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STATE OF THE ART REVIEW
Dyslipidaemias

The dawn of a new era of targeted lipid-lowering therapies

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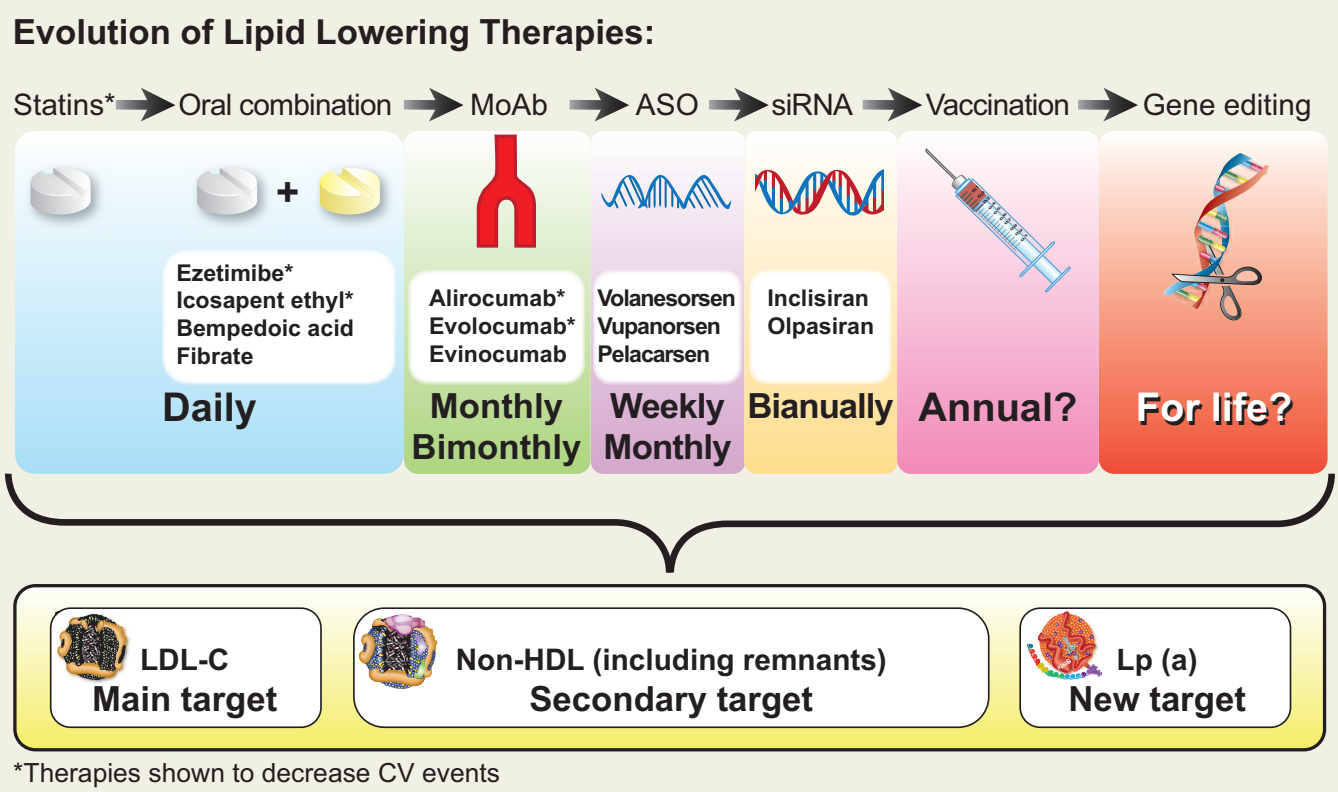
Graphical Abstract



The future evolution of lipid-lowering therapies. The quest for new lipid-lowering therapies enabling less frequent administration is continuing. Outcome trials to show cardiovascular event reduction will determine their clinical application. ASO, antisense oligonucleotide; CV, cardiovascular; IPE, icosapent ethyl; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); MoAb, monoclonal antibodies; siRNA, small-interfering RNA.

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Lipid risk factors for cardiovascular disease depend in part on lifestyle, but optimum control of lipids often demands additional measures. Low-density lipoprotein (LDL) doubtless contributes causally to atherosclerosis. Recent human genetic findings have substantiated a number of novel targets for lipid-lowering therapy including apolipoprotein C-III, angiopoietin-like protein 3 and 4, apolipoprotein V, and ATP citrate lyase. These discoveries coupled with advances in biotechnology development afford new avenues for management of LDL and other aspects of lipid risk. Beyond LDL, new treatments targeting triglyceride-rich lipoproteins and lipoprotein(a) have become available and have entered clinical development. Biological and RNA-directed agents have joined traditional small-molecule approaches, which themselves have undergone considerable refinement. Innovative targeting strategies have increased efficacy of some of these novel interventions and markedly improved their tolerability. Gene-editing approaches have appeared on the horizon of lipid management. This article reviews this progress offering insight into novel biological and therapeutic discoveries, and places them into a practical patient care perspective.

Keywords Lipoproteins • Triglycerides • Lipoprotein(a) • Apolipoprotein C-III • Angiopoietin-like proteins • RNA therapeutics

Introduction

Lipids comprise key modifiable risk factors for atherosclerotic vascular disease (ASVD), a chronic immunoinflammatory process in the arterial wall that causes most cardiovascular (CV) events. The accumulation and retention of apolipoprotein B (apoB)-containing lipoproteins, mainly low-density lipoprotein (LDL) in the arterial intima, accompanies early atherogenesis.[1] An inflammatory response ensues that promotes plaque progression and eventually plaque disruption.[2] LDL particles constitute 90% of apoB-containing lipoproteins in fasting humans, and have become the prime treatment target in clinical practice. But other apoB-containing lipoproteins also contribute causally to atherosclerosis[3] (*Figure 1*). Triglyceride-rich lipoproteins that are <70 nm in diameter such as chylomicron remnants, very low density lipoprotein (VLDL) remnants, and intermediate-density lipoprotein (IDL) can traverse the endothelium, accumulate, and promote atherogenesis. Recent epidemiologic and genetic studies have established that cholesterol-rich remnant particles that accumulate in individuals with hypertriglyceridaemia are atherogenic and contribute to ASVD.[4,5]

In general, LDL-cholesterol (LDL-C) correlates tightly with apoB, but in some circumstances like diabetes, obesity, or very low LDL-C, it may underestimate the risk conferred by other apoB-containing lipoproteins. In these conditions, the simple calculation of non-high-density lipoprotein cholesterol (HDL-C) (total cholesterol – HDL) captures all apoB-containing lipoproteins including remnant cholesterol. The measurement of apoB also yields a more accurate estimation of risk than measurement of LDL concentrations in such individuals. Emerging evidence also suggests that non-HDL-C and apoB reflect residual risk better than LDL in statin-treated patients.[6] Thus, non-HDL-C has become a secondary target in European and other guidelines. Emerging novel therapies can target these non-LDL lipid fractions and promise to provide practitioners with new tools to confront residual risk.

Strong and consistent evidence from monogenic disorders, Mendelian randomization and genome-wide association studies (GWAS), and observational epidemiological, clinical, and interventional investigations have established that LDL satisfies modified Koch's postulates for causing atherosclerosis[7] (*Figure 2*). Without elevated LDL, atherosclerosis would likely be an orphan disease. LDL is the most extensively studied and targeted lipoprotein and remains justifiably the main lipid focus in clinical practice.

Multiple lines of evidence show that the magnitude and duration of exposure to LDL determine the risk of ASVD and its complications.[8] Thus, more and earlier LDL-C reduction provide greater CV prevention. This observation underscores the urgency of identification and early treatment of high LDL. Based on accumulating clinical trial evidence, guidelines and practice have evolved towards the achievement of more stringent LDL-C goals, especially in higher risk patients.[9] Recent studies that lowered LDL-C with combination therapy have not shown a threshold for clinical benefit and have allayed many safety concerns, thus reinforcing the 'lower is better' concept.[10–12]

Recent successes of the trials with non-statin lipid-lowering agents in decreasing CV events have shown that LDL-C lowering by a variety of mechanisms including increased LDL receptor expression or reduced cholesterol adsorption yields CV benefit.[13] Focus has therefore broadened from 'high-intensity statin therapy' to 'high-intensity lipid-lowering therapy' for LDL-C management. This recognition, along with the considerable remaining CV risk even in statin-treated individuals, has accelerated the quest for therapies that reduce atherogenic apoB-containing lipoproteins. Targeted delivery of nucleic acid-based therapies has progressed substantially, enabling safe and effective modulation of causal atherogenic particles, thus ushering in a new era in lipid management (*Graphical Abstract*).

The emergence of new targets for management of dyslipidaemia

In the past decades, advances in genetics, analytical techniques, and increased understanding of signalling molecules have uncovered a diversity of novel targetable mechanisms for lipid-lowering therapy.[14,15] For example, such studies identified the genetic defect in autosomal dominant hypercholesterolaemia as gain of function of proprotein convertase subtilisin/kexin Type 9 (PCSK9).[16–18]

Mendelian randomization approaches can help to differentiate truly causal factors from biomarkers that merely associate with

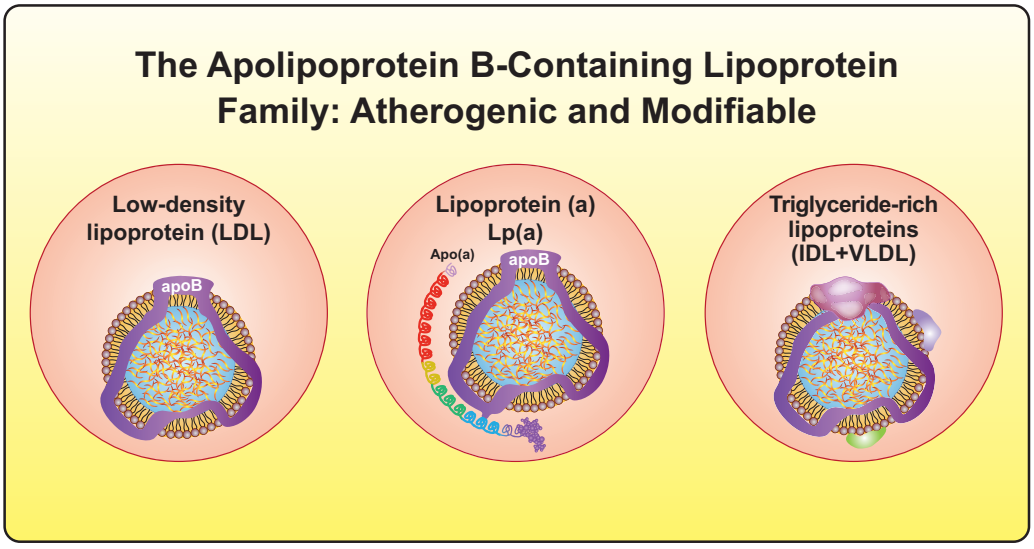

Figure 1 New targets for lipid-lowering therapies. Beyond low-density lipoprotein, lipoprotein(a) and triglyceride-rich lipoproteins or remnant lipoproteins have become actionable targets in lipid management. IDL, intermediate-density lipoprotein; VLDL, very low-density lipoprotein.

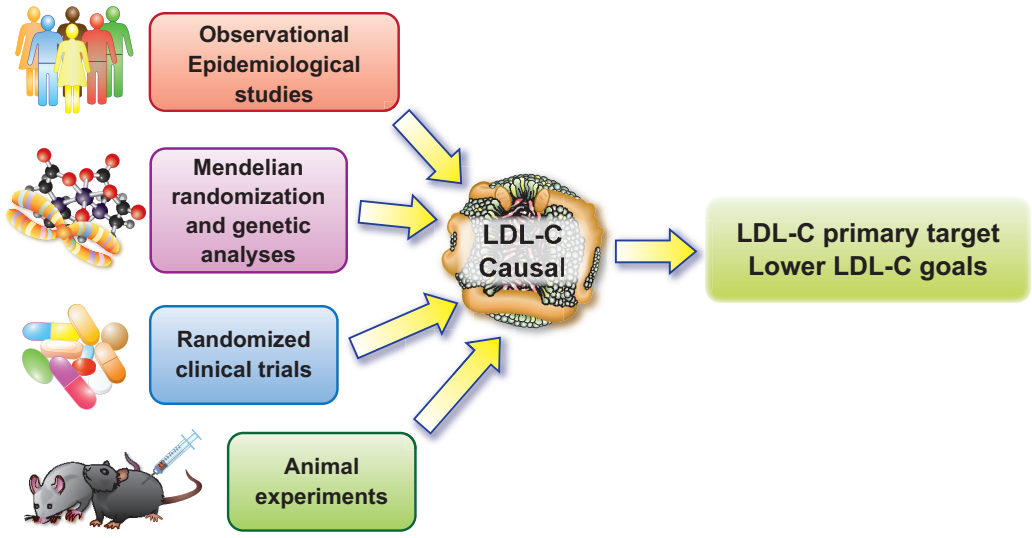

Figure 2 Multiple lines of evidence showing low-density lipoprotein cholesterol is causal for cardiovascular disease. Data that have accrued from observational data, human genetic analyses, randomized clinical trial results, and animal experimentation in multiple species, all concordantly support a causal contribution of low-density lipoprotein to atherosclerosis.

events. The application of Mendelian randomization methodology has validated traditional targets like hydroxymethylglutaryl coenzyme A (HMG-CoA, the enzyme that statins inhibit) and has also identified or buttressed new targets. Examples include NPC1L1, an intestinal transporter inhibited by ezetimibe, apolipoprotein C-III, angiopoietin like-protein (ANGPTL) 3 and 4, apolipoprotein V, and ATP citrate lyase.[19–22] The addition of ezetimibe to simvastatin produced incremental LDL lowering and reduction in events in post-acute coronary syndrome patients in IMPROVE-IT.[23]

Advanced small-molecule, biological, or nucleic acid-based approaches to targeting these newly recognized mediators have undergone rapid development. Monoclonal antibodies and RNA therapeutics have become a reality in the modulation of lipid metabolism. The engineering of antisense RNA and small interfering RNAs (siRNAs) now permits the selective inhibition of the production of specific proteins. These technologies have set the stage for new therapeutic approaches aimed at hepatocytes, key cells in lipid metabolism. Conjugation of RNA therapeutics to a carbohydrate ligand

Table 1 Efficacy, event reduction, and approval status of lipid-lowering therapies

Class of agent	LDL reduction efficacy (%)	Event reduction	Approval status
Statins	30–50	+	+
Ezetimibe	15–20	+ Combined with statin	+
PCSK9 inhibitors	50–60	+ For MoAb combined with statin	+
Bempedoic acid	17–25	Outcome trial in progress	+

This table summarizes the efficacy and approval status for different therapies that target LDL.
LDL, low-density lipoprotein.

with a terminal N-acetyl galactosamine (GalNAc) residue permits engagement of a hepatocyte-specific receptor that can direct drug delivery to these liver cells. This selective targeting can markedly reduce the dose of inhibitory RNAs and limit unwanted effects that plagued early generations of RNA therapeutics, including sometimes serious injection-site reactions.[24] Some of these therapies have already entered testing in Phase 3 trials. Looking forward, the maturation of gene therapies and the recent gene-editing revolution offer exciting new possibilities to treat atherogenic lipoproteins.[25] Such developments promise to advance ASVD management substantially.

Targeting low-density lipoprotein-cholesterol with new tools

Highly efficacious therapies can lower LDL-C. Statins, ezetimibe, or PCSK9 inhibition by monoclonal antibodies have all improved CV outcomes in randomized controlled trials (RCTs), and guidelines include their use.[9] Yet, implementation of these tools in practice has lagged. A recent study in Europe showed that moderate dose statin monotherapy predominated as a mode of lipid-lowering therapy, with only 18% of very high-risk patients getting to goal.[26] In addition to better implementation and adherence strategies, the quest continues for new therapies to lower LDL-C effectively. Most of the emphasis on novel treatments in atherosclerosis have focused on apoB-containing lipoproteins because of their proven causality in ASVD (*Figures 1* and *2*, and *Table 1*).

The finding that PCSK9 regulates LDL homeostasis has provided new opportunities for therapeutic manipulation. PCSK9 acts as a chaperone that delivers the LDL receptor to the lysosome for degradation. PCSK9 reduction promotes recycling of LDL receptors to the cell surface, boosting LDL clearance. The discovery that loss of function mutations in PCSK9 lead to lifelong low LDL-C and decreased CV risk reinforced the development of targeted therapies to inhibit PCSK9.[15] The human monoclonal antibodies evolocumab and alirocumab promote removal of PCSK9 from the circulation. This intervention decreases LDL-C levels by 60% even in statin-treated individuals. Furthermore, in two large, rigorous, RCTs (FOURIER and ODYSSEY OUTCOMES) that enrolled very high-risk patients who were already taking statins, treatment with evolocumab and alirocumab significantly reduced CV events.[27,28] The cost and need for once or twice monthly injections have, however, hampered the widespread use of PCSK9 monoclonal antibodies, despite their high efficacy and excellent tolerability.

Alternative approaches to inhibit PCSK9 either by blocking function or interfering with expression are under development. Current trials are testing reduction of PCSK9 function with small molecules, mimetic peptides, adnectin, or vaccination as well as interfering with PCSK9 expression using antisense oligonucleotides, siRNA, or genome editing with CRISPR[29] (*Figure 3*). PCSK9-specific gene silencing by siRNA with the agent inclisiran has seen rapid translation to clinical use. Such new approaches, if proven to decrease CV events in outcome trials, and if they demonstrate safety, may confer advantages over antibody therapies including improved durability, more convenient dosage regimens, and possibly cost-effectiveness.

The monoclonal antibodies directed against PCSK9 neutralize the target extracellularly, while the approaches that target gene expression act intracellularly. These mechanistic differences could have clinical consequence. Human genetic studies have hinted that germline interference with PCSK9 expression might worsen glucose tolerance, an issue not seen in FOURIER or ODYSSEY OUTCOMES.[30] This issue merits monitoring in the large-scale trials of anti-PCSK9 inhibitors that act intracellularly.

Other efforts to develop PCSK9 inhibitors that act via different mechanisms are in very early stages. Studies of permanent gene-editing have begun for two targets: PCSK9 and ANGPTL3. A recent study has shown in primates that a single infusion of CRISPR base editor delivered by nanoparticles reduces PCSK9 production in the liver and lowers LDL-C by 60% for 8 months after a single treatment.[31] The use of gene-editing methods in the clinic requires resolution of ethical and safety issues.

Inclisiran

The anti-PCSK9 siRNA agent inclisiran delivered preferentially to hepatocytes via GalNAc targeting can be injected only twice or even once a year. It reduces the hepatic synthesis of PCSK9.[32] Inclisiran has a modified nucleic acid backbone that limits its breakdown. One molecule of this siRNA can direct the degradation of multiple mRNA copies serially, producing a durable effect of 3–6 months when administered subcutaneously. When tested in a Phase 2 long-term safety and efficacy trial (ORION-1), inclisiran reduced LDL-C levels up to 53% at 6 months in subjects on the maximum dose of a statin with or without additional lipid-lowering therapy.[33] Except for predominantly mild injection-site reactions occurring in 5% of the inclisiran group, the incidence of adverse events did not differ significantly from placebo during the period of observation.

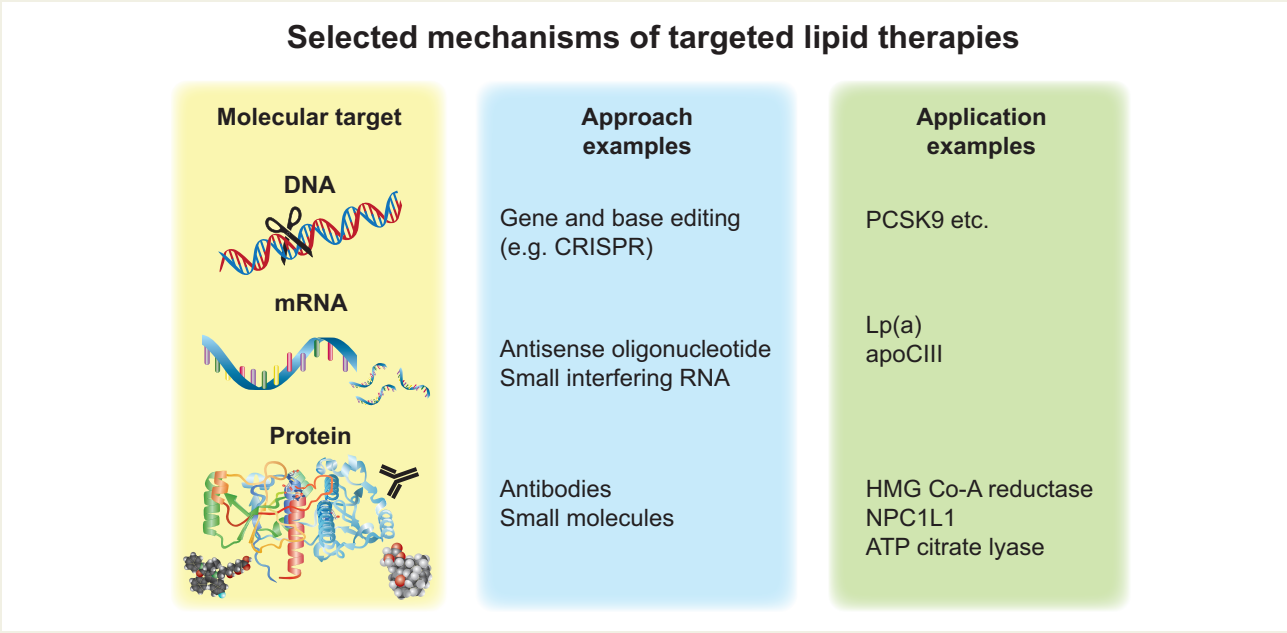

Figure 3 Selected mechanisms of targeted lipid therapies. Current approaches to interventions that modify lipid metabolism include targeting genomic DNA, messenger RNA, or proteins. The strategies available include various strategies ranging from traditional small-molecule medicinal chemistry approaches through biological agents such as monoclonal antibodies, RNA therapeutics, and, on the horizon, gene editing. ApoC-III, apolipoprotein C-III; Lp(a), lipoprotein(a); HMG-CoA, hydroxymethylglutaryl coenzyme A; PCSK9, proprotein convertase subtilisin/kexin type 9.

These findings have received support from two Phase 3 trials that enrolled patients with atherosclerotic CV disease (ORION-10) and patients with atherosclerotic CV disease or an equivalent risk (ORION-11), with elevated LDL-C levels despite maximum tolerated dose statin therapy randomized to inclisiran or placebo.[34] In these trials, inclisiran, administered subcutaneously every 6 months, reduced LDL-C by ∼ 50% and lowered non-HDL-C, apoB, triglycerides, and lipoprotein(a) [Lp(a)] significantly. The treatment did not alter liver or kidney function, creatine kinase, high-sensitivity C-reactive protein (hsCRP), or platelet count compared with placebo. Current studies with inclisiran cover different clinical settings such as homozygous familial hypercholesterolaemia, hepatic or renal impairment, ASVD, and healthy volunteers. An ongoing randomized, double-blind, placebo-controlled trial (ORION-4, NCT03705234) is examining the effects of inclisiran on CV morbidity and mortality. Recent reports show that antidotes can reverse siRNA-mediated gene silencing. Oligonucleotides that target the RNA-induced silencing complex that mediates siRNA inhibition of gene expression can inactivate this apparatus *in vivo*, thus providing an 'antidote' to the usually prolonged action of the siRNA blockade of PCSK9 expression.[35]

The siRNA attack on PCSK9 may offer prolonged duration of action, more convenient dosage regimens, and possibly cost-effectiveness if proven safe and effective in decreasing CV events. European and U.S. regulators have approved the use of inclisiran for the treatment of certain adults with hypercholesterolaemia or mixed dyslipidaemia. Because of consistent and durable LDL-C lowering, inclisiran may increase adherence to lipid-lowering therapy and help more patients to attain and ultimately maintain LDL-C goals.

Bempedoic acid

Bempedoic acid is a small molecule with a novel mechanism of action. It blocks ATP-citrate lyase (ACL), a cytosolic enzyme upstream of HMG-CoA in the pathway for *de novo* cholesterol synthesis.[36] Bempedoic acid also activates adenosine monophosphate-activated protein kinase (AMPK), which regulates phosphorylation of substrates that affect inflammatory signalling and lipid metabolism.[37] Earlier attempts to block ACL were hampered by low bioavailability and cell permeability but have been overcome with the current, once-daily oral formulation. Bempedoic acid is a prodrug that requires activity of very long-chain acyl-CoA synthetase-1 for conversion to an active modulator.[38] The liver, but not most other tissues, contains this enzyme, minimizing the exposure of the active drug to muscle and other extra-hepatic tissues. By virtue of this selective activation in hepatocytes, bempedoic acid should minimize muscle-related side effects, although it acts on the same pathway as statins. Statins can cause muscle symptoms (although much less commonly than generally perceived),[12] and very rarely can cause serious muscle issues including rhabdomyolysis, generally when combined with other agents that raise plasma statin concentrations (e.g. gemfibrozil). Observational studies report a 10–15% incidence of statin-associated muscle symptoms unlike the double-blind RCTs or blinded crossover studies that show lower levels of statin intolerance.[39] Furthermore, recent trials have demonstrated that some of the perceived muscle symptoms may result from the nocebo effect.[40]

Clinical trials have shown that bempedoic acid monotherapy or its addition to background lipid-lowering therapy significantly lowers LDL-C, non-HDL-C, apoB, and hsCRP concentrations.[41] A pooled analysis of 3623 patients with hypercholesterolaemia showed that

bempedoic acid reduced LDL-C between 17% and 25% vs. placebo depending on the background use of statins.[42] Studies have evaluated the LDL-lowering efficacy of bempedoic acid in patients with ASVD, heterozygous familial hypercholesterolaemia, and primary prevention using bempedoic acid either as monotherapy or against a background of different doses of statins and ezetimibe.

In an RCT in patients with atherosclerotic CV disease and/or heterozygous familial hypercholesterolaemia on maximally tolerated lipid-lowering therapy, compared with placebo, bempedoic acid treatment reduced LDL-C by 18%, non-HDL-C by 13.3%, apoB by 11.9%, and hsCRP by 21.5% (CLEAR Harmony).[43] In patients with a history of statin intolerance requiring additional LDL-C lowering, bempedoic acid 180 mg once daily reduced LDL-C by 23.6% and hsCRP by 25.4% when added to ezetimibe with or without additional lipid-lowering therapy (CLEAR Serenity).[44] A recent meta-analysis of 4391 patients and 11 RCTs of bempedoic acid showed that in addition to a reduction in LDL-C and hsCRP, composite CV outcomes and rates of new-onset or worsening diabetes also fell.[45] A multicenter, randomized, double-blind, placebo-controlled trial (CLEAR Outcomes) is evaluating whether bempedoic acid (180 mg daily) can reduce the risk of CV morbidity and mortality in 14 014 patients with statin intolerance.[46]

That bempedoic acid and ezetimibe lower LDL-C by different mechanisms provides a compelling rationale for their combination. In a Phase 3 double-blind trial, a bempedoic acid/ezetimibe fixed-dose combination significantly lowered LDL-C by 38% vs. placebo with a favourable safety profile.[47] A recent small, randomized, double-blind, Phase 2 study evaluated LDL-C lowering with the combination of bempedoic acid, ezetimibe, and atorvastatin (10 mg/day). This oral triple therapy lowered LDL-C levels up to 60.5% compared with placebo with good tolerability.[48] The addition of bempedoic acid to an anti-PCSK9 antibody yielded an incremental drop in LDL of over 25%.[49]

Bempedoic acid treatment had rates of myalgia comparable to placebo, as documented in a pooled analysis of four Phase 3 studies.[50] Bempedoic acid causes mild increases in blood urea nitrogen, creatinine, and uric acid and gout, effects that reverse after treatment cessation. The observed increases in creatinine and uric acid levels likely result from an effect of bempedoic acid on organic anion transporter 2, a renal transporter involved in excretion of creatinine and uric acid.[51] Rare and mild reversible reductions in haemoglobin levels have associated with bempedoic acid. The mechanism is unknown, with no qualitative changes in red blood cells and no evidence that supports plasma dilution as a potential cause.[50]

Unlike the effects of statins on glycaemia, new-onset diabetes and hyperglycaemia occurred less frequently with bempedoic acid compared with placebo.[50] This finding may relate to stimulation of AMPK by bempedoic acid which leads to reduction in gluconeogenesis. AMPK activation also inhibits fatty acid synthesis, an effect not shared by statins.[36]

The US Food and Drug Administration (FDA) approved bempedoic acid and the bempedoic acid/ezetimibe fixed-dose combination for the treatment of hypercholesterolaemia in adults with established CV disease or familial hypercholesterolaemia requiring additional LDL lowering despite maximally tolerated statin therapy. The European Medicines Agency has authorized its use for the treatment of hypercholesterolaemia and mixed dyslipidaemia. These new agents will complement existing lipid-modifying therapy regimens and will facilitate personalized treatment.

Is high-density lipoprotein modulation to mitigate cardiovascular events a lost cause?

Decades of observational epidemiologic data have documented a highly reproducible inverse relationship between HDL-C concentrations and CV outcomes.[52] The heritable components of HDL concentrations depend primarily on small effect variants than on the rarer large effect mutations.[53] Extensive pre-clinical literature documents the effects of HDL and its constituents that could mitigate CV risk. The beneficial effects ascribed to HDL include mediating an efflux of cholesterol from foam cells (reverse cholesterol transport), delivering it to hepatocytes via binding to scavenger receptor B1, endothelial protection, anti-oxidant actions, and combatting inflammation.[54–57] Cholesterol derived from HDL can undergo conversion to bile acids by the hepatocyte and be eliminated in the faeces. Despite the plausibility of the reverse cholesterol transport hypothesis, and the consistent association of low HDL concentrations with poorer prognosis, accumulating human data suggest that high levels of HDL may actually worsen outcomes. Indeed, the relationship of HDL to mortality appears U-shaped, and higher strata of HDL may associate with increased risk of non-CV diseases including infections.[58]

At odds with the abundant observational epidemiologic literature and expansive experimental work, attempts to modulate atherosclerotic risk by raising HDL have met with considerable frustration.[59] Nicotinic acid raises HDL and produces marked HDL raising and LDL lowering when combined with statins. Yet two well-conducted large-scale clinical trials, HPS2-THRIVE and AIM-HIGH, showed no clinical benefit.[60,61] Indeed, in HPS2-THRIVE, the combination of nicotinic acid extended release and a prostaglandin D receptor inhibitor, laropiprant, not only failed to improve CV outcomes, but actually produced a number of unwanted actions.[60]

The inhibition of cholesterol ester transfer protein (CETP) raised great hopes that the robust elevation in HDL that they produce would improve CV outcomes. Yet several rigorous and well-powered studies revealed no clinical benefit of several CETP inhibitors. Indeed, torcetrapib caused hazard including increased mortality that led to premature termination of its Phase 3 trial.[62] An outcome study with dalcetrapib yielded null results.[63] A trial with evacetrapib halted prematurely for futility.[64] The REVEAL trial with anacetrapib showed a very modest clinical benefit with prolonged follow-up, but this effect likely arose from a reduction in LDL rather an increase in HDL.[65] The concept that high concentrations of HDL might deliver rather than remove free cholesterol to macrophages provides one potential contributor to the lack of benefit generally shown by CETP inhibitors.[66] Qualitative alterations in HDL particles not captured in HDL-C measurements might also contribute to the disappointing results of trials of CETP inhibitors. Administration of various preparations of apolipoprotein A reconstituted in phospholipid particles likewise have not shown clinical benefit. A trial investigating this approach in individuals within 90 days of an acute coronary syndrome (AEGIS-II) is currently underway.[67] In sum, multiple attempts to improve CV outcomes by raising HDL pharmacologically by several mechanisms have so far yielded consistent disappointment.

Some contemporary human genetic studies have cast doubt on the ability of HDL to limit CV risk. Mendelian randomization analyses

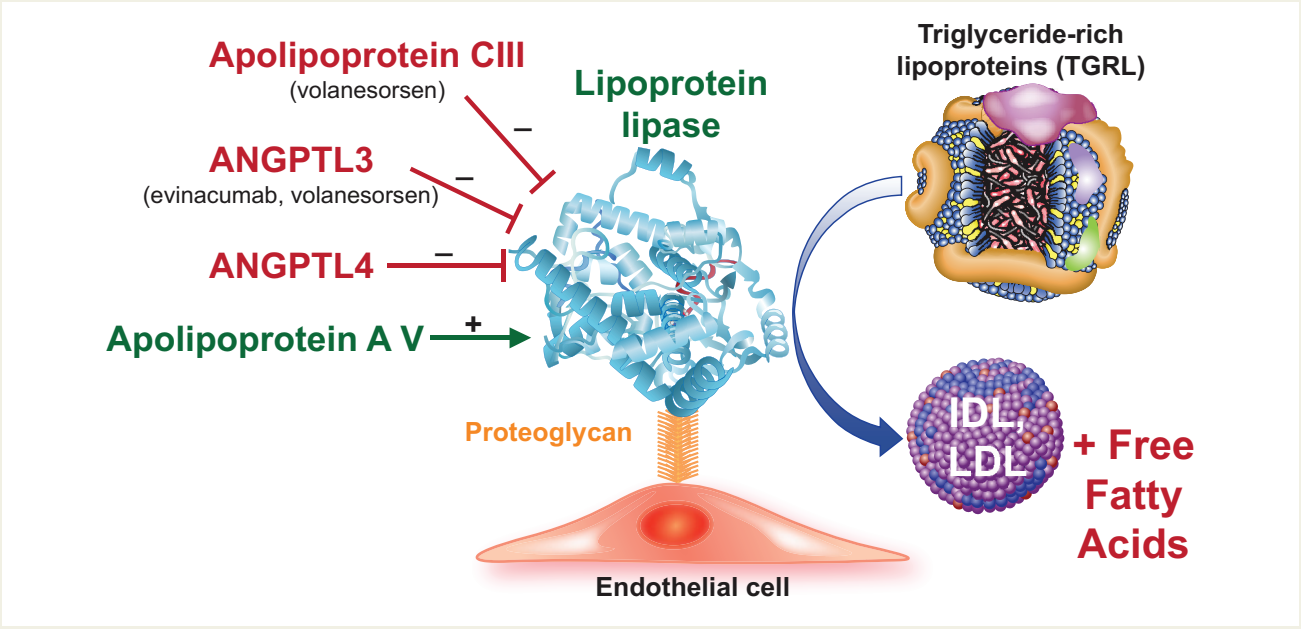

Figure 4 Lipoprotein lipase modifiers. The enzyme lipoprotein lipase (depicted by the ribbon structure) associates with the surface of endothelial cells by binding to proteoglycans. This enzyme trims triglyceride from triglyceride-rich lipoproteins which include remnants of chylomicrons produced by intestinal cells from dietary lipid and very low-density lipoproteins synthesized endogenously by the liver. Lipoprotein lipase-mediated hydrolysis yields free fatty acids and low-density lipoprotein and intermediate-density lipoproteins. The proteins named in red inhibit lipoprotein lipase, and thus raise blood triglyceride-rich lipoprotein concentrations by limiting triglyceride-rich lipoprotein catabolism. The novel therapeutic agents listed inhibit these inhibitors and thus lower triglyceride-rich lipoprotein levels. Apolipoprotein AV activates lipoprotein lipase (shown in green.) Very strong human genetic evidence support the causality of each of the modulatory proteins depicted in regulating triglyceride-rich lipoproteins. ANGPTL, angiopoietin-like protein.

have not shown significant benefits of increased HDL due to inheritance of various variants that raise its concentration.[8] More recent Mendelian randomization analyses, taking pleiotropy into account, have provided evidence for some benefit from lifelong genetically-elevated increases in HDL-C concentration.[68]

Given the complexity of the HDL particle family and the mutable proteome associated with HDL particles, a simple measurement of HDL-C may obscure the potential benefits of particular classes of HDL or particles endowed with particular protein constituents.[69,70] The exploration of therapeutic manipulation to augment the concentrations of particles with beneficial properties remains under investigation. Thus, while HDL has proven to be a frustrating therapeutic target thus far, some experts believe that exploiting the detailed knowledge of the proposed beneficial effects of HDL may yet provide a useful therapeutic avenue.[66]

Triglyceride-rich lipoproteins ascendant as an anti-atherosclerotic target

Triglyceride measurement in clinical laboratories serves a biomarker for a family of triglyceride-rich lipoproteins that include remnant lipoproteins, among them chylomicron remnants, VLDL, and IDL. The atherogenicity of these particles likely resides in their ability to deliver cholesterol to contribute to foam cell formation rather than on their triglyceride content.[71–73] Remnant particles provoke inflammation, as gauged by hsCRP measurements, more potently than LDL itself, another property that may promote atherosclerotic events.[74] In an apparently low-risk population enrolled in the PESA study, higher quantiles of triglycerides associated with augmented ^{18}F-fluorodeoxyglucose uptake in several arterial beds, supporting triglycerides' pro-inflammatory potential.[75]

Triglyceride concentrations in a population tend to vary inversely with HDL levels. Given the convincing observational epidemiologic evidence supporting a protective role of HDL, traditionally investigations of the contribution of triglycerides to CV risk have adjusted this lipoprotein fraction for HDL. This approach greatly attenuated the relation of triglyceride-rich lipoproteins with CV outcomes and led to relegating triglyceride-rich lipoproteins to a lower echelon as a causal CV risk factor. This situation underwent reassessment as evidence accumulated that raising HDL-C by several pharmacologic maneuvers does not improve CV outcomes, as detailed above.[76] Triglyceride concentrations tracked very well with CV events and in long-term follow-up, with CV mortality.[75,77]

Human genetic studies have also renewed interest in the causal role of triglyceride-rich lipoproteins in atherosclerotic events.[78–80] Triglyceride concentrations depend in large part on the activity of the enzyme lipoprotein lipase (LPL) that associates with the surface of microvascular endothelial cells (*Figure 4*). This enzyme releases free fatty acids from the triglycerides, reducing triglyceride

concentrations. Genetic variants that lower LPL activity raise triglyceride-rich lipoprotein particle concentrations. Human genetic studies have shown a consistent increase in CV events in individuals who have inherited variants that boost the action of inhibitors of LPL including apolipoprotein C-III, and ANGPTL3 and 4.[78,79,81] On the other hand, individuals with augmented activity of apolipoprotein A V, which increases LPL activity, have reduced CV risk.[82] Mutations in LPL itself that impair its function also raise CV risk.[80]

The reassessment of the causal role of triglyceride-rich lipoproteins in atherothrombosis has spurred the development of interventions that can reduce their concentration. Agents that reduce apolipoprotein C-III and inhibit ANGPTL3 and 4 are currently undergoing clinical evaluation for event reduction (*Figure 4*). These agents include vupanorsen, an antisense oligonucleotide (targeted to hepatocytes by a GalNAc moiety) that inhibits production of ANGPTL3.[83] Evinacumab, a monoclonal antibody, neutralizes ANGPTL3.[21] The LDL-lowering effects of ANGPTL3 do not depend on the LDL receptor but rather on VLDL/remnant receptors. In patients with homozygous familial hypercholesterolaemia who lack functioning LDL receptors, and thus respond poorly to agents that depend on raising LDL receptor levels, evinacumab reduced LDL-C by 49% in a Phase 3 trial.[84] This result led to the FDA approval of evinacumab as an add-on treatment for adult and paediatric patients aged 12 and above with homozygous familial hypercholesterolaemia. Volanesorsen, an antisense oligonucleotide, targets apolipoprotein C-III, an inhibitor of LPL that elevates triglyceride-rich lipoproteins and may also exert independent proinflammatory effects. In patients with hyperchylomicronaemia, volanesorsen lowered triglycerides by over 70%, but caused injection-site reactions in almost a quarter of patients.[85] A new formulation of antisense oligonucleotide with GalNAc-mediated targeting to hepatocytes limits unwanted actions such as injection-site reactions and thrombocytopaenia seen with earlier generations of RNA therapeutics.[86,87]

Fibric acid derivatives can also raise HDL and lower triglycerides. But fenofibrate has not demonstrated CV benefit in statin-treated patients. Fibric acid derivatives act by stimulating peroxisome proliferator-activated receptor-alpha (PPAR-α). A novel selective PPAR-α modulator, pemafibrate, lowers triglycerides, apolipoprotein C-III, and is currently under investigation in a large-scale clinical outcomes trial, PROMINENT.[88,89] This trial, as opposed to most previous studies with PPAR-α stimulators, will target individuals with elevated baseline levels of triglycerides (>200 mg/dL). PROMINENT includes diabetic individuals both with or without established coronary artery disease.[90]

The increasing recognition of the causality of triglyceride-rich lipoproteins in atherosclerosis supports the recommendation in some guidelines to consider non-HDL-C a secondary target of therapy. In addition to LDL particles, the non-HDL compartment contains remnant cholesterol, now considered atherogenic. ApoB measurements capture all the atherogenic lipoprotein classes and correlate very well with non-HDL-C in a population, but may not be readily available in all practice settings.

Usual clinical practice seldom requires advanced lipid testing such as nuclear magnetic resonance assays, gradient gel electrophoresis, or ultracentrifugation, although their use in selected patients may be appropriate in specialized lipidology clinics.

Lipoprotein(a), a causal risk factor for atherosclerotic events and aortic valve disease: new therapies on the horizon

Lipoprotein(a) denotes a special form of LDL to which an apoprotein known as Apo(a) (unrelated to apolipoprotein A) has bound covalently to the signature protein apoB that encircles the LDL particle (*Figure 1*). Multiple observational epidemiologic studies showed an association of elevated Lp(a) with increased CV risk. Human genetic studies including GWAS and Mendelian randomization investigations have established the causality of Lp(a) not only in atherosclerotic CV disease but also in calcific aortic valve disease.[91–94]

The prevalence of elevated Lp(a) does not follow a bell-shaped Gaussian curve, but rather a skewed distribution. Most individuals have normal levels but there is a long tail of individuals with higher concentrations of Lp(a). Heredity strongly influences Lp(a) concentrations and populations of different ethnicities have distinct distributions and concentrations of Lp(a). African Americans have higher mean Lp(a) concentrations and a bell-shaped distribution.[95]

Measurement and reporting of Lp(a) concentrations has proven daunting. Apo(a) varies in structure depending on inherited genotype. In particular, a looped motif in the secondary structure of Apo(a), known as a kringle (based on its shapes' resemblance to the eponymous Danish pastry), can vary in number quite widely in individuals due to different repeats of the fourth of these kringle domains. Those with fewer kringle IV repeats have higher Apo(a) blood concentrations. The variable structure from person-to-person in Lp(a) has rendered immunoassays very confusing. As the molecular weight of different versions of Lp(a) varies depending on the length of the Apo(a), reporting the concentrations as mass (mg/dL) can be misleading, prompting the current recommendation to report concentrations of Lp(a) in millimoles, obviating the differences in molecular weight. Yet, many clinical laboratories still report in mg/dL. As LDL or apoB measurements incorporate Lp(a), what we read in laboratory reports as LDL includes Lp(a). Various formulas are available for adjusting the LDL concentrations for the fraction contributed by Lp(a). As a person's Lp(a) changes little over time, some current guidelines suggest, and we advocate, measurement of Lp(a) one time in all individuals. Certainly, those with unexplained premature atherosclerotic events, coincident calcific aortic stenosis, those with a family history of elevated Lp(a) or premature CV disease, and patients with suboptimal LDL-C-lowering response to statins merit assessment of Lp(a) concentrations.

Unfortunately, Lp(a) has proven a difficult therapeutic target.[96] The usual panel of pharmacologic agents that lower LDL have little or no effect on Lp(a), with the exception of anti-PCSK9 antibodies that modestly reduce Lp(a). Recent advances in RNA technology have led to the development of hepatocyte-targeted antisense oligonucleotides or siRNA agents that can lower Lp(a) concentrations strikingly.[93,97] The anti-sense oligonucleotide pelacarsen lowers Lp(a) independent of isoform size or genetic variant.[98] An siRNA agent, olpasiran, likewise can decrease Lp(a). Current clinical trials with these novel therapeutics provide optimism that we will have in hand effective therapeutics for this causal CV risk factor. Given the ageing

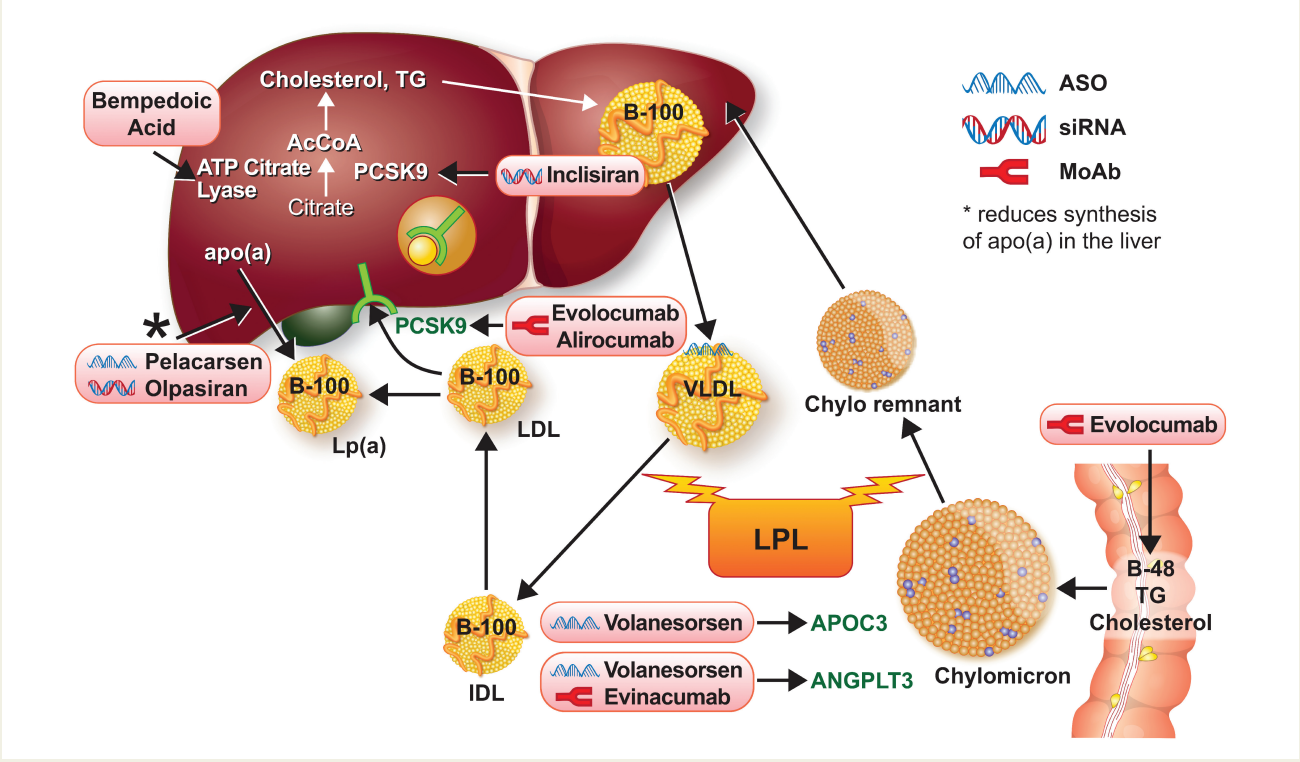

Figure 5 Newer and emerging lipid-lowering therapies target different aspects of lipid metabolism. The statins target hydroxymethylglutaryl coenzyme A reductase. The newer and emerging agents target other aspects of lipid metabolism as shown here. B48 refers to the shorter form of apolipoprotein B produced by RNA editing in the intestine. B100 refers to the longer form produced in the liver. See the list for explanations of other abbreviations.

of the population and the concomitant increase in calcific aortic valve disease, exploring the ability of Lp(a) lowering to prevent or slow the progression of aortic sclerosis/stenosis in those with Lp(a) elevations likewise merits testing.

Shining a new light on omega-3 fatty acids: recent learnings

Since pioneering observational studies in populations with high fish consumption showing associations with reduced CV risk, interest has focused on the hypothesis that marine fatty acids, notably the omega-3 polyunsaturated fatty acids, might mitigate CV risk. Some intervention studies suggested that high consumption of omega-3 fatty acids, either as fish or supplements, could reduce sudden cardiac death, presumably due to limiting lethal ventricular arrhythmias. The GISSI–Prevenzione study suggested a slight benefit of omega-3 fatty acid supplementation on heart failure outcomes.[99] Yet, multiple randomized placebo-controlled clinical trials failed to show a consistent decrease in events in individuals allocated to various omega-3 fatty acid preparations.[100] The non-blinded JELIS study in Japanese individuals, which used a higher dose than most other studies [1.5 g/day of purified eicosapentaenoic acid (EPA)], did show a significant reduction in the primary endpoint.[101]

Recent large-scale trials have yielded mixed results. VITAL, which used 1 g/day of a mixture of EPA and docosahexaenoic acid (DHA), did not meet its primary endpoint.[102] The ASCEND study of diabetic people without known CV disease tested a similar EPA + DHA mixture (1 g/day) and also yielded a null result.[103] STRENGTH used a higher dose (4 g/day) of a mixture of EPA and DHA as free fatty acids, but halted prematurely for futility.[104]

REDUCE-IT, however, reported a striking reduction in first and total CV events in a study of some 20 000 individuals randomly allocated to 4 g/day of purified EPA as ethyl esters (icosapent ethyl).[105] The treatment was well tolerated save for a small but significant signal for atrial fibrillation. The placebo used in ASCEND was olive oil, STRENGTH used corn oil, while REDUCE-IT used mineral oil. The choice of comparators in these studies has generated considerable controversy. In some but not all studies, mineral oil placebo has led to small increases in LDL-C, triglycerides, and hsCRP but did not increase plaque progression by computed tomography angiography .[106]

The difference in outcomes in these two superbly designed and conducted studies with disparate results could be due to differences in the omega-3 fatty acid preparation employed or possibly differences in the placebo. EPA and DHA have differential effects on endothelial function, cellular membranes, inflammation, and LDL-C. EPA and DHA differentially modulate membrane elasticity in the presence of cholesterol.[107] Potential detrimental effects of DHA could explain

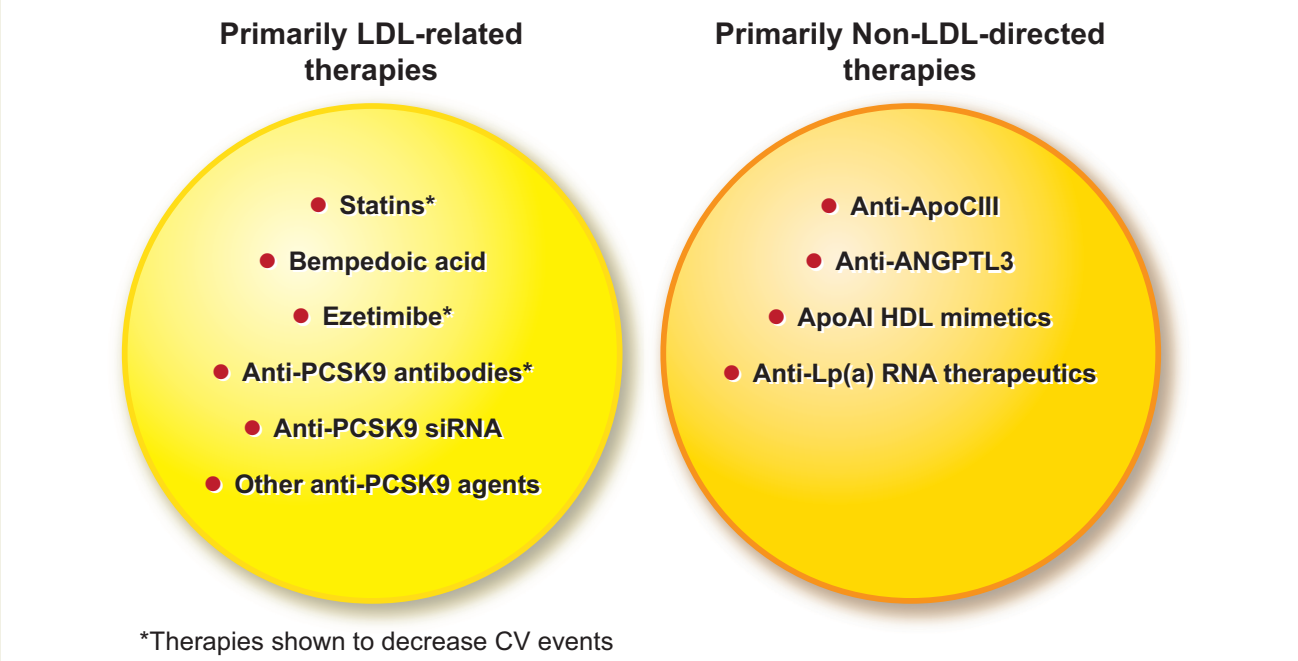

Figure 6 Current and emerging therapies not only deepen our ability to manage low-density lipoprotein, but to target other aspects of lipid risk factors. See text for explanation.

the discrepant results, although increases in DHA levels were modest and did not correlate with events in STRENGTH.[104,108]

Considerable confusion currently reigns regarding the CV effects of omega-3 fatty acids given the disparate results from REDUCE-IT and STRENGTH. Each of these rigorous studies enrolled individuals with similar elevated estimates of CV risk. The US FDA has discounted the mineral oil vs. corn oil comparator issue as a major contributor to the differences between these two studies.[109] The plethora of null trials with omega-3 fatty acids have used mixtures that used lower doses or that contain varying amounts of EPA and DHA, and in the case of many non-prescription products, undefined other components that may include saturated fat and oxidized lipids.[100] The two trials that have shown positive effects on reducing CV events have used purified EPA in higher dose (REDUCE-IT and JELIS). While the achieved EPA concentrations did not correlate with events in an analysis of STRENGTH,[110] they did so in REDUCE-IT. The median achieved levels of EPA in REDUCE-IT (169 μg/mL) were much higher than those in STRENGTH (90 μg/mL) that used the mixture of EPA with DHA. While this controversial area remains unsettled, the preponderance of the data indicates that purified prescription-grade EPA, as ethyl esters, exerts a beneficial effect, while mixtures of omega-3 fatty acids do not. DHA could counter some of the beneficial effects of EPA. That said, DHA is enriched in the retina and central nervous system and may play important protective roles there.

In both REDUCE-IT and STRENGTH, atrial fibrillation increased in the groups treated with omega-3 fatty acids. In REDUCE-IT, much of the atrial fibrillation documented was recurrent rather than new. Yet, in REDUCE-IT, there was no increase in ischaemic strokes. The net benefit on primary and total events in REDUCE-IT suggest an overall protective effect in the study population of high-dose prescription EPA despite the significant increase in atrial fibrillation. While these issues merit further discussion and investigation, icosapent ethyl has received approval in many jurisdictions for reducing CV events in individuals who meet the entry criteria for REDUCE-IT. A further analysis from this trial showed that the benefits of icosapent ethyl increased along with the risk level. The absolute risk reduction was 4% in patients with diabetes without CV disease, and 6% in patients with CV disease without diabetes. The highest benefit with a 10% absolute risk reduction was seen in patients with diabetes and established CV disease.[111]

Although some of the benefit observed in REDUCE-IT might have accrued due to a reduction in triglyceride-rich lipoproteins, there was no heterogeneity in event reduction in individuals with different tertiles of baseline triglyceride concentrations. While the mechanisms of the benefit observed in REDUCE-IT remain uncertain and are probably multiple, the success of REDUCE-IT provides us with a new tool for CV risk reduction, and should stimulate further exploration of icosapent ethyl as CV therapeutics.[112]

New tools permit individual tailoring of therapies

We are entering a new era in lipid lowering (*Figure 5*). Efforts to personalize therapy and target the right patient at the right time include further refinement of risk stratification tools including genetic risk scores and the integration of imaging studies to management decisions.[113,114] Lifestyle measures include a healthy diet, weight control, and incorporation of physical activity into daily life to the fullest

extent possible. Unfortunately, such behaviours alone often do not suffice to achieve control of lipid risk. Many of our patients have physical or financial limitations that deter them from achieving an ideal cardioprotective lifestyle.

Fortunately, we have in hand or close at hand a flourishing armamentarium of lipid-lowering therapies that target new pathways and causal lipoproteins beyond LDL (*Figure 6*). While statins remain the first choice for lipid lowering, the availability of complementary therapies allow for individual tailoring according to the needs of the patient if the CV outcome trials with the novel therapies yield favourable results. Implementation of evidence-based lipid management remains inconsistent, requiring education of both physicians and patients as well as consideration of nuanced behavioural interventions. As a community, we face the additional challenge of achieving equitable distribution of and access to the proven and novel therapies to address dyslipidaemias for all segments of society to confront the continued and growing and now global epidemic of atherosclerotic CV disease.

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Data availability

No new data were generated or analysed in support of this research.

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