Cardiovascular Drugs and Therapy (2021) 35:1269–1279
https://doi.org/10.1007/s10557-020-07082-x

ORIGINAL ARTICLE

Lipid-Lowering Biotechnological Drugs: from Monoclonal Antibodies to Antisense Therapies—a Clinical Perspective

Xiaoming Jia[1] · Jing Liu[1] · Anurag Mehta[2] · Christie M. Ballantyne[1] · Salim S. Virani[1,3,4]

Accepted: 16 September 2020 / Published online: 30 September 2020

Abstract

Purpose While low density lipoprotein cholesterol (LDL-C) remains a key contributor of atherosclerotic cardiovascular disease (ASCVD), additional risk factors identified through epidemiological and genetic studies have ushered in a fertile era of drug discovery in lipid-lowering therapy. Unlike contemporary small molecule medications, many of the novel agents are biologics utilizing monoclonal antibody (mAb) or RNA interference (RNAi) technologies. This report aims to review the evidence to date, focusing on completed and ongoing clinical trials and how these new agents will impact clinical practice.

Methods We review data from pertinent studies on lipid-lowering biologics in clinical use or have translated to human studies and are undergoing clinical trials.

Results Several targets affecting lipid metabolism have been identified to be causally associated with ASCVD including proprotein convertase subtilisin/kexin type 9 (PCSK9), angiopoietin-like protein 3 (ANGPTL3), apolipoprotein C3 (APOC3), and lipoprotein (a) (Lp[a]). Biotechnological modalities that have been developed for these targets include mAb, small interfering RNA (siRNA), and anti-sense oligonucleotide (ASO) agents. Agents such as alirocumab and evolocumab have shown efficacy in risk reduction of ASCVD in cardiovascular outcome trials and have been incorporated into evidence-based practice guidelines. Other agents included in this review are in various stages of clinical trials and have shown significant efficacy in the reduction of lipid parameters.

Conclusion The development of new biologics targeting lipid risk factors will provide clinicians additional tools to reduce the risk for ASCVD. Important factors to consider will be cost-effectiveness and improving methods to personalize treatments to risk factors.

Keywords Novel lipid-lowering therapies · Atherosclerotic cardiovascular disease · Cardiovascular prevention

Introduction

Hyperlipidemia has long been an established risk factor for atherosclerosis, and low-density lipoprotein cholesterol (LDL-C) is recognized as being causally associated with atherosclerotic cardiovascular disease (ASCVD) [1]. The fundamental framework that governed most contemporary cholesterol-lowering agents, which include statins and other small molecule drugs such as ezetimibe and bempedoic acid, is the lowering of LDL-C [2–4].

The advent of genome-wide association and Mendelian randomization instruments have allowed for our expanded understanding into the complex genetic drivers in dyslipidemia and ASCVD, as well as the identification of important genetic mediators including *PCSK9*, *ANGPTL3*, *APOC3*, and *LPA* [5–7]. Some of these genes and their gene products, such as proprotein convertase subtilisin/kexin type 9 (PCSK9), directly affect serum LDL-C level. However, as a result of these genetic linkage studies, other lipids including triglyceride-rich lipoproteins (TRLs) and lipoprotein(a) (Lp[a]) are now also thought to be causally associated with ASCVD [8].

✉ Salim S. Virani
virani@bcm.edu

[1] Section of Cardiology, Baylor College of Medicine, Houston, TX, USA

[2] Division of Cardiology, Emory University School of Medicine, Atlanta, GA, USA

[3] Section of Cardiology, Michael E. DeBakey Veterans Affairs Medical Center, Houston, TX, USA

[4] Health Policy, Quality & Informatics Program, Health Services Research and Development Center for Innovations, Michael E. DeBakey Veterans Affairs Medical Center, 2002 Holcombe Boulevard, Houston, TX 77030, USA

The identification of new therapeutic targets has in turn heralded a new era of drug discovery in lipid-lowering therapy [9]. As the paradigm of the mechanism underlying ASCVD has evolved, so too has the therapeutic design of lipid-lowering medications, and many of the new agents that have been developed or in the pipeline are biologics-based—consisting of monoclonal antibodies (mAb), RNA interference (RNAi), and more recently gene editing technologies (Fig. 1). In the sections below, we highlight these biotechnological drug classes and discuss current clinical evidence on the efficacy and safety of biologic lipid-lowering therapies (Table 1).

Overview of Types of Biotechnological Therapies

Multiple modalities of lipid-lowering biologics have been developed and are under investigation. The two major classes of biotechnological therapies that are either available commercially or under investigation in human trials can be organized into mAbs and RNAi technologies (Fig. 2). A third class, gene editing using CRISPR-Cas9 techniques is being developed to treat hyperlipidemia disorders though studies are still in animal phase [10]. The mAb class of agents acts by binding and inactivating the target protein. Lipid-lowering mAb agents include the PCSK9 inhibitors, alirocumab and evolocumab, both of which are commercially available and have shown efficacy in the secondary prevention of ASCVD as well as management of familial hypercholesterolemia (FH) [11, 12]. Additionally, evinacumab is a mAb against angiopoietin-like protein 3 (ANGPTL3) currently undergoing clinical trials [13]. It is important to note that the above agents are human mAbs. Bococizumab, which is a humanized mAb against PCSK9 with approximately 3% of murine sequence, was shown to elicit high rates of antidrug antibodies in clinical trials, and development of this agent has been subsequently discontinued [14].

A second strategy to inhibit proteins of interest is via gene silencing through RNAi. Gene expression at the transcription level can be disrupted by RNAi biologics either by small interfering RNAs (siRNA) or by antisense oligonucleotides (ASO). SiRNA agents consist of short, double-stranded RNA molecules that bind to RNA-induced silencing complex (RISC), which then targets the complementary mRNA molecules inducing cleavage and degradation. A single siRNA-bound RISC is able to bind and cleave many mRNA transcripts [15]. Meanwhile, ASOs act by directly binding to their complementary messenger RNA (mRNA) molecules, resulting in RNase-mediated degradation [16].

As the liver is central to lipid metabolism, current siRNA and ASO agents are conjugated to *N*-acetylgalactosamine carbohydrates (GalNAc), which binds asialoglycoprotein receptors (ASGPR) on hepatocytes [17]. This strategy facilitates

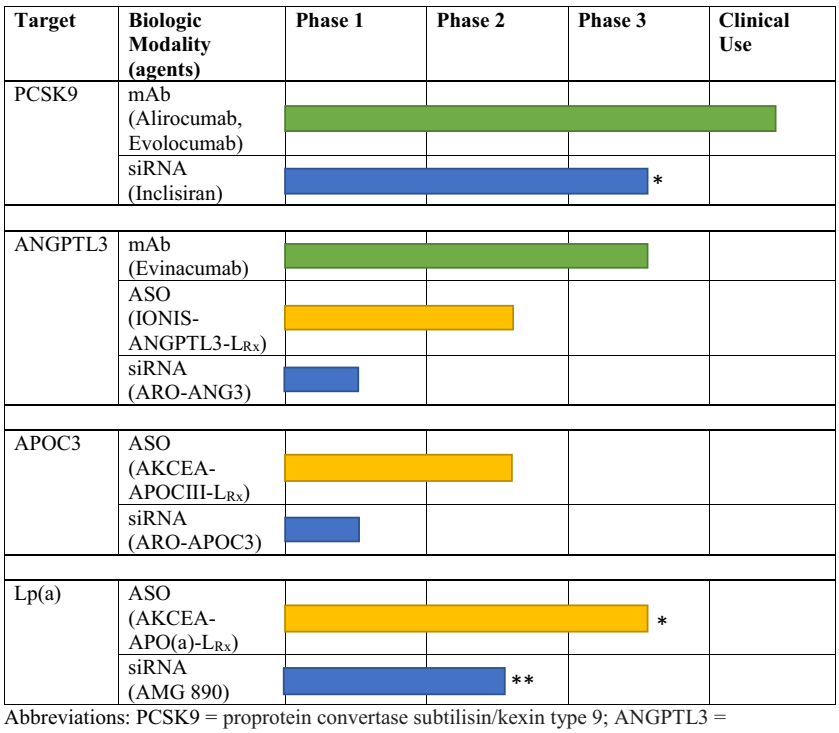

Fig. 1 Lipid-lowering biologics that are in clinical use or are currently under investigation in various phases of clinical trials

Abbreviations: PCSK9 = proprotein convertase subtilisin/kexin type 9; ANGPTL3 = angiopoietin-like protein 3; APOC3 = apolipoprotein C3; lipoprotein (a) = Lp(a); mAb = monoclonal antibody; ASO = antisense oligonucleotide; siRNA = small interfering RNA. *Phase 3 cardiovascular outcomes trial ongoing. **Phase 2 trial ongoing.

Table 1 Summary of lipid-lowering biologics that are currently available or undergoing clinical trials.

	Target	Efficacy	Safety
Monoclonal antibody			
Evolocumab	PCSK9	• 9.8% vs 11.3% (HR 0.85, 95% CI 0.79–0.92) for evolocumab vs placebo with respect to primary endpoint (composite cardiovascular death, MI, stroke, hospitalization for unstable angina, or coronary revascularization) among patients with established ASCVD. • LS mean percentage reduction in LDL-C of 59% in the evolocumab compared with placebo.	• No significant difference in adverse outcomes between treatment and placebo groups.
Alirocumab	PCSK9	• 9.5% vs. 11.1% (HR 0.85, 95% CI 0.78–0.93, $p < 0.001$) for alirocumab vs placebo with respect to primary endpoint (composite of death from CHD, non-fatal MI, fatal or non-fatal ischemic stroke, or hospitalization for unstable angina) among patients with recent ACS. • 54.7% reduction in LDL-C in the alirocumab group compared to placebo at 48 months in on-treatment analysis.	• No significant difference in adverse outcomes between treatment and placebo groups.
Evinacumab	ANGPTL3	• ~ 70% sustained reduction in TG through day 57 in evinacumab group compared with placebo group in the MAD study among healthy volunteers. Dose-dependent reduction in non-HDL-C, LDL-C, and HDL-C also observed. • − 49% LS mean percentage reduction in LDL-C in evinacumab compared with placebo among patients with HoFH.	• No difference in serious treatment emergent adverse events between treatment and placebo groups
Small interfering RNA			
Inclisiran	PCSK9	• LDL-C reduction of 52.3% at 510 days with inclisiran when compared with placebo among patients with ASCVD. • LDL-C reduction of 49.9% at 510 days with inclisiran when compared with placebo among patients with ASCVD or ASCVD risk-equivalents. • Reduction of LDL-C by 47.9% compared with placebo at 18 months among subjects with HeFH.	• No significant difference in serious adverse events were noted between treatment and placebo groups.
ARO-ANG3	ANGPTL3	• Reduced TG by up to 53% from baseline after 16 weeks in single ascending dose study.	• No serious adverse effects noted during the study
ARO-APOC3	APOC3	• Reduced TG by up to 55% after 16 weeks in single ascending dose study.	• No serious adverse events noted during the study
Anti-sense oligonucleotide			
IONIS-ANGPTL3--L_{Rx}	ANGPTL3	• Up to 63.1% reduction in TGs, 36.6% reduction in non-HDL-C, and 25.7% reduction in apoB levels after 6 weeks in multiple dose study.	• No serious adverse effects observed.
AKCEA-APOCIII--L_{Rx}	APOC3	• Reduced TG by up to 73% after 43 days in multiple dose study.	• Similar rates of treatment emergent adverse events between groups. No signal for thrombocytopenia observed.
AKCEA-APO(a)-LRx	Lp(a)	• Mean reduction in Lp(a) of up to 80% from baseline at 6 months in multiple dose study.	• Serious adverse events occurred in 10% patients receiving active therapy and 2% receiving placebo.

PCSK9 proprotein convertase subtilisin/kexin type 9, *ANGPTL3* angiopoietin-like protein 3, *APOC3* apolipoprotein C3; *Lp(a)* lipoprotein (a), *MI* myocardial infarction, *ASCVD* atherosclerotic cardiovascular disease, *LDL-C* low density lipoprotein cholesterol, *TG* triglyceride, *HDL-C* high-density lipoprotein cholesterol, *apoB* apolipoprotein B, *HoFH* homozygous familial hypercholesterolemia, *HeFH* heterozygous familial hypercholesterolemia

drug delivery to the liver and allows for lowering dosing of medication. The redesign resolved issues with adverse side effects including significant thrombocytopenia which was observed during earlier RNAi agents prior to the incorporation of GalNAc when much higher dosages were tested [18, 19]. The current RNAi biologics undergoing clinical trials include inclisiran, a siRNA agent targeting PCSK9 and ASO therapeutics against the mRNAs of apolipoprotein C3 (APOC3), ANGPTL3, and Lp(a). Other siRNA-based therapies include those against ANGPTL3, APOC3, and Lp(a) though limited clinical data have been published regarding these agents [20]. The ASO inhibitor, mipomersen, which targets apolipoprotein B-100 (apoB-100) was an orphan drug previously available for the treatment of homozygous FH (HoFH). However, mipomersen has been associated with significant hepatotoxicity, injection reactions, and flu-like symptoms and at the time of this review has been discontinued from the market [21, 22].

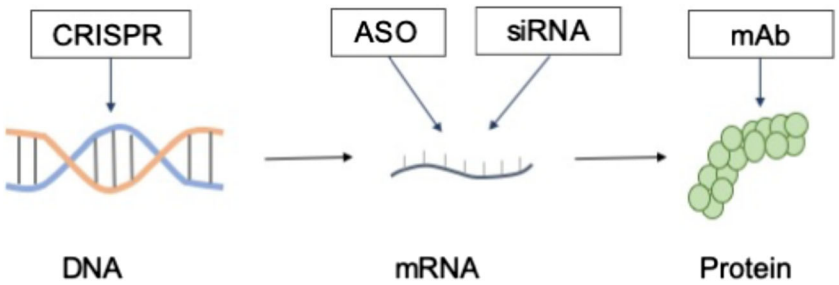

Fig. 2 Molecular targets of novel lipid-lowering biologics include targeting of proteins by monoclonal antibodies (mAb), messenger RNA (mRNA) by antisense oligonucleotide (ASO), and small interfering RNA (siRNA) therapeutics. CRISPR gene-editing technology that works at the DNA level is also being developed

Current Clinical Evidence by Drug Targets

PCSKS9

In humans, the PCSK9 proteins bind to low-density lipoprotein receptor (LDLR), which leads to receptor degradation and lowered LDL cholesterol removal from the circulation. Individuals with loss-of-function PCSK9 genetic variants were found to have lower LDL-C and incidence of coronary heart disease (CHD). Meanwhile, gain-of-function PCSK9 variants lead to increased LDL-C and familial hypercholesterolemia [23]. PCSK9i is a class of monoclonal antibodies that acts to lower LDL-C by inhibiting the PCSK9 proteins and thus preventing LDLR degradation. While PCSK9 inhibitors dramatically reduce LDL-C, these agents do not significantly alter high sensitivity C-reactive protein (hs-CRP), a marker of inflammation [24, 25].

Approved by the FDA in 2015, evolocumab is a fully human monoclonal antibody that inhibits PCSK9 to lower LDL-C cholesterol. The FOURIER study was a randomized, parallel, double-blind, placebo-controlled trial of 27,564 patients with clinical ASCVD (prior MI, non-hemorrhagic stroke, symptomatic PAD) and LDL-C ≥ 70 mg/dL or a non-HDL-C level ≥ 100 mg/dL who were receiving optimized lipid-lowering preferably using high-intensity statin or at least atorvastatin 20 mg with or without ezetimibe. Patients were randomized to evolocumab 140 mg subcutaneous every 2 weeks or 420 mg monthly versus placebo every 2 weeks [12]. Among the trial participants, 69% were on a high-intensity statin, and 30% were on a moderate-intensity statin, and 5.2% were on ezetimibe, with median LDL-C of 92 mg/dl. The primary outcome, a composite of cardiovascular death, MI, stroke, hospitalization for unstable angina, or coronary revascularization, occurred in 9.8% in the evolocumab group compared to 11.3% of the placebo group (hazard ratio [HR] 0.85, 95% confidence interval [CI] 0.79–0.92, $p < 0.001$) at a median follow-up of 2.2 years. Among individual endpoints, individuals in the evolocumab group had lower rates of MI, strokes, and coronary revascularization but did not have significant difference in cardiovascular deaths compared with placebo. At 48 weeks, LDL-C was reduced by a least-squares mean percentage of 59% in the evolocumab group compared with placebo. Absolute reduction in LDL-C was 56 mg/dl in the evolocumab group compared to placebo. Evolocumab was found to be safe with no significant difference in adverse outcomes compared to placebo (serious adverse event [SAE] was 24.8% with evolocumab versus 24.7% with placebo). There have been concerns regarding the very low levels of LDL-C achieved in patients on PCSK9i and potential association with neurocognitive side effects. However, analysis from the EBBINGHAUS study demonstrate no significant difference in cognitive function between those on evolocumab compared with placebo over 19 months follow-up [26]. In post hoc analysis, individuals with high-risk features including more recent MIs, ≥ 2 prior MIs, and presence of residual multivessel coronary artery are at the highest risk for major vascular events and had the greatest risk reduction with evolocumab [27]. In sub-analysis of FOURIER patients with PAD, though the relative risk reduction for clinical outcomes were similar between participants with and without PAD, the absolute risk reduction of evolocumab in patients with PAD were higher given the greater underlying risk [28].

The efficacy and safety of alirocumab was established in the landmark ODYSSEY OUTCOMES trial [11]. The trial randomized 18,924 patients who had an acute coronary syndrome in the preceding 1 to 12 months. Alirocumab was titrated between 75 and 150 mg to keep LDL-C between 25 and 50 mg/dl and to avoid LDL-C levels below 15 mg/dl on a consistent basis. Over a follow-up duration of 2.8 years, the primary outcome (composite of death from coronary heart disease, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, or unstable angina requiring hospitalization) for alirocumab vs placebo was 9.5% vs 11.1% (HR 0.85, 95% CI 0.78–0.93, $p < 0.001$). Among secondary outcomes, alirocumab group to placebo group was found to have lower major coronary heart disease events (8.4% vs. 9.5%, $p = 0.006$), any cardiovascular event (13.7% vs 15.6%, $p < 0.001$) as well as composite of death from any cause/nonfatal MI/nonfatal ischemic stroke (10.3% vs. 11.9%, $p < 0.001$) but not death from CHD, death from CVD cause, or death from any cause. Mean at LDL-C at 48 months was 66

mg/dL in the alirocumab group compared with 103 mg/dL in the placebo group. In on-treatment analysis, there was a 54.7% lower LDL-C in the alirocumab group compared to placebo at 48 months. The rate of adverse events was similar among participants in the alirocumab compared with those in the placebo group (SAE 23.3% vs 24.9% in the alirocumab vs placebo groups, respectively). Of note, a third humanized PCSK9i, bococizumab, was studied in the SPIRE-1 and SPIRE-2 trials. In the SPIRE-1 outcome study, no significant difference in the primary endpoint (non-fatal MI, non-fatal stroke, hospitalization for unstable angina requiring urgent revascularization, or CV death) was observed among enrolled patients with LDL-C greater than 70 mg/dl at 7 months. In the SPIRE-2 trial, at 12 months, there was a 21% reduction in the primary end point among patients enrolled with LDL-C greater than 100 mg/dl, suggesting that longer treatment duration might be beneficial. However, in 2016, the drug's development was discontinued prematurely by sponsors based on the trials' lipid-lowering results [14].

In addition to monoclonal antibodies, small interfering double-stranded RNA has also been developed to inhibit PCSK9 production. Inclisiran, currently under investigation, is a siRNA that works by directly inhibiting the translation of the PCSK9 protein in hepatic cells, thus lowering LDL-C levels in the circulation. The safety and efficacy of inclisiran in patients with heterozygous familial hypercholesterolemia (HeFH), ASCVD, or ASCVD risk-equivalents have been studied in the ORION-9, ORION-10, and ORION-11 trials, respectively. ORION-9, a phase 3, randomized clinical trial, showed that inclisiran sodium 300 mg administered subcutaneously at days 1, 90, 270, and 450 was effective in lowering LDL-C by 47.9% compared with placebo at 18 months (time averaged mean reduction of 44.3% compared to placebo) among subjects with heterozygous familial hypercholesterolemia with LDL-C level > 100 mg/dl at time of enrollment [29]. No significant difference in adverse side effects was noted between inclisiran sodium and placebo groups. In ORION-10, subjects with ASCVD on maximum-tolerated statin therapy and elevated LDL-C cholesterol (LDL-C $\geq$ 70 mg/dl) were randomized to inclisiran 284 mg (equivalent to 300 mg of inclisiran sodium; administered at day 1, day 90, and every 6 months thereafter) versus placebo with 18 months follow-up. At 510 days, there was a between-group difference of − 52.3% ($p < 0.001$) in LDL-C when comparing inclisiran and placebo groups. The time-averaged change in LDL-C was − 53.8% when comparing inclisiran to placebo. Again, no significant difference in serious adverse events was noted between inclisiran and placebo groups. In the ORION-11 trial, patients with ASCVD or ASCVD risk-equivalents were randomized to inclisiran 284 mg injection versus placebo. Mean percent change in LDL-C at 510 days, was − 49.9% ($p < 0.001$) in the inclisiran group compared with the placebo group. The time-averaged reduction in LDL-C for the inclisiran vs placebo groups was − 49.2 ($p < 0.001$). There was no significant difference in adverse events between the groups [30]. Overall, the three ORION trials have established the LDL-C–lowering efficacy and safety of inclisiran in managing patients with HeFH, ASCVD, or ASCVD risk equivalents with elevated LDL-C.

Based on the 2018 American College of Cardiology (ACC)/American Heart Association (AHA) Multisociety Cholesterol guidelines, PCSK9i have a class IIb indication to be considered in patients 30–75 years of age with heterozygous FH and elevated LDL-C level (≥ 100 mg/dL) while on maximally tolerated statin and ezetimibe therapy. In addition, in patients with hypercholesterolemia (baseline LDL-C level of 220 mg/dl or higher) between the age of 40 and 75 years, whose LDL-C remains above 130 mg/dl despite maximally tolerated statin and ezetimibe therapy, PCSK9i may also be considered (IIb indication). In patients with clinical ASCVD who are at very high risk and whose LDL-C remains ≥ 70mg/dl or whose non-HDL-C level remains ≥ 100 mg/dl despite maximally tolerated lipid-lowering agents (statins and ezetimibe), a PCSK9i may be considered after discussion of benefits, safety, and costs between clinician and patient (class IIa indication) [31, 32]. Very high risk is defined as a history of multiple major ASCVD events (recent ACS within the past 12 months, history of MI, history of ischemic stroke or symptomatic PAD) or 1 major ASCVD event plus multiple high-risk conditions (age ≥ 65 years, heterozygous familial hypercholesterolemia, history of coronary revascularization outside of the major ASCVD events, diabetes mellitus, hypertension, chronic kidney disease, current smoking, persistently elevated LDL-C ≥ 100 mg/dL despite maximally tolerated statin and ezetimibe, or history of congestive heart failure).

However, cost remains a concern for PCSK9i [33]. The initial price of these agents made them cost ineffective in many cost-effectiveness analysis models. Newer analysis based on reduced pricing of these agents has found PCSK9i to be more cost effective [34]. It remains to be seen whether the price reduction of evolocumab and alirocumab will impact their scope of use.

ANGPTL3

ANGPTL3 is a protein predominantly found in the liver that acts by inhibiting lipoprotein lipase and endothelial lipase, which are important in TRL metabolism and the regulation of TGs and HDL-C. ANGPTL3 mutations have also been linked to reduced LDL-C, potentially via increased clearance of lipoprotein particles though the precise mechanisms have yet to be elucidated [35, 36]. Loss of function (LOF) mutation in *ANGPLT3* results in familial combined hypolipoproteinemia, characterized phenotypically by low plasma triglycerides, LDL-C, and HDL-C [37]. In the DiscovEHR human genetics study, heterozygous carriers of

LOF variants of ANGPTL3 were associated with 27% lower TG, 9% lower LDL-C, and 4% lower HDL-C compared with noncarriers after adjustment for co-variates [13]. Moreover, the presence of an ANGPTL3 LOF variant was associated with 41% lower odds of coronary artery disease (OR 0.59, 95% CI 0.41–0.85, $p = 0.004$) compared with noncarriers. In mice, treatment of monoclonal antibody against ANGPTL3 was found to reduce total cholesterol (TC) by − 52% ($p < 0001$) and TGs by − 84% ($p < 0.001$) as well as the decrease in atherosclerotic lesion size and necrotic content in atherosclerotic plaques when compared with control.

The mAb, evinacumab, and ASO therapy, IONIS-ANGPTL3-L_{Rx}, against ANGPLT3 are currently undergoing clinical trial investigation. In a phase I, single-ascending-dose trial of evinacumab of 83 healthy human participants with mild to moderately elevated TG (150–450 mg/dL) or LDL-C (≥100 mg/dL) randomized 3:1 to either single dose administration of evinacumab or placebo, the magnitude of reduction in TG, non-HDL-C, LDL-C, and HDL-C were observed in a dose-dependent manner [38]. The greatest reduction in TGs after a single subcutaneous dose was observed in the 250 mg group at 55.5% when compared to placebo. The greatest reduction in TGs for IV dosing was noted in the 10 mg/kg group at 88.0% when compared with placebo. No serious treatment emergent adverse event (TEAE) was observed, with 51.6% vs 42.9% of participants in the treatment vs placebo group experiencing at least 1 TEAE. There were 11.3% vs 0% of subjects in the treatment vs placebo group who experienced elevated alanine aminotransferase levels, and 6.5% vs 0% had increase in aspartate aminotransferase levels.

Similarly, in a phase I multiple ascending dose study of 56 healthy individuals with TG 150–500 mg/dL) or LDL-C ≥ 100 mg/dL randomized 3:1 to evinacumab (subcutaneously at 150/300/450 mg once weekly, 300/450 mg every 2 weeks, or intravenously at 20 mg/kg once every 4 weeks up to day 56), there was a median reduction in TG and VLDL-C of ~ 70% at day 57 observed in the 300 mg SC every week, 450 mg SC every week, and 20 mg/kg IV every 4 week dosing groups [38]. LDL-C reduction was also observed in all evinacumab groups with the greatest reduction at 57 days in the 300 mg SC every week and the 20 mg/kg IV groups (22.0%, $p = 0.0194$ and 25.1%, $p = 0.0074$, respectively). With respect to safety, 67.7% in the treatment group and 75% in the placebo group experienced at least 1 TEAE with headache being the most common. There were no serious TEAEs, death, or discontinuation due to TEAEs during the study.

Meanwhile, in the phase I trial of IONIS-ANGPTL3-L_{Rx}, per weekly SC administration of ASO therapy for 6 weeks resulted in up to 63.1% reduction in TGs, 36.6% reduction in non-HDL-C, and 25.7% reduction in apoB levels [39]. No serious adverse events were documented during the trial. No significant thrombocytopenia, coagulation abnormalities, bleeding episodes, or evidence of liver or renal dysfunction were observed. Three individuals in the treatment arm and three from the control arm developed dizziness or headache.

SiRNA therapeutics against ANGPTL3 are also being developed. In an early phase 1/2a single-ascending dose study of 40 healthy volunteers, ARO-ANG3 administered was found to reduce TG by 47–53% and VLDL-C by 49–51% at 200 mg and 300 mg SC doses after 16 weeks. Meanwhile, LDL-C was found to be reduced by 33–46% from baseline. No serious adverse effects were noted [8].

One potential utilization of ANGPTL3 inhibition is in the treatment of homozygous familial hypercholesterolemia (HoFH). HoFH is a rare condition but carries significant morbidity and mortality [7, 40]. Current lipid-lowering therapies including statins, ezetimibe, and PCSK9i have had limited success in the treatment of HoFH as they work in an LDL-R–dependent fashion. Evinacumab, which likely acts independently of LDL-R pathway, has shown potential as an effective therapy for this difficult to treat disease. In a single group open-label study of 9 patients with HoFH who were already on aggressive lipid-lowering therapy, treatment with evinacumab 250 mg SC on day 1 and then 15 m/kg IV on day 15 resulted in a mean reduction in LDL-C by 49 ± 23% at week 4 [41]. Preliminary data from a phase 3 randomized controlled trial of evinacumab in the treatment of HoFH, which have not yet been published as of the writing of this review, was presented at the American College of Cardiology 2020 Scientific Session [42]. The study enrolled patients with a diagnosis of HoFH (by at least 1 of the following criteria: homozygous mutations in both *LDLR* alleles; homozygous or compound heterozygous mutations in *APOB* or *PCSK9*; double heterozygous mutations or patients with homozygous *LDLRAP1* mutations; untreated TC > 500 mg/dL and TG < 300 mg/dL; and both parents with history of TC > 250 mg/dL or cutaneous or tendinous xanthomas before age 10 years), and with LDL-C ≥ 70mg/dL on stable, maximally tolerated lipid-lowering therapy.

A total of 65 participants were randomized 2:1 to either evinacumab 15 mg/kg IV every 4 weeks or placebo IV every 4 weeks for 24 weeks. The mean baseline LDL-C was 259.5 mg/dL for the evinacumab group and 246.5 mg/dL for the placebo group. At 24 weeks, the least square (LS) mean difference for LDL-C percent change in the evinacumab group versus placebo was − 49.0 ± 8.0%, $p < 0.0001$. The LS mean difference of absolute change in LDL-C was − 132.1 ± 21.5 mg/dL, $p < 0.0001$. Importantly, the significant effect was observed even among individuals with null/null mutations. A significant LS mean reduction was also observed in TC (− 48.4%, $p < 0.0001$), apoB (− 36.9%, $p < 0.0001$), non-HDL-C (− 51.7%, $p < 0.0001$), and triglycerides (− 50.4%, $p < 0.0001$). With regard to safety, there were numerically less TEAEs in the evinacumab compared with the placebo group

(65.9% vs 81.0%). However, there were numerically more, though rare, serious adverse events (SAEs) in the evinacumab group compared with placebo (4.5% vs 0%).

ApoC3

A causal relationship between TRL, including very low-density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and remnant particles, with ASCVD is suggested in Mendelian randomization studies [5, 43]. Though elevated serum TG represent a marker for ASCVD risk, it is not clear if the TGs within these lipoprotein particles directly lead to atherogenesis or if other properties of the TRL including size and cholesterol content are more contributory [44]. For instance, larger TG-rich particles such as chylomicrons are too large to cross arterial walls; smaller TRLs such as remnant particles are thought to be atherogenic [45, 46]. Severely elevated TGs can also lead to acute pancreatitis, which confers significant morbidity and mortality.

ApoC3 is glycoprotein present on VLDL, LDL, Lp(a), and HDL particles. It is a key regulator of TRL metabolism via inhibition of lipoprotein lipase (LPL) activity and interference of hepatic uptake of TRL likely by disruption of binding to LDLR [47, 48]. In genetic studies, heterozygous carriers of LOF mutations of *APOC3* were found to have 46% lower levels of APOC3, 39% lower TG level, and 40% lower risk for CHD compared with non-carriers [49]. In models adjusted for age and sex, each reduction of apoC3 by 1 mg/dL was estimated to be associated with 4% risk reduction in CHD.

Volanesorsen is a subcutaneously injected ASO against apoC3 mRNA. In a phase 2, dose-ranging trial, 57 patients with untreated hypertriglyceridemia (350 to 2000 mg/dL) or treated hypertriglyceridemia (225 to 2000 mg/dL) on stable fibrate therapy were randomized to doses from 100 to 300 mg of volanesorsen or placebo every week for 13 weeks [50]. The mean baseline TG in the untreated cohort was 581 ± 291mg/dL and 376 ± 188mg/dL in the fibrate-treated cohort. Treatment with volanesorsen resulted in a dose-dependent reduction in apoC3 level of up to approximately 80% with a concurrent reduction of TG of approximately 71%. Furthermore, the inhibition of APOC3 by volanesorsen was found to lower apoC3 on apoB-100, Lp(a) and apolipoprotein A-I (apoA-I) lipoproteins, plasma levels of apoC2, triacylglycerols and diacylglycerols as well as increase levels of apoA-I, apoA-2, and apoM, and improved insulin sensitivity [51, 52].

Volanesorsen has further been studied in 2 phase 3 trials in patients with severely elevated TGs. In the APPROACH trial, 66 patients with familial chylomicronemia syndrome (FCS) with median fasting TG 1985 mg/dL were randomized to weekly volanesorsen 300 mg administered subcutaneously vs placebo over 52 weeks. At 3 months, there was a significant mean reduction of apoC3 by 84% observed in the volanesorsen group vs 6.1% increase in the placebo group [19]. Patients on treatment showed a 77% decrease in mean TGs compared with 18% increase in the placebo group. However, there was a significant number of patients in the treatment group with thrombocytopenia (15 of 33 with plt < 100,000 and 2 patients with platelets < 25,000 per microliter). In the COMPASS trial, 113 patients with baseline TG ≥ 500mg/dL were randomized 2:1 to receive subcutaneous volanesorsen vs placebo for 26 weeks. At 3 months, patients on volanesorsen achieved an approximately 73% reduction in TG compared with 2% mean reduction in those treated with placebo [53]. There was further a significant reduction in pancreatitis in the volanesorsen group compared with placebo in the APPROACH and COMPASS trials [54]. A retrospective survey of 22 patients with FCS treated with volanesorsen suggests an improvement in symptoms including steatorrhea, pancreatic pain, and emotional stress [55]. Due to concern over thrombocytopenia, volanesorsen was not approved for commercial use by the Food and Drug Administration but was approved by the European Medicines Agency (EMA).

Another ASO therapy against apoC3 is currently under investigation. This new-generation design incorporates the GalNac conjugate allowing localization of drug to the liver. In multi-dose dose-escalation trial (15 or 30 mg weekly or 60 mg every 4 weeks administered subcutaneously) over 3 months, AKCEA-APOCIII-L_{Rx} was found to reduce TG by up to 73% (in the 30 mg weekly group) after 43 days [18]. Moreover, non-HDL-C was reduced up to 30.7%, LDL-C was reduced up to 21.6%, and HDL-C increased up to 75.8% (all in the 60 mg every 4 weeks group). Overall, the study drug was well tolerated with similar rates of TEAE between groups and without evidence of significant thrombocytopenia.

Finally, ARO-APOC3 is a siRNA biologic that is in early stage clinical trial. In a phase 1/2 a single dose-ranging study, 40 healthy volunteers with fasting TG > 80 mg/dL were randomized to either treatment or placebo. The reduction in TG and VLDL-C was 41–55% and 42–53% respectively after 16 weeks. There were not serious adverse events noted during the study [8].

Lp(a)

Lp(a) has been shown to be a risk factor for atherosclerosis in epidemiology, and Mendelian randomization studies suggest a likely causal association between elevated Lp(a) levels and premature ASCVD [56–62]. In a large epidemiological study, association between Lp(a) and CHD appeared to be curvilinear, with increased relative risk (RR) of CHD estimated to be 1.13, 95% CI 1.09–1.18 per 3.5-fold (1 SD) increase in Lp(a) after adjustment for traditional risk factors including total cholesterol [63]. It is a cholesterol-rich lipoprotein bound by apoB in addition to apoliprotein (a) (apo [a]), which is encoded by the *LPA* gene and contains from 3 to > 50 kringle motifs

similar to those found on plasminogen but does not have any fibrinolytic activity. These molecular properties are thought to contribute to increased atherothrombotic properties. Moreover, Lp(a) has been shown to bind oxidized phospholipids and localize within the arterial wall, contributing to increased inflammation and atherogenesis. Lp(a) level is strongly determined by genetics with genotype accounting for 90% of the plasma concentration [64].

With growing recognition of Lp(a) as a risk factor for ASCVD, testing for Lp(a) has made its way into mainstream cardiology practice. In a European Society of Cardiology (ESC) consensus document, screening for Lp(a) is recommended in those with elevated CVD/CHD risk [64]. The 2018 Multi-society Guideline on the Management of Blood Cholesterol has included Lp(a) ≥ 50 mg/dL or ≥ 125 nmol/L (if measured) as a risk-enhancing factor [65]. Currently, therapies that lower Lp(a) levels are limited. Niacin decreases Lp(a) level but is not always well tolerated and has limited use in patients with diabetes mellitus. PCSK9 inhibitors have also demonstrated some efficacy in reducing Lp(a), but the degree of reduction may not be sufficient in significantly impacting progression of atherosclerosis and reducing ASCVD risk independent of effect on LDL-C [66–68].

The development of an ASO against apo(a), AKCEA-APO(a)-L_{Rx}, provides a potential means of directly targeting Lp(a). In a phase 2 dose-ranging, randomized, placebo-controlled trial involving 286 participants with established CVD and Lp(a) of 150 nmol/L were randomized 5:1 to receive subcutaneous AKCEA-APO(a)-L_{Rx} (20, 40, or 60 mg every 4 weeks; 20 mg every 2 weeks; or 20 mg every week) or placebo for 6 to 12 months [69]. The median baseline Lp(a) levels in the different groups ranged from 205 to 247 nmol/L. A dose-dependent response in decrease in Lp(a) was observed. The maximum mean decrease in Lp(a) was 80% at 6 months, observed with the 20 mg every week dosing. Mean percent decreases of Lp(a) among other dosing regimen ranged from 35 to 72% compared with 6% observed with placebo at 6 months. Adverse events occurred in 90% of patients in the treatment groups and 83% of those in the placebo group. Serious adverse events occurred in 10% of patients receiving active therapy and 2% of those receiving placebo. The most common adverse side effect was injection site reactions. There were no significant changes in platelet, renal, or liver functions observed. Genetic and epidemiological data predicts decrease in risk for coronary artery disease with pharmacologic reduction of Lp(a) among patients with high levels [70]. However, a Mendelian randomization analysis showed that Lp(a) concentration may need to be lowered significantly, approximately by 100 mg/dL in order to achieve the same level in CHD risk reduction as can be achieved by lowering LDL-C by ~ 39 mg/dL [58]. The phase 3 outcome study of AKCEA-APO(a)-L_{Rx}, Lp(a)HORIZON, is currently underway to assess the efficacy and safety of this agent in the reduction of ASCVD risk. The trial has an estimated enrollment of over 7000 participants with established ASCVD, Lp(a) ≥ 70mg/dL at screening, and on optimal LDL-C– lowering therapy [71]. Lastly, the phase 1 study of the siRNA agent targeting Lp(a), AMG890, is being conducted [72].

Future Directions

While LDL-C remains an important contributor to ASCVD risk, genetic studies coupled with large epidemiological data have identified other causal risk factors including TRLs and Lp(a), which have in turn become therapeutic targets for novel drug development. There is a wide array of biotechnological therapies under clinical investigation. The therapies discussed above have demonstrated powerful effect in the reduction of targeted lipid parameters in short-term studies. Outside of PCSK9i mAbs, these agents still require validation in reducing ASCVD risk in cardiovascular outcome trials and must demonstrate safety in long-term studies. Moreover, there remains a need for therapies that address high-risk primary prevention, and future studies are warranted to assess the utility of the above novel agents in this patient population. Aside from the significant reduction in lipids, the advantage of these agents compared with small molecule medications is the duration of effect, which allows for longer duration between doses and has the potential of improving medication adherence. In fact, in the case of gene-editing therapies, patients may only need a single treatment which will last for life.

One important consideration for these new technologies will be cost-effectiveness, which can impact their scope of use. These agents will likely be first used in patients with the highest risk for ASCVD, i.e., high-risk secondary prevention patients or patients with FH. As the field of cardiac prevention continues to evolve, a more personalized approach may include more detailed characterization of a patient's dyslipidemia and ASCVD risk profile through deep phenotyping or genotyping, which can then dictate the regimen of the most effective lipid-lowering therapies.

Compliance with Ethical Standards

Conflict of Interest SSV: research support (Department of Veterans Affairs, World Heart Federation Tahir and Jooma Family); honorarium: American College of Cardiology (associate editor for Innovations, acc.org); steering committee member (Patient and Provide Assessment of Lipid Management [PALM] registry (no financial remuneration). CMB: grants/research support (significant; paid to institution, not individual) and consultant (modest): Abbott Diagnostic, Denka Seiken, Roche Diagnostic. XJ, JL, and AM do not have any conflicts of interest to disclose.

References

1. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. 2017;38(32):2459–72.
2. Baigent C, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, et al. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet. 2010;376(9753):1670–81.
3. Phan BA, Dayspring TD, Toth PP. Ezetimibe therapy: mechanism of action and clinical update. Vasc Health Risk Manag. 2012;8:415–27.
4. Saeed A, Ballantyne CM. Bempedoic acid (ETC-1002): a current review. Cardiol Clin. 2018;36(2):257–64.
5. Musunuru K, Kathiresan S. Surprises from genetic analyses of lipid risk factors for atherosclerosis. Circ Res. 2016;118(4):579–85.
6. Ference BA, Robinson JG, Brook RD, Catapano AL, Chapman MJ, Neff DR, et al. Variation in PCSK9 and HMGCR and risk of cardiovascular disease and diabetes. N Engl J Med. 2016;375(22):2144–53.
7. Brautbar A, Leary E, Rasmussen K, Wilson DP, Steiner RD, Virani S. Genetics of familial hypercholesterolemia. Curr Atheroscler Rep. 2015;17(4):491.
8. Hussain A, Ballantyne CM, Saeed A, Virani SS. Triglycerides and ASCVD risk reduction: recent insights and future directions. Curr Atheroscler Rep. 2020;22(7):25.
9. Macchi C, Sirtori CR, Corsini A, Santos RD, Watts GF, Ruscica M. A new dawn for managing dyslipidemias: the era of rna-based therapies. Pharmacol Res. 2019;150:104413.
10. Furgurson M, Lagor WR. CRISPR: a promising tool for lipid physiology and therapeutics. Curr Opin Lipidol. 2019;30(3):172–6.
11. Schwartz GG, Steg PG, Szarek M, Bhatt DL, Bittner VA, Diaz R, et al. Alirocumab and cardiovascular outcomes after acute coronary syndrome. N Engl J Med. 2018;379(22):2097–107.
12. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, et al. Evolocumab and clinical outcomes in patients with cardiovascular disease. N Engl J Med. 2017;376(18):1713–22.
13. Dewey FE, Gusarova V, Dunbar RL, O'Dushlaine C, Schurmann C, Gottesman O, et al. Genetic and pharmacologic inactivation of ANGPTL3 and cardiovascular disease. N Engl J Med. 2017;377(3):211–21.
14. Ridker PM, Revkin J, Amarenco P, Brunell R, Curto M, Civeira F, et al. Cardiovascular efficacy and safety of bococizumab in high-risk patients. N Engl J Med. 2017;376(16):1527–39.
15. Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. Cell. 2009;136(4):642–55.
16. Shen X, Corey DR. Chemistry, mechanism and clinical status of antisense oligonucleotides and duplex RNAs. Nucleic Acids Res. 2018;46(4):1584–600.
17. Huang Y. Preclinical and clinical advances of GalNAc-decorated nucleic acid therapeutics. Mol Ther Nucleic Acids. 2017;6:116–32.
18. Alexander VJ, Xia S, Hurh E, Hughes SG, O'Dea L, Geary RS, et al. N-acetyl galactosamine-conjugated antisense drug to APOC3 mRNA, triglycerides and atherogenic lipoprotein levels. Eur Heart J. 2019;40(33):2785–96.
19. Blom DJ, O'Dea L, Digenio A, Alexander VJ, Karwatowska-Prokopczuk E, Williams KR, et al. Characterizing familial chylomicronemia syndrome: baseline data of the APPROACH study. J Clin Lipidol. 2018;12(5):1234–43.e5.
20. Hu B, Weng Y, Xia XH, Liang XJ, Huang Y. Clinical advances of siRNA therapeutics. J Gene Med. 2019;21(7):e3097.
21. Akdim F, Stroes ES, Sijbrands EJ, Tribble DL, Trip MD, Jukema JW, et al. Efficacy and safety of mipomersen, an antisense inhibitor of apolipoprotein B, in hypercholesterolemic subjects receiving stable statin therapy. J Am Coll Cardiol. 2010;55(15):1611–8.
22. Fogacci F, Ferri N, Toth PP, Ruscica M, Corsini A, Cicero AFG. Efficacy and safety of mipomersen: a systematic review and meta-analysis of randomized clinical trials. Drugs. 2019;79(7):751–66.
23. Cameron J, Holla OL, Ranheim T, Kulseth MA, Berge KE, Leren TP. Effect of mutations in the PCSK9 gene on the cell surface LDL receptors. Hum Mol Genet. 2006;15(9):1551–8.
24. Ruscica M, Corsini A, Ferri N, Banach M, Sirtori CR. Clinical approach to the inflammatory etiology of cardiovascular diseases. Pharmacol Res. 2020;159:104916.
25. Ruscica M, Tokgozoglu L, Corsini A, Sirtori CR. PCSK9 inhibition and inflammation: a narrative review. Atherosclerosis. 2019;288:146–55.
26. Giugliano RP, Mach F, Zavitz K, Kurtz C, Im K, Kanevsky E, et al. Cognitive function in a randomized trial of evolocumab. N Engl J Med. 2017;377(7):633–43.
27. Sabatine MS, De Ferrari GM, Giugliano RP, Huber K, Lewis BS, Ferreira J, et al. Clinical benefit of evolocumab by severity and extent of coronary artery disease: analysis from FOURIER. Circulation. 2018;138(8):756–66.
28. Bonaca MP, Nault P, Giugliano RP, Keech AC, Pineda AL, Kanevsky E, et al. Low-density lipoprotein cholesterol lowering with evolocumab and outcomes in patients with peripheral artery disease: insights from the FOURIER trial (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk). Circulation. 2018;137(4):338–50.
29. Raal FJ, Kallend D, Ray KK, Turner T, Koenig W, Wright RS, et al. Inclisiran for the treatment of heterozygous familial hypercholesterolemia. N Engl J Med. 2020;382(16):1520–30.
30. Ray KK, Wright RS, Kallend D, Koenig W, Leiter LA, Raal FJ, et al. Two phase 3 trials of inclisiran in patients with elevated LDL cholesterol. N Engl J Med. 2020;382(16):1507–19.
31. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol. 2019;73(24):e285–350.
32. Jia X, Al Rifai M, Birnbaum Y, Smith SC, Virani SS. The 2018 cholesterol management guidelines: topics in secondary ASCVD prevention clinicians need to know. Curr Atheroscler Rep. 2019;21(6):20.
33. Kazi DS, Virani SS. Implications of cost-effectiveness analyses of lipid-lowering therapies: from the policy-maker's desk to the patient's bedside. Prog Cardiovasc Dis. 2019;62(5):406–13.
34. Fonarow GC, van Hout B, Villa G, Arellano J, Lindgren P. Updated cost-effectiveness analysis of evolocumab in patients with very high-risk atherosclerotic cardiovascular disease. JAMA Cardiol. 2019;4(7):691–5.
35. Musunuru K, Pirruccello JP, Do R, Peloso GM, Guiducci C, Sougnez C, et al. Exome sequencing, ANGPTL3 mutations, and familial combined hypolipidemia. N Engl J Med. 2010;363(23):2220–7.
36. Wang Y, Gusarova V, Banfi S, Gromada J, Cohen JC, Hobbs HH. Inactivation of ANGPTL3 reduces hepatic VLDL-triglyceride secretion. J Lipid Res. 2015;56(7):1296–307.
37. Pisciotta L, Favari E, Magnolo L, Simonelli S, Adorni MP, Sallo R, et al. Characterization of three kindreds with familial combined hypolipidemia caused by loss-of-function mutations of ANGPTL3. Circ Cardiovasc Genet. 2012;5(1):42–50.

38. Ahmad Z, Banerjee P, Hamon S, Chan KC, Bouzelmat A, Sasiela WJ, et al. Inhibition of angiopoietin-like protein 3 with a monoclonal antibody reduces triglycerides in hypertriglyceridemia. Circulation. 2019;140(6):470–86.
39. Graham MJ, Lee RG, Brandt TA, Tai LJ, Fu W, Peralta R, et al. Cardiovascular and metabolic effects of ANGPTL3 antisense oligonucleotides. N Engl J Med. 2017;377(3):222–32.
40. Soutar AK, Naoumova RP. Mechanisms of disease: genetic causes of familial hypercholesterolemia. Nat Clin Pract Cardiovasc Med. 2007;4(4):214–25.
41. Gaudet D, Gipe DA, Pordy R, Ahmad Z, Cuchel M, Shah PK, et al. ANGPTL3 inhibition in homozygous familial hypercholesterolemia. N Engl J Med. 2017;377(3):296–7.
42. Raal F, editor Evinacumab in patients with homozygous familial hypercholesterolemia. American College of Cardiology 2020 Scientific Session; 2020.
43. Laufs U, Parhofer KG, Ginsberg HN, Hegele RA. Clinical review on triglycerides. Eur Heart J. 2020;41(1):99–109c.
44. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. JAMA. 2007;298(3):299–308.
45. Nordestgaard BG, Stender S, Kjeldsen K. Reduced atherogenesis in cholesterol-fed diabetic rabbits. Giant lipoproteins do not enter the arterial wall. Arteriosclerosis. 1988;8(4):421–8.
46. Sarwar N, Danesh J, Eiriksdottir G, Sigurdsson G, Wareham N, Bingham S, et al. Triglycerides and the risk of coronary heart disease: 10,158 incident cases among 262,525 participants in 29 Western prospective studies. Circulation. 2007;115(4):450–8.
47. Ginsberg HN, Le NA, Goldberg IJ, Gibson JC, Rubinstein A, Wang-Iverson P, et al. Apolipoprotein B metabolism in subjects with deficiency of apolipoproteins CIII and AI. Evidence that apolipoprotein CIII inhibits catabolism of triglyceride-rich lipoproteins by lipoprotein lipase in vivo. J Clin Invest. 1986;78(5):1287–95.
48. Sehayek E, Eisenberg S. Mechanisms of inhibition by apolipoprotein C of apolipoprotein E-dependent cellular metabolism of human triglyceride-rich lipoproteins through the low density lipoprotein receptor pathway. J Biol Chem. 1991;266(27):18259–67.
49. Crosby J, Peloso GM, Auer PL, Crosslin DR, Stitziel NO, Lange LA, et al. Loss-of-function mutations in APOC3, triglycerides, and coronary disease. N Engl J Med. 2014;371(1):22–31.
50. Gaudet D, Alexander VJ, Baker BF, Brisson D, Tremblay K, Singleton W, et al. Antisense inhibition of apolipoprotein C-III in patients with hypertriglyceridemia. N Engl J Med. 2015;373(5):438–47.
51. Pechlaner R, Tsimikas S, Yin X, Willeit P, Baig F, Santer P, et al. Very-low-density lipoprotein-associated apolipoproteins predict cardiovascular events and are lowered by inhibition of APOC-III. J Am Coll Cardiol. 2017;69(7):789–800.
52. Yang X, Lee SR, Choi YS, Alexander VJ, Digenio A, Yang Q, et al. Reduction in lipoprotein-associated apoC-III levels following volanesorsen therapy: phase 2 randomized trial results. J Lipid Res. 2016;57(4):706–13.
53. Gouni-Berthold I, Alexander V, Digenio A, DuFour R, Steinhagen-Thiessen E, Martin S, et al. Apolipoprotein C-III inhibition with volanesorsen in patients with hypertriglyceridemia (COMPASS): a randomized, double-blind, placebo-controlled trial. Atheroscler Suppl. 2017;28:e1–2.
54. Gelrud A, Digenio A, Alexander V, Williams K, Hsieh A, Gouni-Berthold I, et al. Treatment with volanesorsen (VLN) reduced triglycerides and pancreatitis in patients with FCS and sHTG vs placebo: results of the APPROACH and COMPASS. J Clin Lipidol. 2018;12(2):537.
55. Arca M, Hsieh A, Soran H, Rosenblit P, O'Dea L, Stevenson M. The effect of volanesorsen treatment on the burden associated with familial chylomicronemia syndrome: the results of the ReFOCUS study. Expert Rev Cardiovasc Ther. 2018;16(7):537–46.
56. Clarke R, Peden JF, Hopewell JC, Kyriakou T, Goel A, Heath SC, et al. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. N Engl J Med. 2009;361(26):2518–28.
57. Kamstrup PR, Tybjaerg-Hansen A, Steffensen R, Nordestgaard BG. Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. JAMA. 2009;301(22):2331–9.
58. Burgess S, Ference BA, Staley JR, Freitag DF, Mason AM, Nielsen SF, 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. 2018;3(7):619–27.
59. Saeed A, Sun W, Agarwala A, Virani SS, Nambi V, Coresh J, et al. Lipoprotein(a) levels and risk of cardiovascular disease events in individuals with diabetes mellitus or prediabetes: the Atherosclerosis Risk in Communities study. Atherosclerosis. 2019;282:52–6.
60. Zewinger S, Kleber ME, Tragante V, McCubrey RO, Schmidt AF, Direk K, et al. Relations between lipoprotein(a) concentrations, LPA genetic variants, and the risk of mortality in patients with established coronary heart disease: a molecular and genetic association study. Lancet Diabetes Endocrinol. 2017;5(7):534–43.
61. Virani SS, Brautbar A, Davis BC, Nambi V, Hoogeveen RC, Sharrett AR, et al. Associations between lipoprotein(a) levels and cardiovascular outcomes in black and white subjects: the Atherosclerosis Risk in Communities (ARIC) Study. Circulation. 2012;125(2):241–9.
62. Saeed A, Virani SS. Lipoprotein(a) and cardiovascular disease: current state and future directions for an enigmatic lipoprotein. Front Biosci (Landmark Ed). 2018;23:1099–112.
63. Erqou S, Kaptoge S, Perry PL, Di Angelantonio E, Thompson A, White IR, et al. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. JAMA. 2009;302(4):412–23.
64. Nordestgaard BG, Chapman MJ, Ray K, Borén J, Andreotti F, Watts GF, et al. Lipoprotein(a) as a cardiovascular risk factor: current status. Eur Heart J. 2010;31(23):2844–53.
65. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019;139(25):e1082–e143.
66. Tardif JC, Rhéaume E, Rhainds D, Dubé MP. Lipoprotein (a), arterial inflammation, and PCSK9 inhibition. Eur Heart J. 2019;40(33):2782–4.
67. Ray KK, Vallejo-Vaz AJ, Ginsberg HN, Davidson MH, Louie MJ, Bujas-Bobanovic M, et al. Lipoprotein(a) reductions from PCSK9 inhibition and major adverse cardiovascular events: Pooled analysis of alirocumab phase 3 trials. Atherosclerosis. 2019;288:194–202.
68. Bittner VA, Szarek M, Aylward PE, Bhatt DL, Diaz R, Edelberg JM, et al. Effect of alirocumab on lipoprotein(a) and cardiovascular risk after acute coronary syndrome. J Am Coll Cardiol. 2020;75(2):133–44.
69. Tsimikas S, Karwatowska-Prokopczuk E, Gouni-Berthold I, Tardif JC, Baum SJ, Steinhagen-Thiessen E, et al. Lipoprotein(a) reduction in persons with cardiovascular disease. N Engl J Med. 2020;382(3):244–55.

70. Gudbjartsson DF, Thorgeirsson G, Sulem P, Helgadottir A, Gylfason A, Saemundsdottir J, et al. Lipoprotein(a) concentration and risks of cardiovascular disease and diabetes. J Am Coll Cardiol. 2019;74(24):2982–94.
71. ClinicalTrials.org. Assessing the impact of lipoprotein (a) lowering with TQJ230 on major cardiovascular events in patients with CVD (Lp(a)HORIZON). 2020.
72. ClinicalTrials.org. Safety, tolerability, pharmacokinetics and pharmacodynamics study of AMG 890 in subjects with elevated plasma lipoprotein(a). 2020.