–1039 (2019).
76. Rosenson, R. S. et al. Efficacy and safety of evolocumab in individuals with type 2 diabetes mellitus: primary results of the randomised controlled BANTING study. *Diabetologia* **62**, 948–958 (2019).
77. Ray, K. K. et al. Effect of an siRNA therapeutic targeting PCSK9 on atherogenic lipoproteins. *Circulation* **138**, 1304–1316 (2018).
78. Chan, D. C. et al. Comparative effects of PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibition and statins on postprandial triglyceride-rich lipoprotein metabolism. *Arterioscler. Thromb. Vasc. Biol.* **38**, 1644–1655 (2018).
79. Walker, R. G. & Thompson, T. B. Fibronectin-based scaffold domain proteins that bind myostatin: a patent evaluation of WO2014043344. *Expert. Opin. Ther. Pat.* **25**, 619–624 (2015).
80. Stein, E. A. et al. LDL cholesterol reduction with BMS-962476, an adnectin inhibitor of PCSK9: results of a single ascending dose study [abstract]. *J. Am. Coll. Cardiol.* **63** (Suppl. 12), A1372 (2014).
81. Stein, E. et al. Safety, tolerability and LDL-C reduction with a novel anti-Pcsk9 recombinant fusion protein (Lib003): results of a randomized, double-blind, placebo-controlled, phase 2 study [abstract]. *Atherosclerosis* **287**, e7 (2019).
82. Stein, E. A. et al. Safety, tolerability and LDL-C reduction with LIB003 a novel anti-PCSK9 recombinant fusion protein: results of open-label extension phase 2B study [abstract]. *Circulation* **140** (Suppl. 1), A17222 (2019).
83. Gennemark, P. et al. An oral antisense oligonucleotide for PCSK9 inhibition. *Sci. Transl. Med.* **13**, eabe9117 (2021).
84. Koren, M. J. et al. Etesian: a phase 2B study of the efficacy, safety and tolerability of AZD8233, a PCSK9-targeted antisense oligonucleotide, in patients with dyslipidemia [abstract]. *J. Am. Coll. Cardiol.* **79** (Suppl. 9), 1475 (2022).
85. Zlatev, I. et al. Reversal of siRNA-mediated gene silencing in vivo. *Nat. Biotechnol.* **36**, 509–511 (2018).
86. Hutvágner, G., Simard, M. J., Mello, C. C. & Zamore, P. D. Sequence-specific inhibition of small RNA function. *PLoS Biol.* **2**, e98 (2004).
87. Alleyne, C. et al. Series of novel and highly potent cyclic peptide PCSK9 inhibitors derived from an mRNA display screen and optimized via structure-based design. *J. Med. Chem.* **63**, 13796–13824 (2020).
88. Ballantyne, C. M. et al. Efficacy and safety of the oral PCSK9 inhibitor MK-0616: a phase 2b randomized controlled trial. *J. Am. Coll. Cardiol.* https://doi.org/10.1016/j.jacc.2023.02.018 (2023).
89. Crossey, E. et al. A cholesterol-lowering VLP vaccine that targets PCSK9. *Vaccine* **33**, 5747–5755 (2015).
90. Pan, Y. et al. A therapeutic peptide vaccine against PCSK9. *Sci. Rep.* **7**, 12534 (2017).
91. Momtazi-Borojeni, A. A., Jaafari, M. R., Badiee, A., Banach, M. & Sahebkar, A. Therapeutic effect of nanoliposomal PCSK9 vaccine in a mouse model of atherosclerosis. *BMC Med.* **17**, 223 (2019).
92. Zeitlinger, M. et al. A phase I study assessing the safety, tolerability, immunogenicity, and low-density lipoprotein cholesterol-lowering activity of immunotherapeutics targeting PCSK9. *Eur. J. Clin. Pharmacol.* **77**, 1473–1484 (2021).
93. Musunuru, K. et al. In vivo CRISPR base editing of PCSK9 durably lowers cholesterol in primates. *Nature* **593**, 429–434 (2021).
94. Nishimasu, H. et al. Crystal structure of Cas9 in complex with guide RNA and target DNA. *Cell* **156**, 935–949 (2014).
95. Rosenson, R. S., Shaik, A. & Song, W. New therapies for lowering triglyceride-rich lipoproteins. *J. Am. Coll. Cardiol.* **78**, 1817–1830 (2021).

96. Jong, M. C., Hofker, M. H. & Havekes, L. M. Role of ApoCs in lipoprotein metabolism. *Arterioscler. Thromb. Vasc. Biol.* **19**, 472–484 (1999).
97. Christopoulou, E., Tsimihodimos, V., Filippatos, T. & Elisaf, M. Apolipoprotein CIII and diabetes. is there a link? *Diabetes Metab. Res. Rev.* **35**, e3118 (2019).
98. Giammanco, A., Spina, R., Cefalù, A. B. & Averna, M. APOC-III: a gatekeeper in controlling triglyceride metabolism. *Curr. Atheroscler. Rep.* **25**, 67–76 (2023).
99. Taskinen, M.-R. & Borén, J. New insights into the pathophysiology of dyslipidemia in type 2 diabetes. *Atherosclerosis* **239**, 483–495 (2015).
100. Yao, Z. Human apolipoprotein C-III – a new intrahepatic protein factor promoting assembly and secretion of very low density lipoproteins. *Cardiovasc. Haematol. Disord. Drug Targets* **12**, 133–140 (2012).
101. Taskinen, M.-R. et al. Postprandial metabolism of apolipoproteins B48, B100, C-III, and E in humans with APOC3 loss-of-function mutations. *JCI Insight* **7**, e160607 (2022).
102. Reyes-Soffer, G. et al. Effects of APOC3 heterozygous deficiency on plasma lipid and lipoprotein metabolism. *Arterioscler. Thromb. Vasc. Biol.* **39**, 63–72 (2019).
103. Gaudet, D. et al. Targeting APOC3 in the familial chylomicronemia syndrome. *N. Engl. J. Med.* **371**, 2200–2206 (2014).
104. TG and HDL Working Group of the Exome Sequencing Project, National Heart, Lung, and Blood Institute. Loss-of-function mutations in *APOC3*, triglycerides, and coronary disease. *N. Engl. J. Med.* **371**, 22–31 (2014).
105. Wulff, A. B., Nordestgaard, B. G. & Tybjærg-Hansen, A. *APOC3* loss-of-function mutations, remnant cholesterol, low-density lipoprotein cholesterol, and cardiovascular risk: mediation- and meta-analyses of 137 895 individuals. *Arterioscler. Thromb. Vasc. Biol.* **38**, 660–668 (2018).
106. Taskinen, M.-R., Packard, C. J. & Borén, J. Emerging evidence that ApoC-III inhibitors provide novel options to reduce the residual CVD. *Curr. Atheroscler. Rep.* **21**, 27 (2019).
107. Witztum, J. L. et al. Volanesorsen and triglyceride levels in familial chylomicronemia syndrome. *N. Engl. J. Med.* **381**, 531–542 (2019).
108. Gouni-Berthold, I. et al. Efficacy and safety of volanesorsen in patients with multifactorial chylomicronaemia (COMPASS): a multicentre, double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Diabetes Endocrinol.* **9**, 264–275 (2021).
109. Oral, E. A. et al. Assessment of efficacy and safety of volanesorsen for treatment of metabolic complications in patients with familial partial lipodystrophy: results of the BROADEN study. *J. Clin. Lipidol.* **16**, 833–849 (2022).
110. Tardif, J.-C. et al. Apolipoprotein C-III reduction in subjects with moderate hypertriglyceridaemia and at high cardiovascular risk. *Eur. Heart J.* **43**, 1401–1412 (2022).
111. Baass, A., Paquette, M., Bernard, S. & Hegele, R. A. Familial chylomicronemia syndrome: an under-recognized cause of severe hypertriglyceridaemia. *J. Intern. Med.* **287**, 340–348 (2020).
112. Khetarpal, S. A., Wang, M. & Khera, A. V. Volanesorsen, familial chylomicronemia syndrome, and thrombocytopenia. *N. Engl. J. Med.* **381**, 2582–2584 (2019).
113. Stitziel, N. O. et al. ANGPTL3 deficiency and protection against coronary artery disease. *J. Am. Coll. Cardiol.* **69**, 2054–2063 (2017).
114. Dewey, F. E. et al. Genetic and pharmacologic inactivation of ANGPTL3 and cardiovascular disease. *N. Engl. J. Med.* **377**, 211–221 (2017).
115. Fujimoto, K., Koishi, R., Shimizugawa, T. & Ando, Y. Angptl3-null mice show low plasma lipid concentrations by enhanced lipoprotein lipase activity. *Exp. Anim.* **55**, 27–34 (2006).
116. Shimamura, M. et al. Angiopoietin-like protein3 regulates plasma HDL cholesterol through suppression of endothelial lipase. *Arterioscler. Thromb. Vasc. Biol.* **27**, 366–372 (2007).
117. Ruhanen, H., Haridas, P. A. N., Jauhiainen, M. & Olkkonen, V. M. Angiopoietin-like protein 3, an emerging cardiometabolic therapy target with systemic and cell-autonomous functions. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **1865**, 158791 (2020).
118. Shimamura, M. et al. Angiopoietin-like protein 3, a hepatic secretory factor, activates lipolysis in adipocytes. *Biochem. Biophys. Res. Commun.* **301**, 604–609 (2003).
119. Graham, M. J. et al. Cardiovascular and metabolic effects of ANGPTL3 antisense oligonucleotides. *N. Engl. J. Med.* **377**, 222–232 (2017).
120. Ge, H. et al. Differential regulation and properties of angiopoietin-like proteins 3 and 4. *J. Lipid Res.* **46**, 1484–1490 (2005).
121. Wang, Y. et al. Hepatic ANGPTL3 regulates adipose tissue energy homeostasis. *Proc. Natl Acad. Sci. USA* **112**, 11630–11635 (2015).
122. Chi, X. et al. ANGPTL8 promotes the ability of ANGPTL3 to bind and inhibit lipoprotein lipase. *Mol. Metab.* **6**, 1137–1149 (2017).
123. Sylvers-Davie, K. L. & Davies, B. S. J. Regulation of lipoprotein metabolism by ANGPTL3, ANGPTL4, and ANGPTL8. *Am. J. Physiol. Endocrinol. Metab.* **321**, E493–E508 (2021).
124. Chen, Y. Q. et al. Angiopoietin-like protein 8 differentially regulates ANGPTL3 and ANGPTL4 during postprandial partitioning of fatty acids. *J. Lipid Res.* **61**, 1203–1220 (2020).
125. Gaudet, D. et al. Vupanorsen, an N-acetyl galactosamine-conjugated antisense drug to *ANGPTL3* mRNA, lowers triglycerides and atherogenic lipoproteins in patients with diabetes, hepatic steatosis, and hypertriglyceridaemia. *Eur. Heart J.* **41**, 3936–3945 (2020).
126. Bergmark, B. A. et al. Effect of vupanorsen on non-high-density lipoprotein cholesterol levels in statin-treated patients with elevated cholesterol: TRANSLATE-TIMI 70. *Circulation* **145**, 1377–1386 (2022).
127. Minicocci, I. et al. Mutations in the ANGPTL3 gene and familial combined hypolipidemia: a clinical and biochemical characterization. *J. Clin. Endocrinol. Metab.* **97**, E1266–E1275 (2012).
128. Watts, G. et al. RNA interference targeting hepatic angiopoietin-like protein 3 results in prolonged reductions in serum triglyceride and non-HDL-cholesterol concentrations: first human results with ARO-ANG3. Preprint at *Res. Sq.* https://doi.org/10.21203/rs.3.rs-2097342/v1 (2022).
129. Arrowhead Pharmaceuticals. Arrowhead reports interim clinical data on cardiometabolic candidates ARO-APOC3 and ARO-ANG3. *Arrowhead Pharmaceuticals* https://ir.arrowheadpharma.com/node/15246/pdf (2020).
130. Watts, G. F. et al. Pharmacodynamic effect of ARO-ANG3, an investigational RNA interference targeting hepatic angiopoietin-like protein 3, in patients with hypercholesterolemia [abstract]. *Circulation* **142** (Suppl. 3), A15751 (2020).
131. Watts, G. F. et al. RNAi inhibition of angiopoietin-like protein 3 (ANGPTL3) with ARO-ANG3 mimics the lipid and lipoprotein profile of familial combined hypolipidemia. *Eur. Heart J.* https://doi.org/10.1093/ehjci/ehaa946.3331 (2020).
132. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT04644809 (2022).
133. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT04832971 (2022).
134. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT05256654 (2023).
135. Chadwick, A. C., Evitt, N. H., Lv, W. & Musunuru, K. Reduced blood lipid levels with in vivo CRISPR-Cas9 base editing of ANGPTL3. *Circulation* **137**, 975–977 (2018).
136. Verve Therapeutics. Verve Therapeutics reports preclinical data demonstrating potent editing of ANGPTL3 gene using proprietary GalNAc-LNP delivery technology in non-human primates. *Verve Therapeutics* https://www.globenewswire.com/news-release/2021/11/09/2330092/0/en/Verve-Therapeutics-Reports-Preclinical-Data-Demonstrating-Potent-Editing-of-ANGPTL3-Gene-Using-Proprietary-GalNAc-LNP-Delivery-Technology-in-Non-Human-Primates.html (2021).
137. Bellinger, A. Verve Therapeutics: In vivo CRISPR base editing to treat ASCVD. *Verve Therapeutics* https://ir.vervetx.com/static-files/e1932552-a384-408a-9d4c-2b5424c9cf74 (2022).
138. Banerjee, S. & De, A. Pathophysiology and inhibition of cholesteryl ester transfer protein for prevention of cardiovascular diseases: an update. *Drug Discov. Today* **26**, 1759–1764 (2021).
139. Inazu, A. et al. Increased high-density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation. *N. Engl. J. Med.* **323**, 1234–1238 (1990).
140. Niu, W. & Qi, Y. Circulating cholesteryl ester transfer protein and coronary heart disease. *Circ. Cardiovasc. Genet.* **8**, 114–121 (2015).
141. Nomura, A. et al. Protein-truncating variants at the cholesteryl ester transfer protein gene and risk for coronary heart disease. *Circ. Res.* **121**, 81–88 (2017).
142. Ference, B. A. et al. Association of genetic variants related to CETP inhibitors and statins with lipoprotein levels and cardiovascular risk. *JAMA* **318**, 947–956 (2017).
143. Hovingh, G. K. et al. Cholesterol ester transfer protein inhibition by TA-8995 in patients with mild dyslipidaemia (TULIP): a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet* **386**, 452–460 (2015).
144. Nicholls, S. J. et al. Lipid lowering effects of the CETP inhibitor obicetrapib in combination with high-intensity statins: a randomized phase 2 trial. *Nat. Med.* **28**, 1672–1678 (2022).
145. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT04770389 (2021).
146. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT05202509 (2023).
147. Schmidt, A. F. et al. Cholesteryl ester transfer protein (CETP) as a drug target for cardiovascular disease. *Nat. Commun.* **12**, 5640 (2021).
148. Barter, P. J. et al. Effects of torcetrapib in patients at high risk for coronary events. *N. Engl. J. Med.* **357**, 2109–2122 (2007).
149. Johns, D. G., Duffy, J., Fisher, T., Hubbard, B. K. & Forrest, M. J. On- and off-target pharmacology of torcetrapib. *Drugs* **72**, 491–507 (2012).
150. Schwartz, G. G. et al. Effects of dalcetrapib in patients with a recent acute coronary syndrome. *N. Engl. J. Med.* **367**, 2089–2099 (2012).
151. Tardif, J.-C. et al. Pharmacogenomic determinants of the cardiovascular effects of dalcetrapib. *Circ. Cardiovasc. Genet.* **8**, 372–382 (2015).
152. Tardif, J. C. et al. Pharmacogenetics-guided dalcetrapib therapy after an acute coronary syndrome: the dal-GenE trial. *Eur. Heart J.* **43**, 3947–3956 (2022).
153. Lincoff, A. M. et al. Evacetrapib and cardiovascular outcomes in high-risk vascular disease. *N. Engl. J. Med.* **376**, 1933–1942 (2017).
154. The HPS3/TIMI55–Reveal Collaborative Group Effects of anacetrapib in patients with atherosclerotic vascular disease. *N. Engl. J. Med.* **377**, 1217–1227 (2017).
155. Gotto, A. M. et al. Evaluation of lipids, drug concentration, and safety parameters following cessation of treatment with the cholesteryl ester transfer protein inhibitor anacetrapib in patients with or at high risk for coronary heart disease. *Am. J. Cardiol.* **113**, 76–83 (2014).
156. Nicholls, S. J. & Bubb, K. The mystery of evacetrapib – why are CETP inhibitors failing? *Expert. Rev. Cardiovasc. Ther.* **18**, 127–130 (2020).
157. Berglund, L. & Ramakrishnan, R. Lipoprotein(a). *Arterioscler. Thromb. Vasc. Biol.* **24**, 2219–2226 (2004).
158. Kronenberg, F. & Utermann, G. Lipoprotein(a): resurrected by genetics. *J. Intern. Med.* **273**, 6–30 (2013).
159. Wright, R. S. et al. Pooled patient-level analysis of inclisiran trials in patients with familial hypercholesterolemia or atherosclerosis. *J. Am. Coll. Cardiol.* **77**, 1182–1193 (2021).
160. Raal, F. J. et al. Reduction in lipoprotein(a) with PCSK9 monoclonal antibody evolocumab (AMG 145): a pooled analysis of more than 1,300 patients in 4 phase II trials. *J. Am. Coll. Cardiol.* **63**, 1278–1288 (2014).

161. Gaudet, D. et al. Effect of alirocumab on lipoprotein(a) over ≥1.5 years (from the phase 3 ODYSSEY program). *Am. J. Cardiol.* **119**, 40–46 (2017).
162. Ray, K. K. et al. Lipoprotein(a) reductions from PCSK9 inhibition and major adverse cardiovascular events: pooled analysis of alirocumab phase 3 trials. *Atherosclerosis* **288**, 194–202 (2019).
163. O'Donoghue, M. L. et al. Lipoprotein(a), PCSK9 inhibition, and cardiovascular risk: insights from the FOURIER trial. *Circulation* **139**, 1483–1492 (2019).
164. Szarek, M. et al. Lipoprotein(a) lowering by alirocumab reduces the total burden of cardiovascular events independent of low-density lipoprotein cholesterol lowering: ODYSSEY OUTCOMES trial. *Eur. Heart J.* **41**, 4245–4255 (2020).
165. Tsimikas, S. et al. Antisense therapy targeting apolipoprotein(a): a randomised, double-blind, placebo-controlled phase 1 study. *Lancet* **386**, 1472–1483 (2015).
166. Viney, N. J. et al. Antisense oligonucleotides targeting apolipoprotein(a) in people with raised lipoprotein(a): two randomised, double-blind, placebo-controlled, dose-ranging trials. *Lancet* **388**, 2239–2253 (2016).
167. Tsimikas, S. et al. Lipoprotein(a) reduction in persons with cardiovascular disease. *N. Engl. J. Med.* **382**, 244–255 (2020).
168. Catapano, A. L. et al. 2016 ESC/EAS guidelines for the management of dyslipidaemias. *Eur. Heart J.* **37**, 2999–3058 (2016).
169. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT04023552 (2022).
170. Koren, M. J. et al. Safety, tolerability and efficacy of single-dose AMG 890, a novel siRNA targeting Lp(a), in healthy subjects and subjects with elevated Lp(a) [abstract]. *Circulation* **142** (Suppl. 3), A13951 (2020).
171. O'Donoghue, M. L. et al. Small interfering RNA to reduce lipoprotein(a) in cardiovascular disease. *N. Engl. J. Med.* **387**, 1855–1864 (2022).
172. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT04914546 (2022).
173. Nissen, S. E. et al. Single ascending dose study of a short interfering RNA targeting lipoprotein(a) production in individuals with elevated plasma lipoprotein(a) levels. *JAMA* **327**, 1679–1687 (2022).
174. Prospective Studies Collaboration Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55 000 vascular deaths. *Lancet* **370**, 1829–1839 (2007).
175. Assmann, G., Cullen, P. & Schulte, H. Simple scoring scheme for calculating the risk of acute coronary events based on the 10-year follow-up of the prospective cardiovascular Munster (PROCAM) study. *Circulation* **105**, 310–315 (2002).
176. Di Angelantonio, E. et al. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA* **302**, 1993–2000 (2009).
177. Kannel, W. B., Dawber, T. R., Friedman, G. D., Glennon, W. E. & Mcnamara, P. M. Risk factors in coronary heart disease: the Framingham study. *Ann. Intern. Med.* **61**, 888–899 (1964).
178. Jun, M. et al. Effects of fibrates on cardiovascular outcomes: a systematic review and meta-analysis. *Lancet* **375**, 1875–1884 (2010).
179. Holmes, M. V. et al. Mendelian randomization of blood lipids for coronary heart disease. *Eur. Heart J.* **36**, 539–550 (2015).
180. Frikke-Schmidt, R. et al. Association of loss-of-function mutations in the *ABCA1* gene with high-density lipoprotein cholesterol levels and risk of ischemic heart disease. *JAMA* **299**, 2524–2532 (2008).
181. Voight, B. F. et al. Plasma HDL cholesterol and risk of myocardial infarction: a Mendelian randomisation study. *Lancet* **380**, 572–580 (2012).
182. Ko, D. T. et al. High-density lipoprotein cholesterol and cause-specific mortality in individuals without previous cardiovascular conditions: the CANHEART study. *J. Am. Coll. Cardiol.* **68**, 2073–2083 (2016).
183. Hirata, A. et al. The relationship between very high levels of serum high-density lipoprotein cholesterol and cause-specific mortality in a 20-year follow-up study of Japanese general population. *J. Atheroscler. Thromb.* **23**, 800–809 (2016).
184. Madsen, C. M., Varbo, A. & Nordestgaard, B. G. Extreme high high-density lipoprotein cholesterol is paradoxically associated with high mortality in men and women: two prospective cohort studies. *Eur. Heart J.* **38**, 2478–2486 (2017).
185. Rohatgi, A., Westerterp, M., Von Eckardstein, A., Remaley, A. & Rye, K.-A. HDL in the 21st century: a multifunctional roadmap for future HDL research. *Circulation* **143**, 2293–2309 (2021).
186. Gibson, C. M. et al. Safety and tolerability of CSL112, a reconstituted, infusible, plasma-derived apolipoprotein A-I, after acute myocardial infarction. *Circulation* **134**, 1918–1930 (2016).
187. Kingwell, B. A. et al. Antiatherosclerotic effects of CSL112 mediated by enhanced cholesterol efflux capacity. *J. Am. Heart Assoc.* **11**, e024754 (2022).
188. Nicholls, S. J. et al. Effect of infusion of high-density lipoprotein mimetic containing recombinant apolipoprotein AI Milano on coronary disease in patients with an acute coronary syndrome in the MILANO-PILOT trial: a randomized clinical trial. *JAMA Cardiol.* **3**, 806–814 (2018).
189. Nicholls, S. J. et al. Effect of serial infusions of CER-001, a pre-β high-density lipoprotein mimetic, on coronary atherosclerosis in patients following acute coronary syndromes in the CER-001 Atherosclerosis Regression Acute Coronary Syndrome Trial: a randomized clinical trial. *JAMA Cardiol.* **3**, 815–822 (2018).
190. Diditchenko, S. et al. Novel formulation of a reconstituted high-density lipoprotein (CSL112) dramatically enhances ABCA1-dependent cholesterol efflux. *Arterioscler. Thromb. Vasc. Biol.* **33**, 2202–2211 (2013).
191. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT03473223 (2023).
192. Czarnecka, H. & Yokoyama, S. Regulation of cellular cholesterol efflux by lecithin: cholesterol acyltransferase reaction through nonspecific lipid exchange. *J. Biol. Chem.* **271**, 2023–2028 (1996).
193. Shamburek, R. D. et al. Safety and tolerability of ACP-501, a recombinant human lecithin:cholesterol acyltransferase, in a phase 1 single-dose escalation study. *Circ. Res.* **118**, 73–82 (2016).
194. Gutierrez, M. J. et al. Efficacy and safety of ETC-1002, a novel investigational low-density lipoprotein-cholesterol-lowering therapy for the treatment of patients with hypercholesterolemia and type 2 diabetes mellitus. *Arterioscler. Thromb. Vasc. Biol.* **34**, 676–683 (2014).
195. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT03705234 (2022).
196. Watts, G. et al. Reduced expression of angiopoietin-like protein 3 via RNA interference with ARO-ANG3 produces prolonged reductions in LDL-C and triglycerides in dyslipidemic patients. *J. Clin. Lipidol.* **14**, 599 (2020).

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