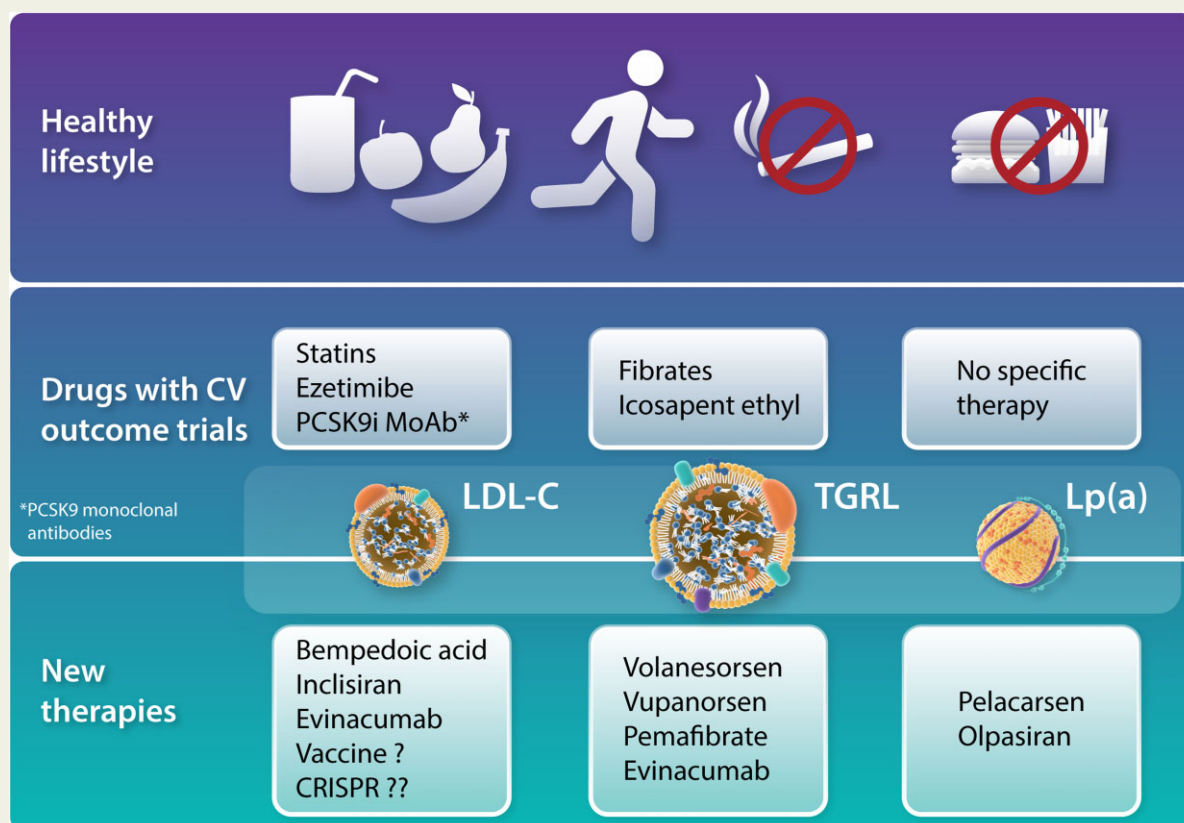


The year in cardiovascular medicine 2021: dyslipidaemia

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Graphical Abstract In addition to a healthy lifestyle, we have different pharmacologic agents to target causal lipoproteins. While statins are the first choice in pharmacotherapy, combination therapy with ezetimibe and PCSK9 monoclonal antibodies have also been shown to decrease cardiovascular outcomes in high-risk patients. Fibrates have been shown to reduce residual risk in the subgroup of patients with high triglycerides and low HDL. Several new therapies are being developed to target the causal lipoproteins by different mechanisms.

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The past year was an exciting time for clinical lipidology when we learnt more about existing therapies as well as therapies targeting novel pathways discovered through genetic studies. LDL cholesterol remained the main target and a variety of drugs to lower LDL cholesterol through different mechanisms were explored. Emerging evidence on the atherogenicity of triglyceride-rich lipoproteins led to renewed interest in lowering them with new treatments. Lp(a) was back in focus with evidence on causality and new targeted therapeutics which dramatically lower Lp(a) levels. We will be able to personalise lipid lowering therapy further with this enriched armamentarium once we have the results of the cardiovascular outcome studies with some of these new agents.

Keywords

Cardiovascular diseases • Lipids • Nucleic acid therapeutics • RNA interference • Antisense • CRISPR • LDL-C. Triglycerides • Lp(a) • Statins • PCSK9 inhibitors

Introduction

Dyslipidaemia is one of the most important causal risk factors for atherosclerotic vascular disease (ASCVD). There is substantial evidence showing that we can alter the trajectory of ASCVD by lowering LDL-C. There is also emerging evidence documenting that triglyceride-rich lipoproteins (TRLs) are atherogenic and reducing them will result in fewer cardiovascular (CV) events. Recently, the interest in lipoprotein(a) [Lp(a)] has been reignited by the evidence that it is causal for ASCVD. A healthy lifestyle is the backbone of lipid lowering but may not be adequate, especially in higher risk patients. The past few years have been very exciting for clinical lipidology with an abundance of novel targets for lipid-lowering therapy being discovered through genetic studies and nucleic acid-based therapies have been developed to suppress the expression of selected genes (*Graphical Abstract*). These developments will significantly enrich our armamentarium of lipid-lowering therapies if proven to decrease ASCVD outcomes.

LDL-C lowering

To reduce the burden of ASCVD in both the individual and the population, the European Guidelines on CV disease prevention have been updated and endorsed by 12 societies recently.¹ Dyslipidaemia management is an important part of the new prevention guidelines. While the ultimate LDL-C goals remained unchanged from the 2019 ESC/EAS dyslipidaemia guidelines,² the treatment decisions are more personalized with a stepwise approach taking the patient profile, comorbidities, and preferences into consideration.¹ Risk stratification has been improved by the new SCORE2 and SCORE OP models which are based on more contemporary data to determine the total CV risk-taking competing non-CV risk into account for elderly and adjusted for different geographical areas. The importance of a healthy lifestyle is emphasized throughout the text. Age-specific risk thresholds have been defined for apparently healthy people, and new risk modifiers have been defined. To communicate the importance of LDL-C reduction, there are charts to calculate average years free of cardiovascular disease (CVD) gained by 1 mmol/L LDL-C reduction in healthy persons.

The relevance of the intensity of LDL-C reduction has been reinforced by the results from the SWEDEHEART registry which investigated the association between LDL-C changes and statin

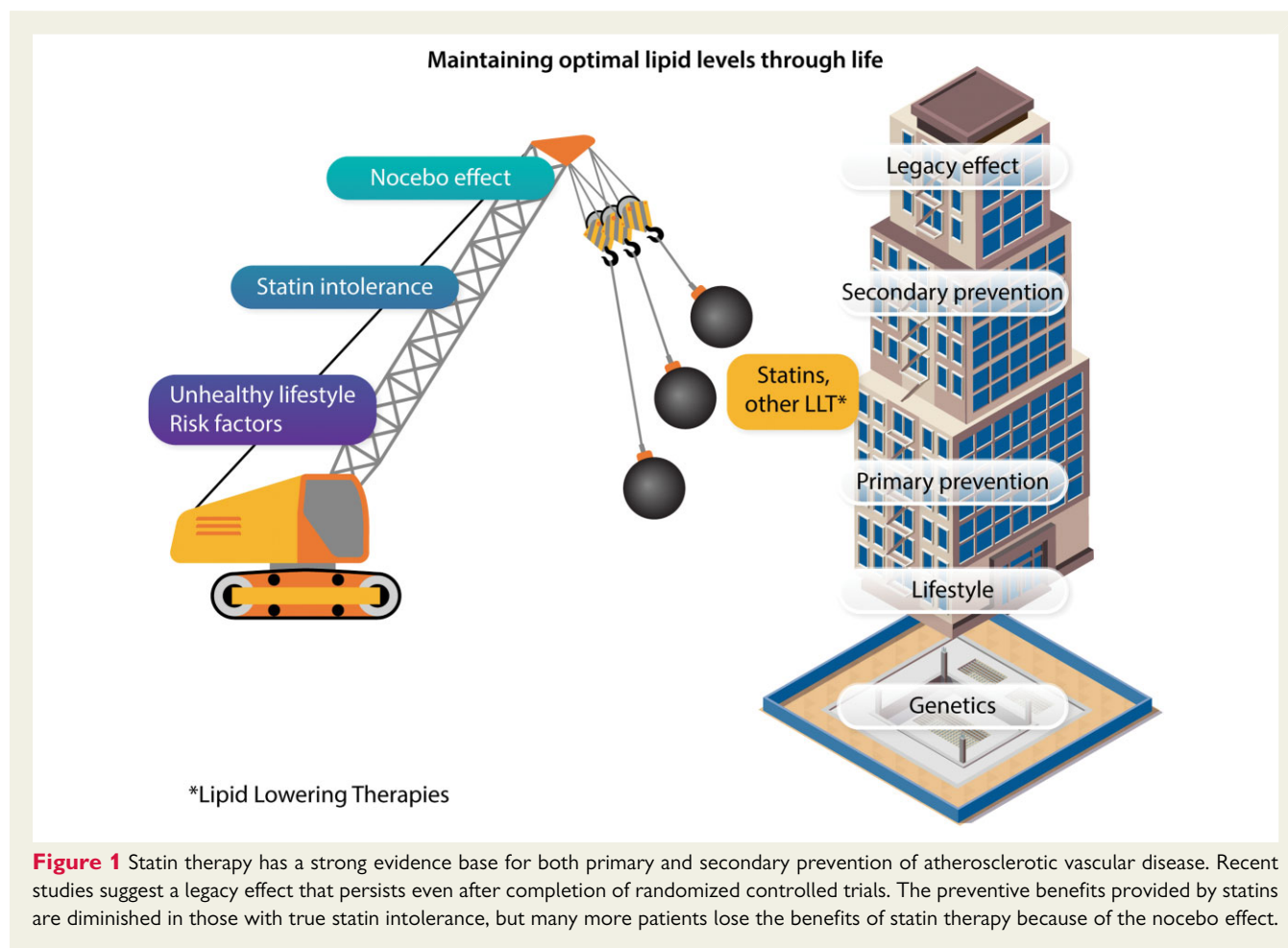
intensity with prognosis after a myocardial infarction in a real-world setting. In 40 607 patients followed for a median of 3.78 years, larger early LDL-C reduction and more intensive statin therapy after MI were associated with a reduced hazard of all CV outcomes and all-cause mortality.³

Lipid lowering is especially important for patients with familial hypercholesterolaemia who are at high risk for ASCVD, but FH is universally underdiagnosed and undertreated as documented by a world-wide registry. The European Atherosclerosis Society Familial Hypercholesterolemia Studies Collaboration global registry reported on 61 612 individuals in 56 countries. This registry demonstrated that most patients were diagnosed late; the median age at diagnosis of familial hypercholesterolaemia was 44.4 years. The median LDL cholesterol was 5.43 mmol/L among patients not taking lipid-lowering medications and 4.23 mmol/L among those taking them. Guideline-recommended LDL cholesterol concentrations were infrequently achieved with single-drug therapy requiring greater use of combination therapies and earlier diagnosis to reduce the global burden of familial hypercholesterolaemia.⁴

Statins

The Heart Outcomes Evaluation Prevention (HOPE)-3 study showed that fixed-dose treatment with low-dose statin therapy, but not blood pressure lowering agents, is superior to placebo in reducing long-term CV events in an intermediate-risk population.⁵ The existence of a legacy effect of the statin and anti-hypertensive therapy given to the HOPE-3 population was tested in a recent 3.1-year follow-up of the original population. During this extended follow-up phase, the subjects originally randomized to rosuvastatin had an additional ~20% relative risk reduction in MACE compared with placebo which continued over the entire 8.7 years of follow-up, suggesting a legacy effect of rosuvastatin therapy which was not seen with anti-hypertensive therapy.

The well-known discrepancy between the absence or low incidence of SAMS reported in randomized controlled trials and observational studies⁶ has prompted the development of trials to define those who report similar symptoms on statins and placebo (*Figure 1*). In 200 individuals who were either considering statin discontinuation or had stopped statin therapy during the previous 3 years because of muscle symptoms, the response to atorvastatin 20 mg daily was compared with placebo over six 2-month



randomly assigned treatment periods. There were no differences in the mean muscle symptom scores between the statin and placebo periods (mean difference statin minus placebo -0.11). At a clinician–patient discussion at the end of the trial, 88% said that the trial had been helpful in their decision-making about whether to restart a statin, and 66% said that they had already or intended to resume taking statins.⁷

Another trial enrolled 60 patients who had previously discontinued statins because of side effects reported within 2 weeks of starting the medication. They entered a double-blind 3-group N-of-1 trial to determine whether symptoms would be induced by atorvastatin 20 mg daily or placebo. Each patient received four bottles containing atorvastatin 20 mg, placebo, and empty bottles, and they were asked to use the content of each bottle for a 1-month period and to use a smartphone app to report symptom intensity ranging from 0 to 100. Among the total group of 60 participants, the mean symptom intensity was 8.0 during no-tablet months, 15.4 during placebo months compared with no-tablet months, and 16.3 during atorvastatin administration compared with no-tablet months. Six months after completion of the trial, 50% had restarted statin therapy and among those who had stopped taking a statin because of side effects, 90% of reported symptoms induced by statin therapy were also induced by the placebo.⁸

The association between statins and adverse events in primary prevention was examined in a systematic review with pairwise, network, and dose–response meta-analyses in 62 trials including a total of 120 456 participants with an average follow-up of 3.9 years. Statin therapy was associated with a mildly increased risk of self-reported muscle symptoms (odds ratio: 1.06) but not with clinically confirmed muscle disorders. Their efficacy in primary prevention outweighed the risk of adverse reported or observed adverse effects. The authors also found no consistent dose–response relationship between different types of statins and the incidence of adverse effects.⁹

Bempedoic acid

Bempedoic acid, a prodrug that acts in the cholesterol biosynthetic pathway, is activated by very-long chain acyl-CoA synthetase-1, an enzyme that is not present in skeletal muscle. It was shown in a double-blind placebo-controlled RCT of 345 adults with hypercholesterolaemia and a history of intolerance of at least two statins to lower LDL-C by a median of 21.4% when compared with placebo and was associated with a favourable safety profile and no increase in reported muscle symptoms.¹⁰ A double-blind clinical trial employing a fixed dose combination of bempedoic acid

180 mg and ezetimibe 10 mg given once daily to high-risk patients showed that this drug lowered LDL-C by a placebo-corrected mean difference of 38% and had similar LDL-C lowering in all subgroups, regardless of the intensity of statin therapy or no statin therapy and had a favourable safety profile.¹¹

In a recent Phase 2, randomized, double-blind, placebo-controlled study, patients were randomized to triple therapy (bempedoic acid 180 mg, ezetimibe 10 mg, and atorvastatin 20 mg) or placebo once daily for 6 weeks. With triple therapy, LDL-C was lowered by 63.6%.¹²

PCSK9 monoclonal antibodies

A 2019 systematic review and meta-analysis of 39 RCTs that included 66 478 patients, of whom 35 938 were treated with the PCSK9 inhibitors, evolocumab, and alirocumab, for a mean follow-up of 2.3 years, showed that the use of these drugs was significantly associated with a lower risk of MI (1.49 vs. 1.93 per 100 patient-years; RR: 0.80), ischaemic stroke (0.44 vs. 0.58 per 100 patient-years; RR: 0.78), and coronary revascularization (2.16 vs. 2.64 per 100 patient-years; RR: 0.83), but not all-cause death or CV death.¹³ The drugs were well tolerated without evidence of an increased risk for adverse effects. The absence of a significant impact on CV death or mortality is likely related to the relatively short follow-up period and the time lag in the effect of LDL-C lowering on these parameters.¹⁴

A pre-specified analysis from the FOURIER trial examined the differential impact of therapy with evolocumab vs. placebo on the incidence of initial and subsequent events in this very high-risk population. The authors reported that evolocumab reduced the incidence of total primary endpoint events by 18% (incidence rate ratio: 0.82), including both first events [hazard ratio (HR): 0.85] and subsequent events (RR: 0.74).¹⁵

Another recent *post hoc* analysis of the FOURIER trial examined the impact of evolocumab therapy on the incidence of first and total acute arterial events. The authors reported 19% fewer acute vascular events (HR: 0.81), including a 17% reduction in first acute coronary events (HR: 0.83), a 23% reduction in first acute cerebrovascular events (HR: 0.77), a 42% reduction in acute peripheral vascular events (HR: 0.58), and a 24% reduction in total first events plus total acute events (incidence event ratio: 0.76). There was a greater magnitude of event reduction over time, with a 16% reduction in the first year, a 24% reduction during the remainder of the study.¹⁶

A recent *post hoc* subgroup analysis from ODYSSEY-OUTCOMES trial questioned which post-ACS patients taking maximally tolerated statin could benefit from the addition of a PCSK9 inhibitor. MACE occurred in 4.2 vs. 3.1 per 100 patient-years among placebo-treated patients with baseline Lp(a) greater than vs. less than or equal to the median Lp(a) value. Among the participants in the higher LDL-C subgroup, MACE occurred 4.7 vs. 3.8 per 100 patient-years among placebo-treated patients with Lp(a) greater than vs. less than the median Lp(a) value, but among those receiving alirocumab, the adjusted treatment HRs were 0.82 and 0.89. The authors concluded that the level of Lp(a) could be used to identify

those post-ACS patients on maximally tolerated statins more likely to benefit from alirocumab therapy.¹⁷

In another subgroup analysis of the ODYSSEY-OUTCOMES, patients receiving alirocumab were classified in pre-specified strata of LDL-C achieved at 4 months of treatment: <25, 25–50, or >50 mg/dL. Treatment HR and absolute risk reduction were similar to those with achieved LDL-C <25 mg/dL (HR: 0.74; absolute risk reduction: 0.92) or 25–50 mg/dL (HR: 0.74; absolute risk reduction: 1.05). The authors concluded that those with achieved LDL-C <25 had a similar reduction in MACE risk to those with achieved levels of 25–50 mg/dL.¹⁸

PCSK9 inhibitors also affect platelet activation and thrombosis. Studies employing a mouse model showed that PCSK9 enhances platelet activation and *in vivo* arterial thrombosis by binding to platelet glycoprotein CD36, resulting in activation of downstream signalling pathways that result in microvascular obstruction and myocardial infarction expansion. These effects were shown to be ameliorated by the PCSK9 inhibitor, evolocumab.¹⁹

The effect of PCSK9 inhibitor therapy on the incidence of deep vein thrombosis and pulmonary embolism (VTE) was evaluated in a meta-analysis of the FOURIER and the ODYSSEY-OUTCOMES trials demonstrating a 31% relative risk reduction in VTE with PCSK9 inhibition compared with placebo (HR: 0.69). When comparing the efficacy of evolocumab to prevent VTE in patients stratified by baseline Lp(a) above and below the median of 37 nmol/L, in the group with Lp(a) levels above the median, evolocumab reduced Lp(a) by 33 nmol/L and VTE risk by 48% (HR: 0.52). Neither of the two large PCSK9 inhibitor trials alone independently demonstrated a statistically significant reduction in VTE events.²⁰

Further evidence of safety of the PCSK9 inhibitors came from a 23-item survey on memory and executive domains done on 22 655 participants of the FOURIER trial provided additional confirmation of the results of the previously performed EBBINGHAUS trial,²¹ showing that evolocumab did not alter cognitive performance when compared with placebo, even in those with extremely low LDL-C levels (<0.5 mmol/L).²² The absence of adverse effects on cognition was further confirmed in a prospective RCT of alirocumab 75 or 150 mg every 2 weeks vs. placebo administered to 2176 patients. The patients were evaluated every 24 weeks for 96 weeks using the Cambridge Neuropsychological Test Automated Battery, and the study showed that alirocumab had no effect on neurocognitive function during the treatment period.²³

Triglyceride-rich lipoproteins

In the past years, there has been increasing interest in the role of TGRLs and their remnants in the development of ASCVD. A consensus statement from the European Atherosclerosis Society has updated the working definition of normo- and hypertriglyceridemic states, as well as a pathophysiologic framework for the generation of excess remnants due to dysregulation of production, lipolysis, and remodelling of TGRL, and defects in the clearance of remnant lipoproteins.²⁴ The potential atherogenicity of TGRL and remnants is examined, as well as therapeutic approaches that have been available for many years or so are presently in

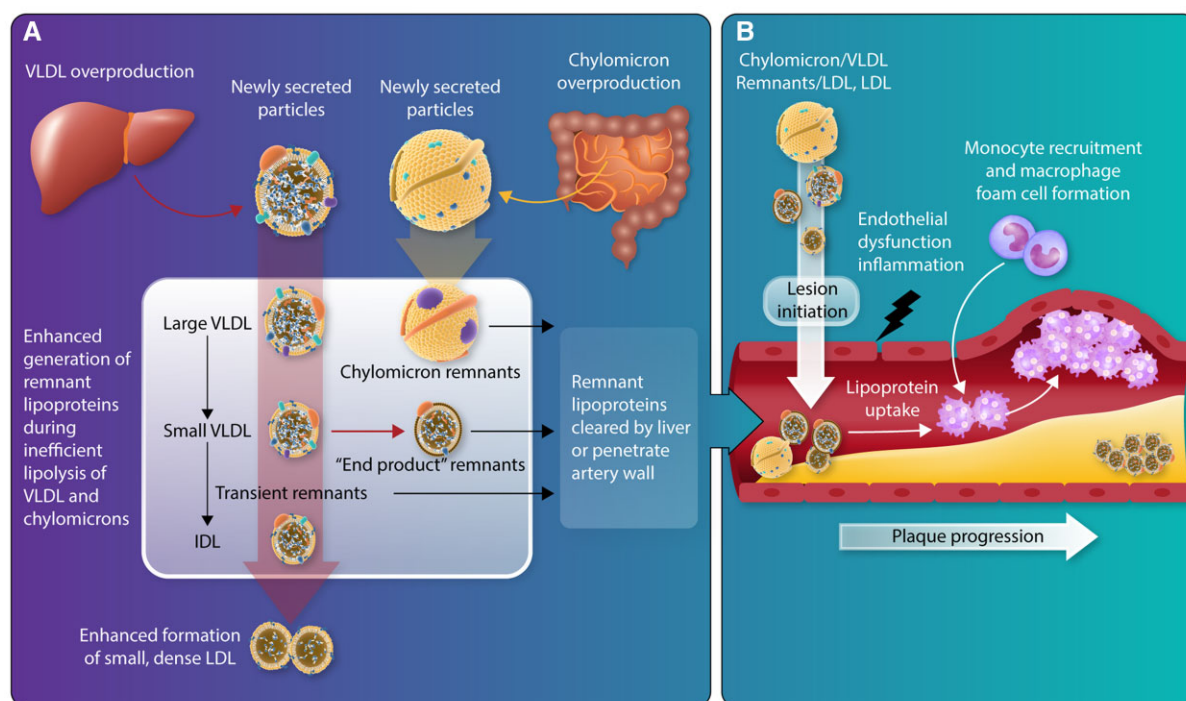


Figure 2 (A) Recent evidence supports a causal association between triglycerides, triglyceride-rich lipoproteins, and triglyceride-rich lipoprotein remnants with cardiovascular events. Overproduction and inefficient lipolysis of both very low-density lipoprotein and chylomicrons lead to increased remnant formation. (B) Triglyceride-rich lipoprotein remnants contribute to the initiation and progression of atherosclerotic lesions. Reproduced with permission from Ginsberg *et al.*²⁴

development (Figure 2). The statement provides a wealth of information regarding the various approaches that have been developed to 'measure' remnants, concluding that we are in great need of both more precise methods and more accurate assays.

In a recent study, cohorts from the ARIC, MESA, and CARDIA were combined to examine the role of remnant cholesterol (RC) in ASCVD events in populations free of disease at baseline.²⁵ Using both direct Cox proportion models and an analysis categorizing groups as discordant or concordant for RC and LDL-C, the authors reported that RC was a stronger predictor of incident ASCVD events and was associated with incident disease independent of LDL-C and apoprotein B. Of note, RC was measured by subtracting HDL-C and estimated LDL-C (using the Martin-Hopkins equation together with the level of plasma or serum TG) from total cholesterol. The concentration of RC is, therefore, numerically equivalent to cholesterol in VLDL. The authors also stressed the independence of RC from apoprotein B in their analyses and concluded that the number of atherogenic particles carrying RC is not the complete story. The apoprotein B measurement used measured whole plasma or serum apoprotein B, not apoprotein B on remnant particles, and the analyses did not adjust for the presence of diabetes, which was much more prevalent in the group with high RC and low LDL. Many of these cautionary notes were provided independently in an editorial that accompanied the paper.²⁶

The Progression of Early Subclinical Atherosclerosis¹⁷ study enrolled 3754 individuals (39% women), mean age of 45 years, free of

clinical ASCVD, and with low or moderate CV risk using the ESC guidelines.²⁷ Baseline determinations were performed for peripheral atherosclerotic plaques, coronary artery calcium scores (CACs), and vascular inflammation by fluorodeoxyglucose PET scans. Peripheral plaques were present in 58%, CAC in 17%, and vascular inflammation in 47% of the participants. When the cohort was divided into tertiles of TGs, there were concentration-dependent increases in the prevalence of peripheral plaque, including the number of vascular beds involved. Triglyceride had no relationship with CACS, but the group with TG > 150 mg/dL had twice the prevalence of vascular inflammation compared with the group with TG < 100 mg/dL.

There is a need to develop better high-throughput methods for the isolation and precise measurement of the TG and cholesterol content of the major lipoproteins and/or their subclasses. Identifying the specific TG carrying lipoproteins that are atherogenic is of critical importance in light of the development of potent therapeutic agents targeting TG levels, either by facilitating the activity of lipoprotein lipase or by the removal of remnants.^{28,29}

Omega-3 fatty acids

Two large-scale, randomized ASCVD outcome trials with high dosages of omega-3 fatty acids, the REDUCE-IT and STRENGTH trials, have shown discrepant results in terms of CV outcomes.

The administration of 4 g/day of Icosapentethyl (IPE) significantly reduced the risk of a primary endpoint event by 25% in the REDUCE-IT trial.³⁰ A pre-specified analysis of the REDUCE-IT trial showed that IPE also reduced first and total coronary revascularizations.³¹ To explain the mechanism of the clinical benefit of IPE, a subgroup analysis of the EVAPORATE study looked at the effect of IPE on whole-heart coronary atherosclerotic burden. Patients on IPE had significant reductions in coronary plaque burden >18 months with 55% lower per cent atheroma volume for total plaque and 61% lower for total non-calcified plaque compared with placebo ($P < 0.010$).³²

On the other hand, in the STRENGTH trial, the administration of 4 g/day of EPA + docosahexaenoic acid (DHA) failed to reduce the risk of major adverse CV events.³³ To further investigate the benefit of $n-3$ fatty acid supplementation in secondary prevention, the OMEGA-3 fatty acids in Elderly with Myocardial Infarction (OMEMI) trial was performed. A total of 1027 patients with a mean age of 75 ± 3.6 years were randomized to 1.8 g $n-3$ PUFA (930 mg EPA and 660 mg DHA) vs. placebo (corn oil) on top of the usual treatment. There was no decrease in CV events and an increase in atrial fibrillation.³⁴

Different hypothesis have been put forward to explain these discrepancies, including the possible negative effects of mineral oil used as placebo, differential effects of EPA and DHA and even the possibility of EPA and DHA counter-regulating each other.³⁵ A possible explanation for the contrasting results of REDUCE-IT vs. STRENGTH was put forward by a cohort study mimicking trial designs. Data from the Copenhagen General Population Study were used to identify cohorts that met key trial inclusion criteria for REDUCE-IT and STRENGTH trials. The difference in ASCVD incidence that could be explained by changes in TGRLs, LDL and hs-CRP was examined in these two cohorts. They found that the contrasting results of REDUCE-IT vs. STRENGTH could partly be explained by a difference in the effect of comparator oils.³⁶ In REDUCE-IT, mineral oil was associated with a 10.2% increase in LDL-C, 7.8% increase in apo B, and 32% increase in hs-CRP, whereas corn oil in STRENGTH had none of these effects. However, both the US FDA and EMA have discounted the mineral oil vs. corn oil comparator issue as a major contributor to the differences in outcomes between these two studies.³⁷

Other publications tried to explain this discrepancy by focusing on the differential effects of DHA and EPA on membrane structure, inflammatory biomarkers, endothelial function, and tissue distributions.³⁸ A recent *in vitro* study used micropipette aspiration of model membranes to measure membrane strain in response to applied force. They found that EPA and DHA differentially modulate membrane elasticity in the presence of cholesterol, and these changes have the potential to affect a wide range of physiological responses.³⁹

Another study looked at the common and differential effects of EPA and DHA supplementation on systemic inflammation, monocyte inflammatory response and the synthesis of specialized pro-resolving lipid mediators which reinforce the resolution of inflammation. Twenty-one individuals with chronic inflammation received two phases of 10-week supplementation with 3 g/day EPA or DHA. Plasma markers of inflammation, PUFA-derived lipid mediators, and cytokine expression were measured. EPA and

DHA supplementation differently modulated monocyte inflammatory response by differently regulating cytokine expression.⁴⁰

A cross-sectional analysis including 64 symptomatic patients who underwent coronary CTA looked at pericoronary adipose tissue (PCAT) attenuation. They found significantly higher values of EPA (1.00 vs. 0.78%) in patients with lower PCAT attenuation, whereas all other fatty acids showed no difference. Moreover, a significant negative correlation was seen between PCAT attenuation and EPA (CC: 0.38; $P = 0.002$). This led to the conclusion that high levels of EPA are associated with lower PCAT attenuation on coronary CTA, suggesting a different composition of PCAT, potentially caused by a lower degree of coronary inflammation.⁴¹

Lipoprotein(a)

Since Berg⁴² reported his discovery of Lp(a), there has been a steady increase in publications and most recently on newly targeted therapeutics that will dramatically lower Lp(a) levels and, hopefully, reduce ASCVD in individuals with the top 20–30% of circulating Lp(a) levels.

The epidemiology of Lp(a) and CVD

It is well established that circulating concentrations of Lp(a) are genetically regulated up to 80–90% and that this regulation is due to variation in the LPA gene coding for apolipoprotein (a). This has allowed for studies using Mendelian randomization, with the number of KIV-2 repeats as an excellent genetic instrument, to provide strong evidence for the causality of Lp(a) for ASCVD and to also strengthen the validity of traditional epidemiologic approaches using serum or plasma concentrations of Lp(a).

Using serum Lp(a) levels, Patel et al.⁴³ demonstrated a strong and continuous risk for ASCVD associated with increasing Lp(a) levels in 460 506 middle-aged participants in the UK Biobank followed for a median of 11.2 years. They demonstrated racial differences in the median Lp(a) concentrations: Whites—19, South Asians—31, Blacks—75, and Chinese—16 nmol/L. For all groups combined, there was a linear increase in risk for ASCVD of 11% for each increase in Lp(a) of 50 nmol/L (HR: 1.11) ethnic origin. Of note, the secondary prevention group had an attenuated risk, with an HR of 1.04 compared with an HR of 1.10 in the primary prevention group. Some of this seemed to be linked to statin use in individuals with pre-existing ASCVD. Using a Lp(a) of ≥ 150 nmol/L to define a 'high' level, 12.2% met this criterion in the primary prevention group and 20.3% in the secondary prevention group, demonstrating marked enrichment of 'high' Lp(a) in that group.

Somewhat different findings for ancestry were published by Satterfield et al.⁴⁴ using genetically predicted Lp(a) concentrations and Mendelian randomization in several cohorts, confirming other published results for major ASCVD categories in the European Ancestry Group. For individuals of African Ancestry, only PAD and abdominal aortic aneurysm were associated with Lp(a), whereas CAD and cerebrovascular disease were not. The differences in the statistical approaches used in these papers and the unique association of Lp(a) levels to allele size in people of African vs.

European Ancestry might account for these differences. The number of participants of African Ancestry in the Satterfield's paper was more than 10 times greater.

At the other end of the spectrum of circulating Lp(a) concentrations, Langsted *et al.*⁴⁵ examined 109 440 participants in the Copenhagen General Population Study to determine if low levels of plasma Lp(a) were related to adverse health outcomes. The only concordant association was for Lp(a) levels and genotypes with ASCVD. The hazard ratio for cancer was 1.06 (95% confidence interval: 0.97–1.15) for the fourth to the first quartile of Lp(a) levels and 1.05 for infections (0.99–1.10). The authors reported mixed data for a possible inverse relationship between Lp(a) and risk for diabetes, an important issue that has shown an inconsistent association in prior publications (Figure 3).

Of interest on this issue, in ODYSSEY-OUTCOMES, 10 mg/dL lower Lp(a) was associated with a 4% increase in incident diabetes in the placebo group over the course of the trial.⁴⁶ Although there was no effect of alirocumab-mediated lowering of Lp(a) (23%) on incident diabetes in the overall treated group, there was a significant interaction between baseline Lp(a) levels and alirocumab treatment-associated incident diabetes ($P=0.006$) with patients starting with higher levels of Lp(a) having both greater reductions in Lp(a) and more incident diabetes mellitus.

New genetic findings for Lipoprotein (a)

Despite the clear, robust role of the number of KIV-2 repeats in apo(a) in determining the level of circulating Lp(a), there can be large differences between individuals who have the same or very similar low-molecular-weight isoforms; several snps have been identified that contribute to inter-individual differences in Lp(a) levels despite similar numbers of KIV-2 repeats. Schachtl-Reiss *et al.* previously identified two splice site variants in KIV-2 region that reduced protein expression and therefore Lp(a) concentrations (4924G > A and 4733G > A). They now report the effects of these two variants on Lp(a) levels and risk for ASCVD in 4763 participants in the German Chronic Kidney Disease study; when both are present, Lp(a) concentrations are reduced by 31 mg/dL. They also identified a surrogate snp for 4733G > A in the UK Biobank data set; carriers of 4733G > A has a 9% reduction in CAD and carriers of both splice variants (compound heterozygotes for 4924G > A and 4733G > A) had a 12% reduction in CAD. Importantly, the carrier frequency for 4733G > A ranged from 1.5% in Africans to 35.1% in European.⁴⁷

In a related paper, the authors characterized variable number of tandem repeat (VNTR) polymorphisms to gain new insights into phenotype–genotype relationships. They looked at common DNA variants within the KIV-2 VNTR in an effort to better characterize the heritability of Lp(a) that has previously been explained by the number of KIV-2 repeats. They identified 17 protein-altering variants, with the two most impactful variants within the KIV-2 region; one of those was the 4925G > A described previously. Importantly, they were able to explain 83% of total variance in Lp(a) levels, significantly better than previous studies, and were able to demonstrate that the significantly increased Lp(a) levels in African Ancestry participants in the UK Biobank result from a lower frequency of Lp(a) reducing variants

and a higher frequency of an Lp(a)-raising variant in the 5'UTR of the gene.⁴⁸

The problem of Lipoprotein(a) cholesterol

As a cholesterol-carrying lipoprotein, the level of Lp(a) impacts the levels of LDL-C as presently measured. Even beta-quantification (BQ), the gold standard for determining LDL-C, is impacted by Lp(a), which is in the density > 1.006 fraction of plasma. Lipoprotein(a) contributes, therefore, a portion of the calculated LDL-C after HDL-C is selectively measured and subtracted from the total cholesterol. We have always ignored the contribution of IDL-C, which is in the BQ calculation, as well as the Friedewald, Martin–Hopkins, and NIH equation to estimations of LDL, despite the fact that it is a more substantial source of cholesterol in the large majority of the population than is Lp(a). However, the fact that Lp(a) does not respond to most treatments that lower both IDL and LDL, and the strong sense that the atherogenicity of Lp(a) is not simply because it can deliver cholesterol to arteries makes the case separately measuring Lp(a)-C and LDL-C with specific and reproducible assays. While such assays were in development, investigators have been using 30% as the cholesterol, by weight, in Lp(a) measured with mg/dL assays. Studies focusing on both errors in ASCVD-risk prediction and misclassification of patient's status regarding LDL-C goals or the diagnosis of familial hypercholesterolaemia have been published.⁴⁹ Yeang *et al.*⁵⁰ have developed a specific, sensitive, and reproducible assay with high-throughput capacity to measure Lp(a)-C demonstrating that there were reductions in LDL-C after correction for Lp(a)-C, with greater reductions occurring when Lp(a) was elevated. This assay will increase the accuracy of cohort studies and clinical guideline recommendations where LDL-C is central to the outcome. However, the demonstration that Lp(a)-C ranged from 5.8 to 57.3% of Lp(a) total mass indicates that neither clinical research nor clinical practice can depend on an approximation of 30% for Lp(a)-C when the desired goal is to obtain the 'true' LDL-C.⁵¹

Nucleic acid-based therapies

In recent years, the physiological mechanism of gene silencing, a post-transcriptional process by which cells regulate gene expression by turning off a selected gene, has been largely adopted and based on this mechanism, selective targeting of genes playing key roles in lipid metabolism developed with the use of both antisense oligonucleotides and small interfering RNA (siRNA). New-generation ASOs and siRNAs exhibit greater nuclease resistance, binding affinity, cell permeation, efficacy, and reduced off-target effects, which translate into a reduced incidence and severity of adverse events that were instead observed with the first-generation antisense drugs (Figure 4). In particular, the conjugation of siRNAs or ASOs to *N*-acetylgalactosamine (GalNAc) ligands has become a primary strategy for hepatocyte-targeted delivery with administration schedules, from once a week to twice a year (Figure 5). A major issue with the first-generation ASOs was thrombocytopenia which was not observed with siRNA-based

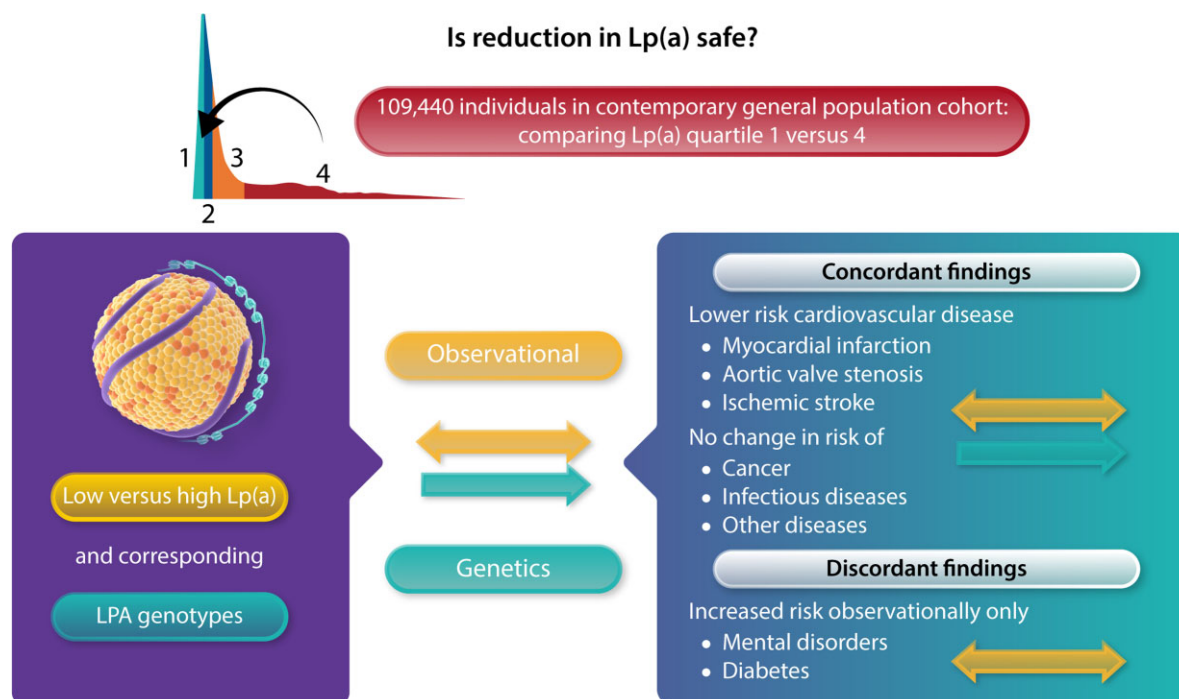


Figure 3 The safety of low lipoprotein(a) is addressed in a large population cohort. Low levels of lipoprotein(a) and corresponding LPA genotypes associate with decreased MI, ischaemic stroke, and aortic stenosis with no change in the risk of cancer and infectious disease where lowest and highest quartiles of lipoprotein(a) are compared. Reproduced with permission from Langsted et al.⁴⁵

therapeutics. Nucleic acid-based therapeutics for the treatment of hypercholesterolaemia include both ASOs targeting apolipoprotein B (apoB), apolipoprotein CIII (apoC-III), angiopoietin-like 3 (ANGPTL3), or apolipoprotein(a) [apo(a)], and siRNAs targeting PCSK9 or apo(a).

Inclisiran

Inclisiran, a siRNA targeting PCSK9, was approved by EMA in December 2020 for the treatment of adults with primary hypercholesterolaemia or mixed dyslipidaemia. In the ORION-10 and ORION-11 trials, inclisiran (284 mg) or placebo have been administered by subcutaneous injection on Day 1, Day 90, and then every 6 months for a period of 540 days in patients with atherosclerotic CV disease (ORION-10 trial) and patients with atherosclerotic CV disease or an atherosclerotic CV disease risk equivalent (ORION-11 trial) having elevated LDL-C levels despite receiving statin therapy at the maximum tolerated dose.⁵² Inclisiran lowered LDL-C levels by ~50% compared with placebo, with the rate of adverse event being similar in both groups. ORION-9 trial, which evaluated the effect of inclisiran (300 mg) or matching placebo in a population of heterozygous FH patients, showed a 47.9% reduction in LDL-C levels,⁵³ that was independent of the underlying genetic defect. The analysis of data from these three trials showed that inclisiran administered twice yearly is effective in reducing LDL-C levels, safe, and well tolerated.^{54,55} Inclisiran also reduces LDL in homozygous FH (HoFH) but with a greater variability,⁵⁶ and this will be evaluated in a larger

population in the ORION-5 trial.⁵⁷ A clinical outcome trial (ORION-4) is testing the effect of inclisiran or placebo in a population of patients with CV disease after a median follow-up of 5 years.⁵⁸ A pre-specified safety analysis of the phase 2 ORION-1 trial could not detect any adverse effects on measures of inflammation, immune activation, platelet count, or clinical immunogenicity over at least 6-month treatment.⁵⁹

Vupanorsen

Vupanorsen is a GalNAc-conjugated antisense drug-targeting ANGPTL3 mRNA that was shown to impact favourably lipid/lipoprotein profile in patients with diabetes, hepatic steatosis, and hypertriglyceridaemia, without inducing significant alterations in platelet counts.⁶⁰ This ASO may represent a potential strategy for the control of residual CV risk.

The observations from Mendelian randomization studies suggest that massive Lp(a) reductions (70–100 mg/dL) are required to reduce CV risk, leading to the development of antisense-based therapies that include both an ASO and an siRNA.

Pelacarsen

Pelacarsen is an ASO against apolipoprotein(a) that reduces Lp(a) levels up to 80% with good tolerability and allows 98% of subjects receiving the ASO to reach on-treatment levels of <125 nmol/L (<50 mg/dL).⁶¹ Whether this Lp(a) reduction translates into a clinical benefit will be addressed in the ongoing Lp(a) HORIZON outcomes trial. Recently, a GalNAc-conjugated

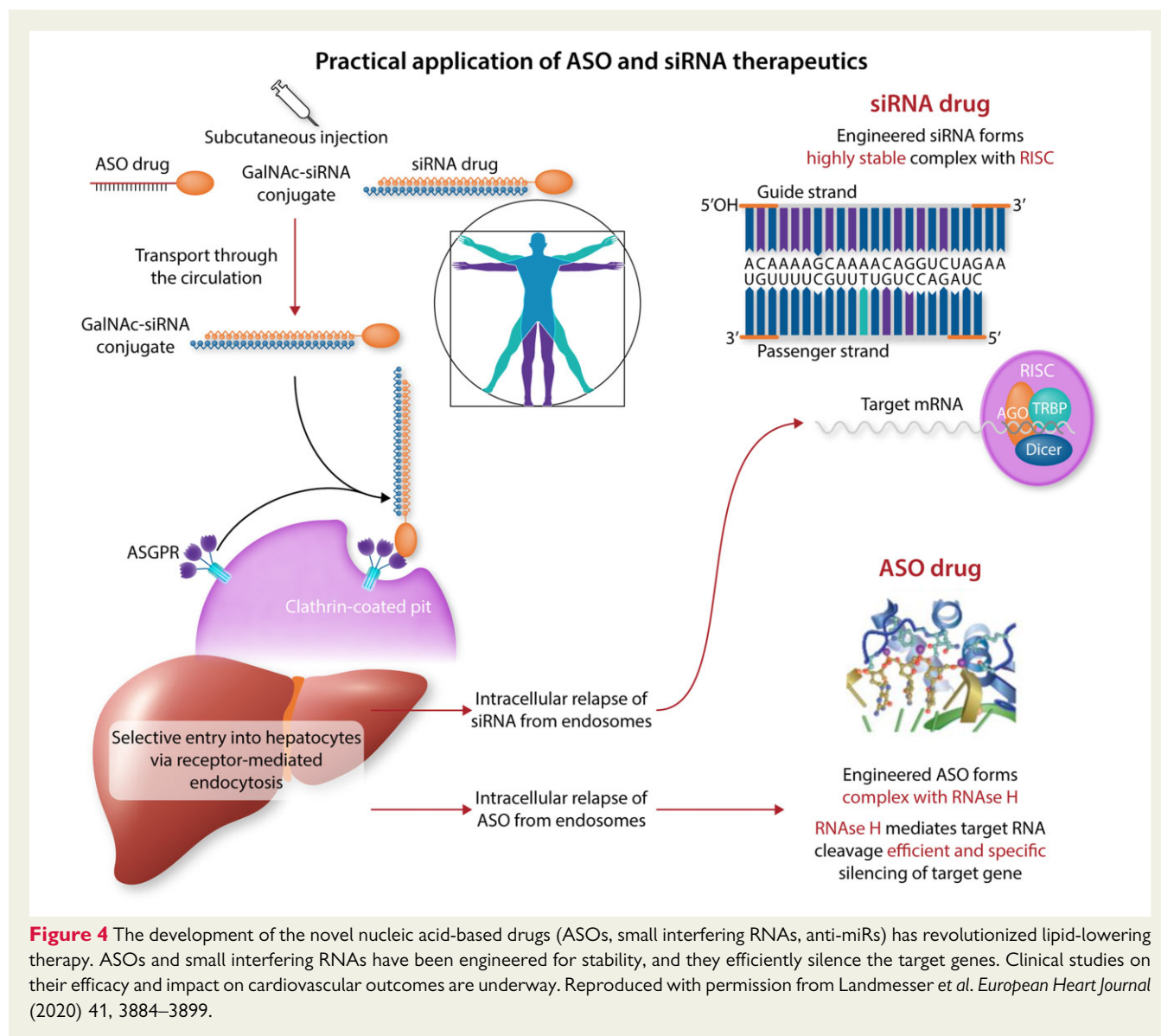


Figure 4 The development of the novel nucleic acid-based drugs (ASOs, small interfering RNAs, anti-miRs) has revolutionized lipid-lowering therapy. ASOs and small interfering RNAs have been engineered for stability, and they efficiently silence the target genes. Clinical studies on their efficacy and impact on cardiovascular outcomes are underway. Reproduced with permission from Landmesser *et al. European Heart Journal* (2020) 41, 3884–3899.

siRNA targeting apo(a) (olpasiran) was shown to reduce Lp(a) by >90% at doses ≥ 9 mg, with reductions persisting 3–6 months. A Phase II study is now assessing the efficacy, safety, and tolerability of olpasiran in 240 subjects with Lp(a) >60 mg/dL (>150 nmol/L).⁶²

The inhibition of ANGPTL3 with a monoclonal antibody (evinacumab), resulting in a marked reduction in TG and LDL-C levels, was shown to reduce LDL-C levels by ~50% in HoFH patients independently of the type of genetic defects, due to its LDLR-independent mechanism of action.⁶³ A small study in four patients suggests that evinacumab markedly increases IDL and LDL apoB catabolic rates, thus increasing apoB-containing lipoprotein removal.⁶⁴ Furthermore, the addition of evinacumab to an intensive lipid-lowering therapy resulted in a profound plaque reduction⁶⁵ (76 and 85% after 6 months of evinacumab) in two young HoFH patients. Evinacumab may therefore represent an effective approach for the treatment of patients that have a poor

response to classical LDL-C-lowering therapies, especially when bearing null LDLR mutations. A major limitation was the i.v. route of administration of evinacumab. A subsequent study has compared subcutaneous and i.v. administration of evinacumab and observed that the mAb is effective in reducing LDL-C levels regardless of the route of administration.⁶⁶ Evinacumab significantly reduced TG levels in subjects with either moderate or severe hypertriglyceridaemia, with an expected increase in LDL-C levels in both cohorts likely due to the enhanced conversion of VLDL and IDL to LDL particles.⁶⁷

Alternative approaches for PCSK9 inhibition

The discovery of novel very small, non-biological, *in vitro* synthesized ligands that bind PCSK9 with low-nanomolar affinity,

GalNAc-siRNA conjugates facilitate selective hepatic uptake

- **Asialoglycoprotein receptor (ASGPR)**
- Highly expressed in hepatocytes only
- High rate of uptake



- siRNA conjugated to N-acetylgalactosamine
- Subcutaneous administration
- Targeted delivery to hepatocytes

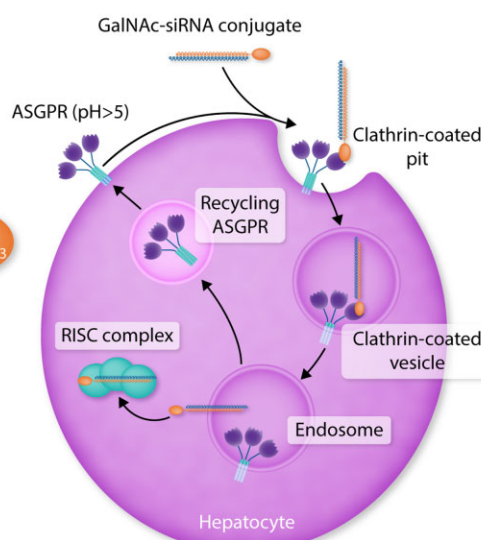


Figure 5 The development of delivery systems based on the GalNAc (ligand)–ASGPR (receptor) interaction to the liver has enabled efficient and specific drug delivery upon subcutaneous injection. *N*-acetylgalactosamine–small interfering RNA conjugates facilitate rapid hepatic uptake thus preventing off-target toxicity.

resulting in the disruption of PCSK9 activity, may represent a valuable tool for the development of oral inhibitors of PCSK9.⁶⁸ An affinity-based screen of 1013 *in vitro*-translated macrocyclic peptides led to the identification of high-affinity PCSK9 ligands being able to increase robustly hepatic LDLR expression and to reduce plasma cholesterol levels in mice.⁶⁸

There is a compelling need to develop pharmacological approaches that can increase patient compliance with reduced frequency of treatment. Vaccines can be an opportunity to provide a long-lasting inhibition of PCSK9. Recently, it has been reported that an immunotherapeutic targeting PCSK9 can induce a strong PCSK9-reactive antibody response which translated into reductions in LDL-C levels of 11.2 and 13.3% from baseline at Weeks 20 and 70, respectively.⁶⁹ The immune response was readily reactivated by a booster immunization.

Most known pathogenic point mutations in humans are C•G to T•A substitutions. Adenine base editors can efficiently mediate the conversion of A•T to G•C and enable corrections; this approach has been used to introduce a splice site mutation in *PCSK9*, resulting in PCSK9 inhibition and LDL-C level reduction in mice (95 and 58%, respectively) and macaques⁷⁰ (32 and 14%), without off-target mutations in genomic DNA. On the hand, *in vivo* CRISPR base editing of *PCSK9* using lipid nanoparticles was shown to induce a near-complete knockdown of hepatic PCSK9 after a single infusion in cynomolgus monkeys with concomitant reductions in circulating PCSK9 and LDL-C (~90% and ~60%, respectively).⁷¹ Another approach consists in the PCSK9 knockdown in non-human primate liver by adeno-associated virus¹⁷-delivered meganuclease leading to a sustained reduction in circulating PCSK9 and LDL-C through the course of the study concomitant with stable gene editing of the *PCSK9* locus.⁷² A low-frequency of

off-target editing was observed, and no evident adverse changes in histopathology of the liver were detected.

An interesting recent *in silico* study showed that the *PCSK9* rs11591147 TT genotype is protective, whereas the GG genotype is more susceptible to CAD progression.⁷³ Furthermore, patients with the *PCSK9* rs11591147 TT genotype have significantly lower LDL-C levels compared with patients with the GG genotype. The analysis of *PCSK9* promoter DNA methylation showed that TT genotype was associated with a hypermethylation status, lower mRNA expression, and lower PCSK9 blood levels compared with the GG genotype. This observation indicates novel treatment possibilities, such as pharmacogenetic and promoter DNA methylation-related drug interventions targeting PCSK9 for CAD management.

PCSK9 inhibition may promote the infiltration of T cells within tumours, thus increasing the tumour susceptibility to immune checkpoint therapy. Accordingly, *PCSK9* gene deletion in murine cancer cells significantly reduced or prevented their growth in a cytotoxic T-cell-dependent manner and increased the efficacy of an anti-PD1 therapy; in addition, evolocumab synergized with anti-PD1 therapy in suppressing tumour cell growth.⁷⁴ The underlying mechanism is the involvement of PCSK9 in disrupting the recycling of major histocompatibility complex I at cell surface, which results in the inhibition of cytotoxic T-cell infiltration within the tumour.

In conclusion, there has been an explosion of new information on lipid-lowering therapies in the past years. The possibility to target proteins with nucleic acid-based therapies has opened up a new era in lipid lowering, where we will have more powerful tools and a wide variety of medications to choose from to truly personalize dyslipidaemia management.

Conflict of interest: L.T. has received honoraria or lecture fees from Abbott, Janssen, Amgen, Bayer, Daiichi Sankyo, MSD, Mylan, Novartis, Novo Nordisk, Sanofi, Servier, Pfizer, Recordati; participation on a data safety monitoring board or advisory board for Abbott, Amgen, Novartis, Novo Nordisk, Sanofi, Daiichi Sankyo, Mylan, and Pfizer. L.T. was the Past President of the European Atherosclerosis Society and the Past President of the Turkish Society of Cardiology. C.O. and H.N.G. have no disclosures related to this paper. A.L.C. has received honoraria, lecture fees, or research grants from Akcea, Amgen, Astrazeneca, Eli Lilly, Genzyme, Kowa, Mediolanum, Menarini, Merck, Pfizer, Recordati, Sanofi, Sigma Tau, Amryt, and Sandoz. The research work of A.L.C. is supported by Ministero della Sanità ricerca corrente, and he was the Past President of the European Atherosclerosis Society.

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