There is a wealth of evidence showing the beneficial effects of lowering low-density lipoprotein-cholesterol (LDL-C) levels by statins on cardiovascular disease (CVD) outcome. The meta-analysis of intervention trials has shown that every 1 mmol/L decrease in LDL-C is associated with an estimated 22% reduction of CVD events and a 10% reduction of all-cause mortality.1 However, a large residual disease burden remains, even in patients treated with statins and other CVD risk modifying interventions. This residual risk has a large impact on both individuals as well as on global healthcare costs and, as a consequence, novel targets for therapy are eagerly sought.2 High-density lipoprotein-cholesterol (HDL-C) has been considered one of the targets for therapy given the unequivocal evidence of an inverse association between HDL-C levels and CVD risk.3 It is of note that this association is also found in patients who are treated with statins, irrespective of the attained LDL-C level.4,5 This epidemiological association is supported by several biological plausible mechanisms.

Abstract: Cardiovascular disease (CVD) remains a major burden for morbidity and mortality in the general population, despite current efficacious low-density lipoprotein-cholesterol–lowering therapies. Consequently, novel therapies are required to reduce this residual risk. Prospective epidemiological studies have shown that high-density lipoprotein-cholesterol (HDL-C) levels are inversely correlated with cardiovascular disease risk, and this initiated the quest for HDL-C–increasing therapies. Consequently, several different targets in HDL metabolism have been identified. Initial studies addressing the effect of cholesteryl ester transfer protein inhibition on cardiovascular disease outcome have been discontinued for reasons of futility or increased mortality. As of yet, 2 cholesteryl ester transfer protein inhibitors are still in phase III studies. Other HDL-based interventions, such as apolipoprotein A1–based compounds, ABC-transporter upregulators, selective peroxisome proliferator–activated receptor modulators and lecithin-cholesterol acyltransferase–based therapy, hold great promise for the future. The aim of this review is to provide a comprehensive overview of HDL-targeted pharmaceutical strategies in humans, both in early development as well as in late stage clinical trials. (Circ Res. 2014;114:193-204.)

Key Words: cardiovascular diseases ■ lipoproteins, HDL ■ reverse cholesterol transport

Novel Therapies Focused on the High-Density Lipoprotein Particle

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Regulation of High-Density Lipoprotein Metabolism [Circ Res. 2014;114:143–156]

Novel Therapies Focused on the High-Density Lipoprotein Particle
High-Density Lipoprotein and Atherosclerosis Regression: Evidence From Preclinical and Clinical Studies
Population Genetics
Cholesterol Efflux and Reverse Cholesterol Transport
Proteomics and Particles

Alan Tall and Daniel Rader, Editors
Among the large number of antiatherogenic properties ascribed to HDL, its role in reverse cholesterol transport (RCT) is commonly considered to be crucial. In RCT, free cholesterol contained within macrophages in the vessel wall is taken up by the HDL-particle and subsequently transported to the liver for excretion into bile. In addition to RCT, HDL has anti-inflammatory, antioxidant, and antithrombotic properties. These biological activities are mainly studied in vitro and in animal models, and translation of the epidemiological findings to a direct causal role for HDL-C in atherosclerosis has been difficult, partly because of the fact that virtually all CVD risk factors (i.e., obesity, the metabolic syndrome, smoking, and physical inactivity) are associated with low HDL-C levels. The hypothesis that HDL-C directly confers biological protection against atherosclerosis has, as of yet, never been proved and, as a consequence, HDL-C has been argued to be merely a CVD biomarker rather than an active player in atherogenesis. This notion has gained support because therapies with an established HDL-C increasing effect were shown not to result in the anticipated decrease in CVD risk. In particular, 3 large outcome trials were prematurely terminated for futility (Athero-thrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health Outcomes [AIM-HIGH] trial, Heart Protection Study 2-Treatment of HDL to Reduce the Incidence of Vascular Events [HPS2-THRIVE] study, the Dalcetrapib CVD Outcome Study [DAL-OUTCOMES]), despite significant HDL-C increases.

In addition, data from Mendelian randomization studies have shown that common variants that raise HDL-C levels are not associated with a proportionally lower CVD risk. It is of note that variants with an effect on LDL-C levels were associated with altered CVD risk in the anticipated direction, which further strengthens the notion that LDL-C is directly involved in atherosclerosis. Recent case–control studies have shown that cholesterol efflux capacity of apo-B–depleted serum is inversely associated with CVD, independent of HDL-C levels. This finding lends support for pharmaceutical strategies targeting HDL-particle functionality, rather than HDL-C concentration alone. However, it should be noted that the role of cholesterol efflux capacity is controversial because it was recently shown that enhanced cholesterol efflux was associated with incident risk for myocardial infarction, stroke, or death in a longitudinal cohort study of stable patients with CVD. The cause of the paradoxical findings is incompletely understood.

Despite the equivocal role of HDL-C levels in atherosclerosis, several HDL-targeted therapies are still in different stages of development. The results of the studies addressing the effects of these therapies are crucial not only from a clinical perspective but also to increase our understanding of the relevance of players in HDL metabolism for CVD outcome.

The aim of this review is to provide a comprehensive overview of the pharmaceutical strategies, in different stages of development, to increase HDL-C in humans. An overview of the HDL-targeted drugs and their current stages of development are provided in the Table.

### Antiatherogenic Properties of HDL: The Basis for Novel Therapeutics

The beneficial effects of HDL in protecting against the development of CVD are thought to be mediated predominantly through induction of cellular cholesterol efflux and RCT from peripheral tissues to the liver. Apolipoprotein A1 (apoA-1) is secreted by the liver and the small intestine and upon secretion in the circulation, apoA-1 interacts with the ATP-binding cassette transporter A1 (ABCA1) located in the liver and peripheral macrophages/foam cells, which results in the uptake of cholesterol by apoA-1. This interaction leads to the formation of nascent preβ discoid HDL-particles. The enzyme lecithin-cholesterol acyltransferase (LCAT) esterifies the free cholesterol located at the surface of the newly formed HDL-particle. Because cholesteryl esters are hydrophobic, LCAT activity therefore results in trapping of cholesterol in the core of the HDL-particle, thereby generating capacity for additional cholesterol uptake at the surface of the lipoprotein. The accumulation of cholesteryl esters in the core transforms the lipid poor discoid preβ particles into larger spherical HDL-2 and HDL-3 particles. These particles are still capable of acquiring free cholesterol from peripheral macrophages/foam cells by interaction with the ATP-binding cassette transporter G1 (ABCG1). Two established and independent physiological mechanisms are responsible for the subsequent clearance of cholesteryl esters transported by HDL. First, cholesteryl esters from HDL-3 particles can be selectively taken up via the hepatic scavenger receptor B1. Alternatively, cholesteryl esters are cleared via the cholesteryl ester transfer protein (CETP)-mediated pathway. CETP is responsible for the shuttling of cholesteryl esters from HDL to apoB-containing particles in return for TGs. The apoB-containing particles are then taken up by LDL-receptors on hepatocytes. On hepatic uptake, cholesteryl esters are excreted into bile or converted to bile acids. A review on the role of RCT in atheroprotection has recently been published by Rosenson et al. Virtually all HDL-targeted therapies currently in development in humans do target one of the abovementioned pivotal players in RCT. In addition to its pivotal role in RCT, HDL exerts important anti-inflammatory, antioxidant, and antithrombotic properties.
Established Drug Therapies

Statins
Statins are the cornerstone in the treatment of patients at risk for CVD. As such, current guidelines proclaim their almost universal use in patients at risk for CVD. In addition to LDL-C lowering, statins also do have a small effect on HDL-C levels. In a large meta-analysis, it was shown that this increase was dose and statin dependent, with HDL-C increases ranging from 2.3% to 7.9%.23 Interestingly, this effect was not correlated with the effect size of the LDL-C reduction. In fact, baseline HDL-C and TG levels were the best independent predictors of statin-induced HDL-C elevations. There is controversy concerning the effect of this HDL-C increase on CVD outcome data,24,25 and this might be related to the differences in HDL-C levels at baseline in these studies because it has been suggested that only patients with low HDL-C baseline values benefit from the statin-induced HDL-C increase.23,26,27

Nicotinic Acid
Nicotinic acid has been in clinical use since 1954 and has been the topic of a plethora of clinical studies without ever reaching clear consensus on its clinical use. Although the extended release (ER) formulation of nicotinic acid (ER niacin) has been shown to increase HDL-C levels and lower TG, LDL-C, and lipoprotein(a) levels, its clinical use is severely hampered by its common side-effect of cutaneous flushing.28 Over the past decades, several small-scaled clinical studies in both patients with and without statin therapy have been performed, and some have suggested a beneficial effect of ER niacin on cardiovascular risk. The recent AIM-HIGH9 trial investigated the effect of 2 doses of ER niacin compared with placebo in 3414 patients with established CVD in addition to statin and ezetimibe treatment. After 3 years of follow-up, the study was prematurely terminated for futility (hazard ratio 1.02; 95% CI [0.87–1.21]; \( P=0.79 \)). However, a recent meta-analysis of 11 randomized controlled trials (9959 patients in total, including AIM-HIGH) did report a significant reduction in CVD composite end points in niacin-treated patients (odds ratio 0.75, 95% CI [0.49–0.89]; \( P=0.0007 \)).29 Last December, Merck & Co announced that the HPS2-THRIVE study was also prematurely terminated after a median follow-up of 3.9 years for both futility as well as an increased adverse event rate in the ER niacin/laropiprant arm.10

A total of 25673 patients with high CVD risk were randomized to either placebo or ER niacin in combination with laropiprant, a specific antagonist of the prostaglandin D2 receptor. The CVD event rate was similar in both groups, despite an average 0.25 mmol/L decrease in LDL-C, 0.16 mmol/L increase in HDL-C, and 0.37 mmol/L decrease in TG in the ER Niacin/laropiprant-treated patients.30 However, it should be noted that during the ER niacin/laropiprant run-in phase (7–10 weeks) 33.1% of patients withdrew from treatment before randomization, mainly because of side-effects. During the study, an additional 25% of patients discontinued from active treatment compared with 17% in the placebo arm. Apart from known side-effects such as flushing, gastrointestinal complaints, and an increased incidence of diabetes mellitus, the authors also reported an increased incidence of major bleeding and infection rates, in addition to myopathy and rhabdomyolysis (which was more common among participants in China).31 Patients enrolled in HPS2-THRIVE had peculiar baseline characteristics: the LDL-C (1.64 mmol/L) and TG (1.43 mmol/L) levels were low, and HDL-C levels were not far from normal (1.14 mmol/L). It is questionable

<table>
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<tr>
<th>Class</th>
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<tr>
<td>ApoA-1 based</td>
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<td>Intravenous apoA-1</td>
<td>MDCO-216</td>
<td>Intravenous</td>
<td>Phase I</td>
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<td>CSL-112</td>
<td></td>
<td>Intravenous</td>
<td>Phase IIa (completed)</td>
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<tr>
<td>CER-001</td>
<td></td>
<td>Intravenous</td>
<td>Phase I</td>
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<td>Autologous HDL delipidation</td>
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<td>apoA-1 mimetics</td>
<td>FX-5A</td>
<td>intravenous</td>
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<tr>
<td>ETC-642</td>
<td></td>
<td>intravenous</td>
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<tr>
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<td>RVX-208</td>
<td>oral</td>
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<td>LXR agonists</td>
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<td>K-877</td>
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Table. Overview of Classes of HDL-Based Agents Tested in Humans
whether in real-life clinicians would consider prescribing 2 g of ER niacin/laropiprant to patients with similar lipid profiles. Nevertheless, with 2 large outcome trials being prematurely terminated for futility and taking the high prevalence of side-effects into account, ER niacin is unlikely to become a significant player in CVD risk reduction therapy.

**Fibrates**

Fibrates are fibric acid derivates that activate peroxisome proliferator-activated receptor alpha (PPAR-α), a nuclear receptor that regulates many aspects of lipoprotein metabolism. Fibrates have been in use during the past 4 decades and, in general, a decrease of TG levels by 30% to 50% and an increase in HDL-C levels by 5% to 15% have been noted. Fibrates also decrease LDL-C levels in some individuals, but this effect has not been reported consistently.  

Several studies have been performed to assess the efficacy of fibrates on CVD end points, but the exact significance remains elusive. Jun et al performed a large meta-analysis comprising 45,508 individuals and found a significant reduction in CVD event rates associated with fibrate therapy, but no decrease in CVD-related or all-cause mortality. Lee et al also performed a meta-analysis on subgroups within the rather heterogeneous studies and found a statistically significant cardiovascular risk reduction in patients with elevated TG and low HDL-C levels, with a stronger beneficial effect for individuals meeting both criteria. However, in patients with normal TG and HDL-C levels, there was no significant reduction in cardiovascular events compared with placebo. All but one of these studies compared the effect of fibrate monotherapy with that of placebo. In the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study, Ginsberg et al investigated the effect of fenofibrate on top of statin therapy in 5,518 patients with type 2 diabetes mellitus in a placebo-controlled trial and found no significant effects of fenofibrate on cardiovascular outcome. This finding is highly relevant given the fact that any future lipid-lowering therapy will be started in conjunction with statins.

All together, one can conclude that there is scant evidence on the efficacy of fibrates on CVD outcomes, once started on top of statin therapy. This is underlined by the recently updated statement by the European Medicines Agency that fibrates should not be used as first line treatment except for patients with severe hypertriglyceremia and patients for whom statin therapy is contraindicated or who do not tolerate statin therapy. This is underlined by the recently updated statement by the European Medicines Agency that fibrates should not be used as first line treatment except for patients with severe hypertriglyceremia and patients for whom statin therapy is contraindicated or who do not tolerate statin therapy. It is of note that more specific and more potent PPAR-α agonists are currently in development, which will be discussed further on in this review.

**Future Therapies in Development**

**ApoA-1–Based Therapies**

ApoA-1 is the major structural protein of the HDL-particle, and the interaction of apoA-1 with membrane bound ABCA1 initiates cholesterol efflux, which is the initial step in RCT. ApoA-1 plays a pivotal role in RCT and has been shown to exert direct anti-inflammatory effects. Patients with molecular defects in the gene encoding apoA-1 are characterized by increased atherosclerosis and CVD risk, and studies in rodents showed atherosclerotic lesion regression on apoA-1 infusions. These results have resulted in a strive to generate apoA-1–oriented pharmacotherapeutic strategies. In this section, we summarize the data from human studies on (1) HDL/apoA-1 intravenous infusion therapy, (2) apoA-1 mimetic peptides, and (3) RVX-208, a novel apoA-1 inducing agent.

**ApoA-1–Based Infusion Therapy**

Several particle-based therapies are in development for intravenous administration in humans. It is hypothesized that apoA-1 infusion may be beneficial in the setting of an acute coronary syndrome because even single IV administration in mice showed rapid changes in plaque composition.  

The rationale to study the effect of this specific lipoprotein particle is based on the observation that carriers of the apoA-1 Milano mutation are characterized by low prevalence of CVD events, despite markedly decreased HDL-C levels. The potential cardioprotective characteristics of this mutated apoA-1 have been ascribed to a greater cholesterol efflux potential as well as to anti-inflammatory and plaque-stabilizing properties. These potential beneficial effects of apoA-1 Milano, however, are controversial because others found no differences in terms of RCT between wild-type and apoA-1 Milano transgenic mice.

The ApoA-1 Milano Trial addressed the effects of apoA-1 Milano infusion in 57 patients who had an acute coronary syndrome (ACS). These patients were randomized to 5 weekly infusions with either placebo or ETC-216 (the former name of the compound) and, before and after 5 infusions, intravascular ultrasonography (IVUS) of the coronary arteries was performed. Infusion of ETC-216 was associated with a significant decrease in plaque size and atheroma volume compared with placebo. Additional analyses of IVUS data also provided support for a beneficial effect of apoA-1 Milano infusion on vessel wall remodeling. Nicholls et al subsequently showed a significant reduction in external elastic membrane volume that correlated with the change in atheroma volume. Although the observed effect sizes were small in absolute terms, these morphological changes are remarkable given the short duration of treatment. A phase Ib/Ia study in humans is currently ongoing with MDCO-216, and an IVUS outcome study is in the preparatory phase. (ETC-216 was renamed MDCO-216 by the new license owner The Medicines Company.)

**CSL-111 and CSL-112**

CSL-111 is a reconstituted HDL-particle comprising both human apoA-1 and soybean phosphatidylcholine. This infusible compound has been studied in the Effect of rHDL on Atherosclerosis-Safety and Efficacy (ERASE) trial, in which 183 patients were randomly assigned to 4 weekly infusions of placebo, 40 mg/kg or 80 mg/kg of CSL-111. The infusions were given within 2 weeks after the patient had an ACS. Before randomization and 2 weeks after the last infusion, IVUS of the coronary arteries was performed. The 80 mg/kg treatment arm was prematurely discontinued because a high incidence of transaminase elevations (with some patients exceeding 100× the upper limit of normal)
was observed. A significant change from baseline in atheroma volume (−3.4%; $P=0.001$) and plaque volume (−5.3%; $P<0.001$) was observed in the 40 mg/kg arm, but this difference did not reach statistical significance ($P=0.39$ and $P=0.47$, respectively) compared with placebo. The authors conclude that there is a suggestion of benefit of CSL-111 infusions and substantiate this by showing significant differences between CSL-111 and placebo for secondary outcomes on angiographic end points. However, the higher incidence of reversible liver transaminase elevations resulted in the discontinuation from further development.

Based on the results of CSL-111, a second-generation compound is in development (CSL-112). However, the specific composition of the particle is not yet published. Results from 2 phase I studies in 93 healthy individuals were presented at the American Heart Association scientific sessions in November 2012.49,50 The single-dose study used doses up to 135 mg/kg. In the multiple-dose study, subjects received either once weekly infusions for 4 weeks of low or high dose CSL-112, a twice weekly infusion schedule for 4 weeks of low dose CSL-112 or placebo infusions (n=9 per group). CSL-112 infusion resulted in a rapid and significant 36-fold increase of the concentration of proβ-HDL-particles, which was reflected by an increased cholesterol efflux capacity mainly through the ABCA1 pathway, as measured by De la Llera-Moya et al.50 Moreover, infusion of CSL-112 induced an increase in HDL-C levels, which remained elevated up to 72 hours after the infusion, whereas no effect on TG and LDL-C levels was seen. Importantly, infusion of CSL-112 was not accompanied by clinically important transaminase or bilirubin elevations. Recently, a phase Ia multicenter study in stable patients with CVD has been completed, and the results are eagerly awaited (ClinicalTrials.gov identifier: NCT01499420). Currently, a multicenter international outcome and safety study in patients with ACS is in the preparatory phase.

**CER-001**

CER-001 is an engineered small HDL protoparticle, comprising recombinant apoA-1 and phospholipids, designed to mimic nascent proβ-HDL. Preβ-HDL-particles are small cholesterol-poor HDL-particles with a high affinity for ABCA1-driven cholesterol efflux from macrophages.51 In an atherosclerosis-prone mouse model, CER-001 infusions resulted in significant reductions of atherogenic plaque size, lipid content, and macrophage content.52 Results of a phase I study in dyslipidemic human volunteers showed that a single administration of CER-001 was safe and well tolerated. A single CER-001 infusion resulted in a marked increase in total cholesterol (up to 80% from pretreatment levels) as well as cholesterol in the HDL-fraction (up to 130% from pretreatment levels), suggesting mobilization of cholesterol from extravascular sources, an effect that was observed even at low doses.53

Currently, several phase II clinical studies are ongoing. In the Can HDL Infusions Significantly Quicken Atherosclerosis Regression (CHI-SQUARE) trial, 504 patients with ACS were randomized to receive 6 weekly infusions of either placebo or 3 dose regimens of CER-001. The primary outcome measure is the change in total athero volume as assessed by IVUS (ClinTrials.gov identifier NCT01201837).

Two additional phase II trials are currently ongoing to assess the effect of chronic weekly CER-001 in homozygous (ho) and heterozygous (he) patients with familial hypercholesterolemia (ClinTrials.gov identifier NCT01412034 and NCT01515241, respectively). The primary outcome for these studies is defined as the percentage change in carotid plaque volume as assessed by 3T magnetic resonance imaging. These trials provide further insights into the therapeutic mechanism of apoA-1–based infusion therapies both as a plaque-stabilizing agent in the acute treatment of ACS and as chronic treatment in extreme high-risk patients, with low HDL-C or high LDL-C levels.

**Delipidated HDL-Infusion**

In an attempt to optimize RCT, an extracorporal method was developed to delipidate HDL-particles derived specifically from plasma, resulting in a massive increase of proβ-like particles. This strategy was proven to be effective in cynomolgus monkeys, where infusion of delipidated plasma resulted in a decrease of diet-induced atherosclerosis.54 Waksman et al.55 tested the efficacy of 7 weekly delipidated HDL plasma infusions in 28 patients with ACS. After selective HDL delipidation, the proβ–HDL-particle concentration was increased by 28-fold. IVUS was performed in relatively small number of patients (n=26), and an important trend in terms of effect size was observed for reduction of total atheroma volume. Potential benefits of this novel method of HDL-infusion are the autologous nature of the particles, which would limit toxicity, and the fact that proβ–HDL-particles, with great cholesterol efflux generating potential, are reinfused. A multicenter international IVUS study is currently in the preparatory phase.

**ApoA-1 Mimetic Peptides**

ApoA-1 mimetic peptides are molecular compounds that mimic the secondary helix-like structure of apoA-1 without sharing primary amino-acid homology. ApoA-1 mimetic peptides are smaller than native recombinant apoA-1 and are therefore easier to produce, a characteristic that is of particular interest for drug development. In animal studies, treatment with apoA-1 mimetic peptides has been shown to result in increased levels of proβ–HDL-particles that promote cholesterol efflux and reduce atherosclerotic plaque formation, potentially by the anti-inflammatory and antioxidant nature of these particles.56,57 Several compounds have been developed by replacing nonpolar amino acids with an increasing number of phenylalanine (F) or alanine (A) residues and L- versus D-stereoisomer configuration to influence lipid affinity and hydrophobic characteristics of the peptide. For the outcome of the studies with these mimetic peptides, we refer to a recent review by Osei-Hwedieh et al.58

**FX-5A**

Only few studies have been performed to test apoA-1 mimetic peptides in humans. Recently, Kinemed has received a National Institutes of Health clinical development grant to aid the rapid development of the apoA-1 mimetic FX-5A for infusion therapy after ACS.59 FX-5A is based on the initial compound 5A. Administration of 5A complexed with phospholipids in apoE−/− mice resulted in increased HDL cholesterol and phospholipid content, together with an increase...
in cholesterol efflux. Three weekly infusions for 13 weeks in apoE−/− mice resulted in a 29% (6.6±0.5 versus 9.3±1.2; P=0.05 F-5A complexed with phospholipids) and 54% (3.2±0.4 versus 6.9±0.6; P=0.001; F-5A complexed with sphingomyelin/phospholipid combination) smaller aortic lesion area, compared with saline control. In addition, 5A complexed with 1-palmitoyl-2-linoleoyl phosphatidylcholine exerted anti-inflammatory and antioxidant characteristics in a rabbit model. The first administration of FX-5A in humans is planned with 5 weekly infusions in patients with ACS.

**ETC-642**

ETC-642 is an apoA-1 mimetic peptide that was in early stage clinical development for short-term subacute treatment after ACS to reduce cardiovascular events. ETC-642 is intravenously administered as a peptide–phospholipid complex. In a hyperlipidemic rabbit model, ETC-642 inhibited the progression of atherosclerosis as assessed by IVUS. The effect of ETC-642 on cholesterol efflux was comparable with recombinant HDL, and ETC-642 exhibited anti-inflammatory effects comparable with apoA-1. Currently, there are no planned or ongoing clinical studies in humans.

**Oral ApoA-1 Mimetics**

The invasive nature of the administration of full-length infusible apoA-1 can be considered as a barrier for large-scale clinical application, and this has driven the quest for small apoA-1 mimetic peptides that can be taken orally. In a placebo-controlled pilot study by Bloedon et al., a single administration of 4 doses of the oral apoA-1 mimetic peptide D-4F was studied in high-risk patients with CVD (total n=40, n=8 per treatment arm), but no effect on lipid parameters was observed. However, a significant improvement of the HDL-inflammatory index was reported, despite the presence of low plasma concentrations of D-4F. This index is a reflection of the capacity of HDL to inhibit LDL-induced monocyte chemotactic activity. In a subsequent placebo-controlled study by Watson et al., the apoA-1 mimetic peptide L-4F was administered daily either intravenously for 7 days or subcutaneously for 28 days. Higher plasma concentrations of L-4F were measured compared with the D-4F results in the study by Bloedon et al, but this did not result in any effects on plasma lipid levels or on the HDL-inflammatory index.

Interestingly, a recent report was published by the same research group, who engineered transgenic tomatoes expressing the apoA-1 mimetic peptide 6F. The tomatoes were processed to a powder that was administered to LDLR−/− mice on a Western diet. A significant reduction in aortic atherosclerotic lesions was found in the mice receiving L-6F compared with mice treated with a powder derived from tomatoes engineered with an empty vector. Strikingly, intact L-6F was found in the small intestines of the animals, whereas no L-6F was measured in plasma, which suggests an intestinal specific effect of this novel apoA-1 mimetic, as was previously reported for 4F. To the best of our knowledge, only preclinical studies have been performed and, to date, no further studies in humans have been announced.

**ApoA-1 Induction**

RVX-208 is an orally administered agent that has been shown to induce endogenous apoA-1 synthesis. RVX-208 inhibits the bromodomain of a bromodomain and extra terminal protein that inhibits apoA-1 transcription and plays an important role in epigenetics by detecting the presence of histone acetylation. Therefore, RVX-208 has an effect on the expression of the apoA-1 gene at a molecular level. Cynomolgus monkeys were treated with RVX-208, which resulted in a significant increase of apoA-1 (60%) and HDL-C (97%) levels and a 60% increase of α1 HDL subparticles. Comparable results were obtained in a 1-week phase I study in humans. The ApoA-1 Synthesis Stimulation Evaluation in Patients Requiring Treatment for Coronary Artery Disease (ASSERT) trial was the first large-scale clinical trial evaluating the effect of increasing doses of RVX-208 for 12 weeks in 299 statin-treated patients with coronary artery disease. Compared with baseline, plasma apoA-1 levels did increase significantly and dose dependently to up to 5.6% in the patients treated with RVX-208 (P=0.035 for trend), but this was not statistically significant when compared with placebo. RVX-208 treatment also resulted in a significant increase in HDL-C levels (up to 8.3%) compared with placebo (P=0.02 for trend). The largest increases were observed in the last 4 weeks of the trial, without reaching a plateau phase, suggesting potential further HDL-C increases with prolonged treatment. None of the other plasma lipid parameters did change significantly on RVX-208 administration. A significant dose-dependent trend was observed for transaminase increases, with 18 patients having >3x upper limit of normal and 8 patients >8x upper limit of normal. The transaminase increase returned to baseline within 2 weeks after treatment cessation in all patients, and none were accompanied by bilirubin increases. After the ASSERT trial, 2 subsequent placebo-controlled studies have been announced. In the Study of Quantitative Serial Trends in Lipids with Apolipoprotein A-I Stimulation (SUSTAIN), 172 patients on stable statin therapy with low levels of HDL-C will be treated for 24 weeks, and the percentage change in HDL-C will be assessed. In SUSTAIN, topline results reveal that all prespecified end points were reached with high statistical significance (ClinTrials.gov identifier NCT01423188). In the ApoA-1 Synthesis Stimulation and Intravascular Ultrasound for Coronary Atheroma Regression Evaluation (ASSURE) trial, 310 patients with coronary artery disease will be treated for 26 weeks, and nominal change of percentage of atheroma volume will be assessed by IVUS compared with baseline (ClinTrials.gov identifier NCT01067820). It is of note that the transaminase increase in the ASSERT trial was primarily observed in simvastatin-treated patients, and patients on simvastatin will therefore be excluded from the other phase IIb studies.

On June 27, 2013, Resverlogix reported that the ASSURE trial did not meet its primary end point of −0.6% atheroma volume regression. The RVX-208–treated group had −0.4% regression in atheroma volume (P=0.08). Secondary end points of total atheroma volume regression and increases in ApoA-1 and HDL-C were reached. Analysis of the full data set is awaited to assess the consequences for future development of RVX-208.
Upregulation of ABC Transporters to Induce Cholesterol Efflux

ABCA1 and ABCG1 are transmembrane transport proteins responsible for the transfer of free cholesterol from peripheral cells onto (nascent) HDL-particles. As such, they play an important role in RCT, and consequently they should be considered as potential targets for therapy.

Liver X Receptor Agonists

Liver X Receptors (LXRs) are nuclear receptors with downstream effects on the expression of several lipid related genes of which ABCA1 and ABCG1 upregulation are pivotal. LXRs form heterodimers with the nuclear transcription factor retinoid X receptor and, on heterodimerization, they bind to LXR response elements in target genes, thereby affecting gene expression. Calkin and Tontonoz71 recently published an extensive review on this subject.

LXR agonists exhibit a significant decrease of atherosclerotic plaque formation in apoE−/− and LDLr−/− mouse models.71 In addition, LXR agonists have been shown to exert anti-inflammatory characteristics.73,74 However, the beneficial effects of LXR agonists in animal studies have been largely overshadowed by a concomitant increase of hypertriglyceremia and hepatic steatosis.74 Recent efforts have led to the development of novel, more specific LXR-α agonists to overcome the negative effects on TG and liver metabolism. These compounds are still in preclinical investigation.76–78

MicroRNA 33

ABCA1 and ABCG1 expressions are partly regulated by microRNA 33 (miR-33). MicroRNAs are short noncoding RNA sequences that have an impact on the transcription of several genes, by causing either repression or destabilization of target mRNA. MiR-33 has been shown to reduce HDL-C levels by the inhibition of hepatic ABCA1 expression. In macrophages, miR-33 inhibition of ABCA1 and ABCG1 expression resulted in reduced cholesterol efflux to HDL.79 An antisense oligonucleotide has been developed that effectively silences miR-33 and increases ABCA1 and ABCG1 expressions. The net result, enhanced cholesterol efflux, induced regression of atherosclerotic plaques in LDLr−/− mice.80 Subsequent studies in nonhuman primates showed that 12-week administration of antisense oligonucleotides to miRNA-33 resulted in a 50% increase in HDL-C and a decrease in VLDL by inducing ABCA1 expression as well as other proteins involved in fatty acid metabolism.81 No human studies have been announced to date.

All together, LXR agonists and miR-33 antisense therapy hold promise for the future. However, it should be noted that animal models do not represent human biology, and that human data should be awaited to assess the potential of these novel pathways.

Selective PPAR Modulators

Fibrates have been shown to induce beneficial effects on lipid metabolism but, as described above, the effects on CVD outcome are controversial. The lipid-lowering effect of fibrates is mediated by activation of the PPAR-α pathway, but fibrates are rather weak activators of this pathway. More specific PPAR-α agonists with a greater binding potency are in development. Of these novel compounds, K-877 is currently at the most advanced stage. In a phase II trial, 224 individuals with fasting TG ≥200 mg/L and HDL-C <50 mg/L for men and <55 mg/L for women were randomized to either K-877, fenofibrate, or placebo. A 12-week administration of K-877 resulted in a substantial decrease of TG, large VLDL-C, (very) small LDL-C, and increase of HDL-C levels compared with placebo and fenofibrate treatment. A rise in creatinine and homocysteine levels was not observed in K-877-treated patients, although this effect has been observed in some fibrate-treated individuals.82 Phase III studies in Japanese patients and Phase II dose-finding study in white patients are currently ongoing.

Additional dual PPAR-α/γ and -β/δ agonists are in development. α/γ- and β/δ-receptors have isotype-specific functions and induce partially overlapping expression patterns.83 These new dual PPAR agonists have promising beneficial effects in the treatment of the metabolic syndrome and nonalcoholic steatohepatitis.84 Some of these new drugs, like GFT505, have been shown to increase HDL-C by 9.3% in dyslipidemic patients.85 No clinical outcome studies with these compounds, however, are in the planning stage.

Lecithine-Cholesterol Acytransferase

Lecithine-cholesterol acyltransferase (LCAT) mediates the esterification of free cholesterol located at the surface of lipoprotein particles. The hydrophobic cholesteryl esters then migrate to the core of the particle, which does result in an increase of the size of the particle. In this way, LCAT creates a driving force for free cholesterol uptake by HDL, and it has been argued that LCAT plays a major role in RCT.77,99 LCAT deficiency gives rise to 2 distinct disorders, familial LCAT deficiency and fish-eye disease. Familial LCAT deficiency is characterized by HDL deficiency, renal impairment, anemia, and corneal opacification, whereas fish-eye disease causes HDL-C deficiency only. The effect of LCAT deficiency on CVD risk is subject to debate. Recently, a 42% prevalence of coronary artery disease (16 out of 38) was noticed in heterozygous carriers of LCAT loss of function mutations with HDL-C levels below the 5th percentile for age and sex.86 This finding was in line with previous reports from the same group.77,78 However, other studies reported no association between LCAT mutations and LCAT levels and CVD risk.13,89 Consequently, despite decades of research, the final verdict on LCAT and its role in atherosclerosis is still out.99

Currently, recombinant LCAT (ACP-501) replacement therapy is in an advanced stage of development. In a phase I study, comprising a total of 16 stable patients with coronary artery disease, it was shown that a single intravenous administration ACP-501 was safe and well tolerated. Compared with baseline, HDL-C levels increased by 0.40 mmol/l in the group who were exposed to the highest dose. As expected, this HDL-C increase was accompanied by a substantial shift from small to larger, more mature HDL-particles.90 Further studies are anticipated to be focussed on the treatment of ACS and on chronic enzyme replacement therapy in patients with familial LCAT deficiency.

CETP Inhibitors

CETP is secreted primarily by liver and adipose tissue and, on entering circulation, CETP mediates the transfer of cholesteryl
esters from HDL to apolipoprotein-B–containing particles (mainly LDL and VLDL) in exchange for TGs. Hence, CETP-inhibition will result in retainment of cholesteryl esters in HDL and a decrease of the cholesterol content within the atherogenic apo-B–containing particles.

Large Mendelian randomization studies and meta-analyses have shown that CETP variants resulting in lower CETP activity are associated with elevated HDL-C levels, lower LDL-C levels, and a low CVD risk. In addition, pharmacological CETP-inhibition in rabbits resulted in reduced atherosclerotic plaque formation. Taken together, there is ample evidence to support CETP-inhibition in humans.

To date, 5 CETP-inhibitors have been tested in humans, of which 2 phase III trials were terminated prematurely (torcetrapib, dalcetrapib). Two large CVD outcome trials are still in the recruiting phase: the Randomized Evaluation of the Effects of Anacetrapib Through Lipid-modification (REVEAL) and the Assessment of Clinical Effects of Cholesteryl Ester Transfer Protein Inhibition With Evacetrapib in Patients at a High-Risk for Vascular Outcomes (ACCELERATE) studies.

The first phase III CVD outcome trial (ILLUMINATE) was performed with torcetrapib and was prematurely terminated because of excess of overall mortality and major cardiovascular events in the torcetrapib-treated patients. The Dal-OUTCOMES trial was discontinued for futility, without evidence of increased harm by dalcetrapib treatment. In this section, we summarize the implications of the discontinued trials with torcetrapib and dalcetrapib, discuss the rationale for additional CETP-inhibitor development, and present an update on the 3 CETP-inhibitors currently tested in phase II and III trials.

Torcetrapib

Torcetrapib was the first CETP-inhibitor investigated in a large phase III study. In the ‘Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic events’ (ILLUMINATE) trial, 15,607 patients at high CVD risk were randomized to either torcetrapib or placebo on top of atorvastatin treatment. Torcetrapib treatment resulted in a remarkable 72.1% increase in HDL-C and a 24.9% decrease in LDL-C. However, unexpectedly, total mortality and major CVD event rates were significantly higher in the torcetrapib-treated patients, leading to premature cessation of the trial. The cause of this excess in mortality has been the subject of much debate, and the off-target vasopressor effect of torcetrapib is commonly accepted as one of the underlying mechanisms for this detrimental outcome. This vasopressor effect is driven by adrenal aldosterone and cortisol release as well as endothelin 1 upregulation. Post hoc analyses showed that the increased all-cause mortality and CVD-related mortality was mainly restricted to those patients in whom a large decrease in potassium and increase in bicarbonate levels was found. Subsequent preclinical studies showed a similar effect of torcetrapib in mice without CETP, and in vitro studies showed that torcetrapib induced aldosterone and cortisol release from adrenocortical cells. It is of note that no effect on blood pressure or aldosterone levels was observed for dalcetrapib, anacetrapib, or evacetrapib. In further support of CETP independent effects on blood pressure, Sofat et al reported no association between several CETP polymorphisms and blood pressure in a cohort of 67,687 individuals.

All together the most plausible explanation for the excess mortality caused by torcetrapib is an off-target vasulotoxic effect, independent of CETP-inhibition.

Dalcetrapib

The dal-OUTCOMES trial, the first placebo controlled phase III randomized controlled trial investigating the effect of dalcetrapib in 15,871 statin-treated patients, was terminated early in May 2012 for futility. Compared with placebo, dalcetrapib treatment resulted in a 30% increase of HDL-C levels, without a significant effect on LDL-C levels. There was, however, no significant benefit in overall and CVD-related mortality and major cardiovascular events (hazard ratio 1.04, 95% CI [0.93–1.16]; P=0.52), which formed the basis for the advice of the Data Safety Monitoring Board (DSMB) to terminate the trial.

Apart from cardiovascular event rates, the efficacy of dalcetrapib on surrogate end points has been studied. The dal-PLAQUE study used multimodality imaging (magnetic resonance imaging and positron emission tomography -computed tomography) to test the effect on vessel wall structure and inflammation of 2-year dalcetrapib treatment (n=64) compared with placebo (n=66). A small change in total vessel area was observed in the dalcetrapib-treated patients (−0.01 mm²; 90% CI [−0.73 to −0.00]; P=0.04), without a substantial difference in positron emission tomography -computed tomography measured grade of vascular inflammation. In a subgroup of patients with low baseline HDL-C, dalcetrapib treatment resulted in beneficial effects on endothelial function. However, in the subsequent dal-VESEEL study, specifically designed to test this hypothesis, there was no evidence supporting the beneficial effect of dalcetrapib on endothelial function.

Importantly, no significant difference in blood pressure or aldosterone levels emerged in any of the trials mentioned above, and there was no evidence for increased mortality or major adverse event rate associated with dalcetrapib treatment, despite a 50% reduction of CETP activity. This further underlines a CETP independent mechanism for the observed torcetrapib toxicity.

The dal-OUTCOMES study was powered to detect a 15% relative risk reduction for major cardiovascular events based on the observed HDL-C increase of 30%. The failure of dalcetrapib to reduce cardiovascular events in this trial has raised serious questions on the direct causal effects of HDL-C on atherosclerosis and CVD risk.

Rationale for Additional CETP Inhibitor Development

Currently, 3 other CETP-inhibitors are in different stages of clinical investigations (anacetrapib, evacetrapib, and TA-8995). The rationale for this is that, unlike dalcetrapib, these CETP-inhibitors have been shown to have beneficial effects on other lipid parameters, such as LDL-C and lipoprotein(a). The currently tested drugs anacetrapib, evacetrapib, and TA-8995 are therefore considered much more potent than dalcetrapib.

Anacetrapib

The Determining the Efficacy and Tolerability of CETP Inhibition with Anacetrapib (DEFINE) trial was performed

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to investigate the safety and efficacy of anacetrapib in 1623 patients with high cardiovascular risk and prespecified HDL-C levels <1.6 mmol/L. After 76 weeks, LDL-C levels were decreased by 36.2% and HDL-C increased by 138.8% compared with placebo-treated patients. Treatment with anacetrapib was safe and well tolerated, and no effect on blood pressure, electrolyte, or aldosterone levels was observed. In addition, in vitro studies showed that HDL isolated from anacetrapib-treated patients was characterized by normal or even enhanced ability to induce cholesterol efflux from macrophages. A large phase III end point study, Randomized Evaluation of the Effects of Anacetrapib Through Lipid-modification (REVEAL), is ongoing, and ≈30,000 patients with established cardiovascular disease have been enrolled in this trial. These patients are either randomized to 100 mg of anacetrapib or placebo on top of current best-practice guidelines. The predicted follow-up for this study is 4 to 5 years, and completion of the trial is expected to be in 2017 (ClinicalTrials.gov Identifier: NCT01252953).

Given the design of the trial, one can again expect LDL-C levels to be low, just like HPS2-THRIVE. It is questionable whether this specific population, with such ideal lipid levels and low risk, should be exposed to anacetrapib, and whether this therapy should not be reserved for patients at high CVD risk, that is, those elevated TG and low HDL-C levels to be low, just like HPS2-THRIVE. It is questionable whether this specific population, with such ideal lipid levels and low risk, should be exposed to anacetrapib, and whether this therapy should not be reserved for patients at high CVD risk, that is, those elevated TG and low HDL-C levels or those with high LDL-C levels despite aggressive lipid-lowering therapy.

**Evacetrapib**

The most recently developed CETP-inhibitor currently undergoing a phase III CVD outcome trial is evacetrapib. In a phase II study, 398 patients were randomized to 10 treatment arms to receive either placebo or a range of doses of evacetrapib both as monotherapy as well as in combination therapy with statin regimes. After 12 weeks, a dose-dependent increase in HDL-C of 53.6% to 128.8% for monotherapy and 78.5% to 88.5% in combination with statin therapy was observed. Evacetrapib monotherapy resulted in a dose-dependent LDL-C plasma level decrease of −13.6% to −35.9% and in combination with statins of −11.2% to −13.9% compared with statin monotherapy. Evacetrapib was well tolerated and evacetrapib treatment was not associated with increased blood pressure, aldosterone, cortisol, or electrolyte changes.

The Assessment of Clinical Effects of Cholesterol Ester Transfer Protein Inhibition With Evacetrapib in Patients at a High-Risk for Vascular Outcomes (ACCELERATE) phase III CVD outcome trial is currently recruiting an estimated 11,000 patients at high CVD risk to randomize to either evacetrapib 130 mg or placebo on top of optimal lipid-lowering treatment (ClinicalTrials.gov Identifier: NCT01687998).

**TA-8995**

The CETP-inhibitor TA-8995 is being developed by Dezima Pharma for the treatment of the dyslipidemia and was previously developed by Mitsubishi Tanabe. A first-time-in-human single ascending dose study has been performed in healthy volunteers and also included assessment of food-effect, sex-effect, and age-effect. Single oral doses of TA-8995 were well tolerated, and pharmacokinetic properties were consistent with once-daily dosing. There were no consistent or clinically important differences in the pharmacokinetics or pharmacodynamics of TA-8995 related to ethnicity, age, or sex. A multiple-dose study in healthy subjects has also been performed. All doses were considered safe and well tolerated with no serious adverse events. After cessation of dosing, TA-8995 was fully eliminated in the washout period. TA-8995 maximally inhibited CETP in healthy volunteers and, as expected, increased HDL-C levels up to 140% and decreased LDL-C levels by 44% at steady state. TA-8995 is about to be evaluated in a Phase II clinical trial. The trial is a multicenter, randomized, double blind, placebo controlled, parallel group study of TA-8995 in patients with mild dyslipidemia, alone and in combination with statin therapy. It is being performed in Europe, and recruitment is expected to start Q3 2013.

**Conclusions**

The large residual burden of disease in patients with CVD necessitates the search for novel therapeutics. In the past decade, HDL-C increasing therapies were at the center of research attention. However, results from clinical outcome studies have been disappointing, questioning whether an increase of cholesterol in the HDL-particle should be the primary aim. Despite the failure of 2 large CVD outcome trials addressing the role of CETP-inhibitors, safer and more potent CETP-inhibitors are currently investigated in large clinical trials. Nevertheless, a definite answer to the question whether HDL-C increase, per se, is beneficial in CVD risk reduction will not be derived from these trials because CETP-inhibitors both increase HDL-C and decrease LDL-C levels. This answer is more likely to be derived by studies addressing the effect of the more specific HDL increasing therapies such as HDL/apoA-1–based infusions. The preliminary data are promising, but a definite statement can only be provided by well-designed end point trials. In addition, one could expect a shift in paradigm; whereas traditionally the main focus was to raise HDL-C levels, in the future HDL functionality might become a surrogate marker. It should be noted, however, that controversial data on the role of efflux capacity in CVD risk has been published in recent years. The recent failures in clinical trials have clearly taught us to be cautious and not to follow presumptions based on epidemiology, but rather follow clinical evidence when it comes to decision making.

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